Hodgkin lymphoma in Iceland

A clinico-pathological study

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Thesis for the degree of Master of Science

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October 2010

Hodgkin eitilfrumukrabbamein á Íslandi

Klínísk og meinafræðileg rannsókn

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Ritgerð til meistaragráðu í læknisfræði

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Læknadeild

Heilbrigðisvísindasvið Háskóla Íslands

Október 2010

Prentun: Háskólaprent ehf.

Reykjavík, Ísland, 2010

Abstract

Indroduction

Hodgkin lymphoma (HL) is a rare malignancy with an incidence of 2-3 per 100.000 in the developed world. It is classified into five categories that differ in their clinical presentation and morphology. The malignant cell, the Reed-Sternberg cell, is of B-cell origin and composes approximately 1% of the tumour mass. HL has in the last 50 years gone from being a universally fatal disease to a highly curable one, with over 80% five year survival. The mainstay of therapy is chemotherapy, sometimes followed by local radiation therapy.

Methods and Material

All patients diagnosed with HL in the years 1990-2005 were identified in the Icelandic Cancer Registry. Clinical and treatment related information was collected and lymph node biopsies examined. Tissue microarray blocks were made and immunohistochemical staining performed for CD30, CD15, CD20, CD45, CD3, CD5, LMP1, MUM1, bcl-2 and bcl-6. Survival analysis was done using Kaplan-Meier curve and groups compared using the log-rank test. Multivariate analysis was done using the Cox proportional hazard model. Comparison of groups was made with the Chi-squared-test or the Fisher's-Exact test, comparison of means was done with the Student's-t-test. Results were considered significant if $p<0.05$.

Results

There were 105 patients diagnosed with HL in Iceland, making the age standardized incidence 2.05 per 100.000. A bimodal age curve was seen with one peak for young adults and the other after 70 years. The male:female ratio was 3:2. The most common histological subtype was nodular sclerosis. Expression of antigens in classical HL was as follows: CD30 95%, CD15 52%, CD20 15%, LMP1 23%, MUM1 87% and bcl-2 36%. MOPP/ABVD was the most used chemotherapy in the first half of the period and ABVD in the second half. The form of radiation therapy changed, with radiation field and dose decreasing with time. Seven patients had high dose chemotherapy with autologus stem cell transplantation. Overall five year survival was 81%. In univariate analysis the following factors were associated with worse survival: female gender, age 60 years and higher, stage III/IV disease, infradiaphragmatic disease, other subtype than nodular sclerosis and mixed cellularity, and lack of MUM1 expression. In the multivariate analysis only age and bulky disease were associated with worse survival and CD15 was a possible positive prognostic factor.

Conclusion

HL in Iceland is similar to HL in other Western countries with regard to both clinical and histological factors. Treatment modalities have followed what is considered the accepted standard of care. Results from the immunohistochemical staining are similar to other reports however results are highly variable between studies, as is criteria of interpretation. Low mortality rate in the study made it hard to find prognostic factors and only increasing age was found to be the strong negative prognostic factor although bulky disease was a probable negative prognostic factor. MUM1 and CD15 expression were possible positive prognostic factors but need to be validated in a larger cohort.

Ágrip

Inngangur

Hodgkin eitilfrumukrabbamein (Hodgkin lymphoma, HL) er sjaldgæft krabbamein og er aldursstaðlað nýgengi 2-3 per 100.000 íbúa í hinum vestræna heimi. Það er flokkað í fimm flokka sem eru ólíkir varðandi meinafræðilega og klíníska hegðun. Æxlisfruman, Reed-Sternberg fruma, er af B frumu uppruna og myndar hún innan við 1% af æxlinu. Á síðastliðinni hálfri öld hefur HL farið frá því að vera ólæknandi í að vera oftast læknanlegur sjúkdómur með fimm ára lifun yfir 80%. Helsta meðferðin er samsett krabbameinslyfjameðferð stundum ásamt staðbundinni geislameðferð.

Efni og aðferðir

Upplýsingar fengust frá Krabbameinsskrá Íslands um alla þá sem greindust með HL frá 1990-2005. Sjúkraskrár voru yfirfarnar og klínískum og meðferðartengdum upplýsingum safnað. Vefjasýni úr eitlum voru yfirfarin, gert *tissue microarray* og ónæmisfræðilegar litanir á sýnum framkvæmdar fyrir CD30, CD15, CD20, CD45, CD3, CD5, LMP1, MUM1, bcl-2 og bcl-6. Einþáttalifunargreining var gerð með Kaplan-Meier aðferð og hópar bornir saman með log-rank prófi. Fjölþáttalifunargreining var gerð með Cox líkani. Samanburður á hópum var gerður með Kí-kvaðrat prófi eða Fisher's-Exact prófi. Samanburður á meðaltölum var gerður með tvíhliða t-prófi. Niðurstöður voru taldar marktækar ef $p<0.05$.

Niðurstöður

Alls voru 105 sjúklingar greindir með HL og aldursstaðlað nýgengi því 2.05 per 100.000 íbúa. Rannsóknin sýndi að á Íslandi er nýgengi hæst hjá ungum fullorðnum og eftir 70 ára aldur. Kynjahlutfallið var þrír karlar fyrir hverjar tvær konur. Algengasti vefjaundirflokkurinn var *nodular sclerosis*. Tjáning fyrir mótefnavökum í klassískum HL sjúkdómi var eftirfarandi: CD30 95%, CD15 52%, CD20 15%, LMP1 23%, MUM1 87% og bcl-2 36%. MOPP/ABVD lyfjameðferð var algengust á fyrri hluta tímabilsins en ABVD á seinni hluta tímabilsins. Notkun geislameðferðar breyttist á tímabilinu, þannig að bæði geislasvæðin og geislaskammturinn minnkuðu. Háskammtalyfjameðferð og stofnfrumuígræðsla var gerð í sjö sjúklingum. Fimm ára lifun var 81%. Í einþáttalifunargreiningu voru eftirfarandi þættir tengdir verri lifun: kvenkyn, aldur 60 ára og eldri, sjúkdómur á stigi III/IV, sjúkdómur neðan þindar, undirflokkar aðrir en *nodular sclerosis* eða *mixed cellularity* og tap á MUM1 tjáningu. Í fjölþáttalifunargreiningu voru eingöngu hækkandi aldur og fyrirferðarmikill sjúkdómur tengdir verri lifun en CD15 tjáning var mögulegur verndandi þáttur.

Ályktanir

HL á Íslandi er svipaður sjúkdómur og HL í öðrum vestrænum löndum hvað varðar klíníska og meinafræðilega þætti. Meðferðin hefur fylgt því sem er viðurkennt á hverjum tíma. Niðurstöður úr ónæmisfræðilegum litunum fylgja að mestu niðurstöðum eins og sést hafa í öðrum rannsóknum. Þær niðurstöður eru þó mjög mismunandi og þyrftu aðferðir við túlkun að vera betur staðlaðar. Lág dánartíðni olli því að erfitt var að finna forspárþætti fyrir horfur. Hækkandi aldur var þó sterkur neikvæður forspárþáttur og fyrirferðarmikill sjúkdómur mögulegur neikvæður áhættuþáttur. Tjáning á MUM1 og CD15 voru hugsanlegir jákvæðir forspárþættir en klínískt gildi þeirra þyrfti að sannreyna í stærri rannsókn.

Acknowledgements

First and foremost I would like to thank my supervisor, Bjarni A. Agnarsson for his help on designing the study, his helpful comments and guidance throughout the research and writing the thesis. Then I would like to thank Friðbjörn Sigurðsson and Brynjar Viðarsson in my masters committee for very valuable advice and help in writing the thesis.

A big thanks goes to Kristrún Ólafsdóttir the chief histotechnologist for doing the tissue microarray and immunohistochemical staining. She was exceptionally helpful in her guidance in the laboratory.

Then I would like to thank Anna Helga Jónsdóttir and Jóhanna Sigmundsdóttir from Tölfræðimiðstöð Háskólans but they really did go beyond their duty in trying to solve some complex statistical problems we were facing.

For financial support I would like to thank Samtök krabbameinsrannókna á Íslandi, Krabbameinsfélag Reykjavíkur, Vísindasjóður Landspítala and Milli himins og jarðar.

For technical assistance and help in preparing the manuscript I would like to thank my friends Guðný Stella Guðnadóttir and Hulda Rósa Þórarinsdóttir as well as my sisters Ólöf Kristjánsdóttir and Sigrún Kristjánsdóttir.

To my dear partner, Magnús Þórarinsson, I thank you for your patience and thoughtfulness. To my wonderful parents and inlaws, who supported me and my family in so many ways, I thank you so much for your support.

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List of abbreviations

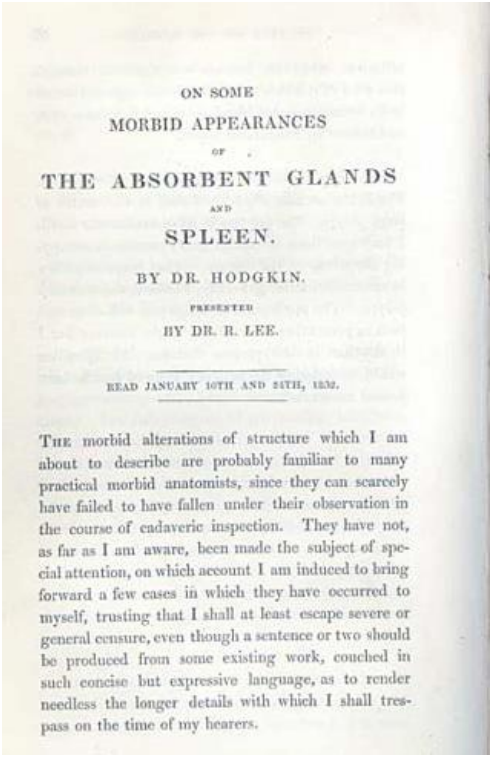
ABVD	Doxorubicin, bleomycin, vinblastine, dacarbazine
ASCT	Autologous stem cell transplantation
BEACOPP	Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone
BEAM	Carmustine, etoposide, cytarabine, melphalan
CHL	Classical Hodgkin lymphoma
CHOP	Cyclophosphamide, doxorubicin, vinblastine and prednisone
CLL	Chronic lymphocytic leukemia
DAB	Diaminobenzidinetrahydrochlorine
DexaBEAM	Dexamethasone, carmustine, etoposide, cytarabine, melphalan
EBNA	Epstein-Barr virus nuclear antigen
EBV	Epstein-Barr virus
ESMO	European Society for Medical Oncology
H&E	Hemotoxylin and eosin
HDCT	High-dose chemotherapy
HL	Hodgkin lymphoma
ICE	Ifosfamide, carboplatin, etoposide
IQR	Interquartile range
L&H	Lymphocytic/histiocytic
LD	Lymphocyte depleted
LMP	Latent membrane protein
LP	Lymphocyte predominant
LR	Lymphocyte rich
MC	Mixed cellularity
MIME	Methyl-GAG, ifosfamide, methotrexate, etoposide
MOPP	Mechlorethamine, vincristine, procarbazine, prednisone
MOPP-ABVD	Mechlorethamine, vincristine, procarbazine, prednisone -doxorubicin, bleomycin, vinblastine, dacarbazine
MUM1	Multiple myeloma 1/interferon regulatory factor 4
NCCN	National Comprehensive Cancer Network
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B
Non-HL	Non-Hodgkin lymphoma
NS	Nodular sclerosis
PET	Positron emission tomography
REAL	Revised European-American Lymphoma
WHO	World Health Organization

1 Introduction

1.1 History

In 1832 Thomas Hodgkin, a British pathologist and physician, described the clinical presentation and gross anatomy of seven patients in his paper "On Some Morbid Appearances of the Absorbent Glands and the Spleen". He observed that all the patients had in common an enlargement of the lymph nodes that was frequently associated with swelling of the spleen (1). Figure 1 and figure 2 show Hodgkin's portrait and his original report.

Figure 1. Thomas Hodgkin (1798-1866) (2).



ON SOME

MORBID APPEARANCES

OF

THE ABSORBENT GLANDS

AND

SPLEEN.

BY DR. HODGKIN.

PRESENTED

BY DR. R. LEE.

READ JANUARY 10TH AND 24TH, 1832.

THE morbid alterations of structure which I am about to describe are probably familiar to many practical morbid anatomists, since they can scarcely have failed to have fallen under their observation in the course of cadaveric inspection. They have not, as far as I am aware, been made the subject of special attention, on which account I am induced to bring forward a few cases in which they have occurred to myself, trusting that I shall at least escape severe or general censure, even though a sentence or two should be produced from some existing work, couched in such concise but expressive language, as to render needless the longer details with which I shall trespass on the time of my hearers.

Figure 2. Thomas Hodgkin original report, "On some morbid appearances of the absorbent glands and spleen" (1).

Sixteen years later Sir Samuel Wilks, unaware of Hodgkin's work, published a more detailed clinical and pathological description of the disease (3). Some of his patients where the same as in Hodgkin's paper and when he realized that he named the disease Hodgkin's disease (4). These papers were based on clinical symptoms and autopsy reports in which gross anatomy was described but little attention was given to the microscopic appearance. Around 1900 several descriptions of the multinucleated giant cell typical of Hodgkin disease were published. Its description was however credited to and named after Carl Sternberg and Dorothy Reed who gave the cell a more definite and thorough histopathological description, hence the name Reed-Sternberg cell (Figure 3) (5, 6). One of the unusual aspects of Hodgkin disease was that the malignant cell represents only a minority of the tumour mass and lies in a reactive background of lymphocytes, neutrophils, eosinophils, histiocytes and fibroblasts (7).

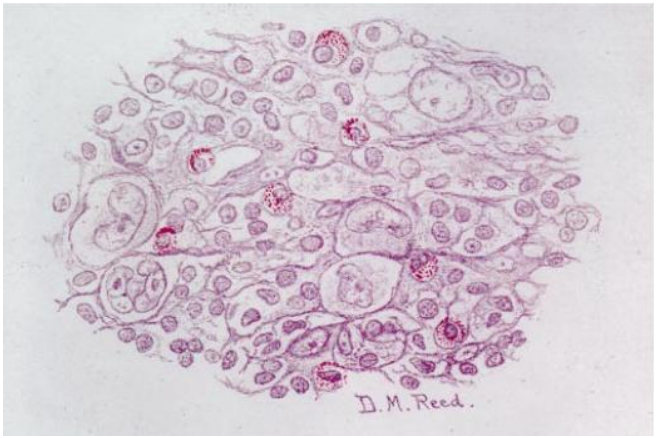


Figure 3. Hand drawn picture of the multinucleated giant cell, by Dorothy Reed (2).

1.1.1 Classification of Hodgkin lymphoma

In 1947 Jackson and Parker published the first histological classification of Hodgkin disease (2). Their classification had three subtypes or paragranuloma, granuloma and sarcoma, but as it had little clinical relevance it was replaced in 1966 when Lukes and Butler proposed a new more clinically relevant classification (8). That classification divided Hodgkin disease into six groups: a) lymphocytic and/or histiocytic (L&H) nodular, b) lymphocytic and/or histiocytic (L&H) diffuse, c) nodular sclerosis, d) mixed, e) diffuse fibrosis and f) reticular. This classification was then altered at a conference in Rye in New York USA, and contained four subtypes: lymphocyte predominant (LP), nodular sclerosis (NS), mixed cellularity (MC) and lymphocyte depleted (LD) (Rye classification) (9). In 1993 the Revised European-American Lymphoma (REAL) classification was presented at the International Lymphoma Study Group meeting in Berlin, Germany. The meeting was held to find a universally accepted classification scheme for lymphomas. The REAL classification divides Hodgkin disease into lymphocyte predominant HL and classical HL (CHL), which include nodular sclerosis, mixed cellularity and lymphocyte depleted with lymphocyte rich (LR) as a temporary subgroup. Those two main categories differ in morphology, immunophenotype and clinical course (10). In 2001 the currently used classification from the World Health Organization (WHO) was introduced (Table 1). It accepted the two major subdivisions as in the REAL classification, it made the lymphocyte rich category an independent subgroup and the term Hodgkin disease was replaced by Hodgkin lymphoma (HL) (11).

Table 1. The World Health Organization classification of Hodgkin lymphoma (7).

Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis
Mixed cellularity
Lymphocyte rich
Lymphocyte depleted

1.1.2 Staging of Hodgkin lymphoma

A need for an anatomic staging system emerged with evolution of treatment and diagnostic procedures. Thus in 1950 Vera Peters introduced a three step staging approach of HL: stage I involving a single lymph node or a region, stage II involving two or more proximal lymph nodes regions on either the upper or lower trunk and stage III involving lymph nodes in two or more regions of both the upper and lower trunk (12). A four step staging system was first introduced at the Rye conference, where anatomic definitions of lymph node regions were made for the purpose of staging. All stages were subclassified as A or B according to constitutional symptoms, B for those with fever, night sweats or pruritus and A for those without those symptoms (2, 9, 12). The presently used staging system was proposed in 1971 at the Ann Arbor conference in Michigan USA (Table 2). Each stage was subdivided as before into A and B although pruritus was excluded as it showed little clinical relevance. B symptoms were defined as: 1) unexplained night sweats, 2) persistent or recurrent fever with temperature >38°C for the last month and 3) weight loss defined as loss of ≥10% of body weight during the last six months. The letter E was designated for patients with localized/adjacent lymph node extralymphatic disease. Staging included both clinical staging and pathological staging with laparotomy and splenectomy (13).

The tools to evaluate the extent of the disease have changed overtime. In 1952 Kinmoth introduced lymphangiography of the lower extremities which proved to be of great value in assessing the extent of the disease in the retroperitoneal and pelvic lymph nodes and conventional X-rays were used to determine the presence of a mediastinal mass (14). Today, computerized tomography is a routine study in the staging of HL, abolishing the need for lymphangiography and pathological staging in HL (15).

Table 2. The Ann Arbor staging of Hodgkin lymphoma (16).

Stage	Definition
I	Involvement of a single lymph node region or lymphoid structure (e.g. spleen, thymus, Waldeyer's ring)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes are lateralized). The number of anatomic sites should be indicated by a suffix (e.g. II_3)
III	Involvement of lymph node regions or structures on both sides of the diaphragm
III_1	With or without splenic, hilar, celiac, or portal nodes
III_2	With paraaortic, iliac, or mesenteric nodes
IV	Involvement of extranodal site(s) beyond those designated E
Annotations	
A: No B symptoms	
B: Fever more than 38°C for the last month, drenching night sweats, or weight loss more than 10% of the body weight in the last six months before diagnosis	
X: Bulky disease: more than 1/3 widening of the mediastinum at T5-6, or maximum of nodal mass more than 10 cm	
E: Involvement of a single extranodal site, or contiguous or proximal to known nodal site of disease	
CS: Clinical stage	
PS: Pathological stage	

1.1.3 Evolution of treatment

X-rays were discovered in 1896 and only six years later reports about their utilization in treating Hodgkin disease were published. For the next twenty years crude X-rays were used to treat lymphoma patients. An initial response was noticed but side effects such as skin burns and ulcerations were prominent resulting in suboptimal control of the disease. With technical development results improved and with the development of the linear accelerator in 1956 extended survival and even cure for some patients with localized disease was possible with high-dose extended field radiation (2, 17). Radiation fields commonly extended to adjacent lymph node areas (extended field radiation therapy) and radiation fields were commonly defined as the mantle zone, inverted Y or total nodal radiation (Figure 4). The mantle zone included the neck, mediastinum and axilla (all areas above the diaphragm) and the inverted Y included the abdominal aorta, the inguinal lymph nodes with or without the spleen (all areas below the diaphragm). For areas on both sides of the diaphragm so called total nodal radiation was used. Nowadays the radiated area is usually limited to the lymph node group affected and is called involved field radiation therapy. Currently radiation therapy is mostly used as a consolidation therapy following chemotherapy (17). A major advantage of minimizing the radiation fields and dose are to decrease the late complications associated with radiation therapy, such as secondary malignancies and increased risk of cardiovascular disease. The risk of secondary solid malignancies starts to increase ten years after radiation therapy and the most common cancers are of breast, lung, gastric and thyroid origin. Smoking adds to the risk of developing lung cancer. The younger the patient is when receiving radiation the bigger the risk is of developing secondary solid tumour. There is however no increased risk of treatment related leukemia associated with radiation. Radiation therapy also leads to a higher risk of cardiovascular disease, including coronary artery disease, valvular disease, myocardial dysfunction, pericardial disease and defects in the electrical conduction system of the heart (18).

A by-product of World War II was the development of the highly toxic nitrogen mustard that later became the first modern anti-tumour drug. Its anti-tumour effect was discovered when sailors who had been exposed to mustard gas developed bone marrow failure. Patients with HL

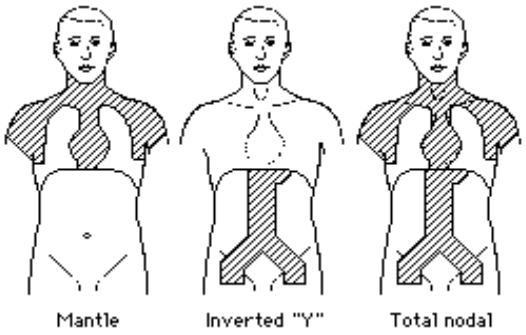


Figure 4. Extended field radiation therapy: mantle zone, inverted Y and total nodal radiation (picture adapted from www.meded.virginia.edu/.../wcd/hodgclinic.cfm).

showed a good clinical response to nitrogen mustard but disease relapse was almost universal (19). Over the next years other anti-tumour drugs where developed, but the biggest problem was the short-lived clinical response. Prognosis for HL was however greatly influenced by the development of the multidrug combination MOPP (nitrogen mustard, vincristine, procarbazine and prednisone), introduced in the mid 1960s by DeVita and colleagues (20). Using MOPP 84% of patients with advanced disease went into complete remission and the ten year relapse-free survival was 66% (21). Another major advancement was the ABVD chemotherapy regimen (doxorubicin, bleomycin, vinblastine, dacarbazine) introduced by Bonadonna and colleagues in the late 1970s (22, 23). The ABVD regimen was found to be both more effective and less toxic than MOPP. ABVD is the standard therapy for HL today to which all other therapies are compared. The MOPP and ABVD chemotherapies will be described in more detail later.

1.2 Pathology and different subclasses

As shown in Table 1, HL has two main subgroups. The first is classical Hodgkin lymphoma, which further divides into NS, MC, LR and LD, and the other is lymphocyte predominant HL (7). These subgroups will now be discussed separately with regard to special features and morphology.

1.2.1 Nodular lymphocyte predominant Hodgkin lymphoma

LP differs from the classical type of HL with regard to morphology, histology, phenotype and clinical course. The morphology is usually nodular, at least in part, but can be diffuse. The malignant cell in LP is called L&H (lymphocytic/histiocytic) cell, and is regarded as a variant of the Reed-Sternberg cell. The L&H cell is large and usually has one large nucleus which is multilobulated and the cells are therefore also termed "popcorn" cells. The nucleoli are smaller than in the classical Reed-Sternberg cells. The reactive infiltrate consists mainly of small lymphocytes with some histiocytes and plasma cells. Eosinophils and neutrophils are rare. The typical phenotype for the L&H cell, CD30-/CD15-/CD20+/CD79a+/bcl-6+, is different from the classical phenotype of the Reed-Sternberg cell (5, 7) (antigens will be discussed later). LP accounts for 5% of all HL, has a unimodal age distribution in the fourth decade, patients are predominantly male and most present at stage I or II. Its course is indolent and spontaneous regression may occur. The ten years overall survival is more than 80% but there is a high rate of late relapse. Advanced stage has an unfavourable prognosis and progression to large B-cell lymphoma has been reported (7).

1.2.2 Classical Hodgkin lymphoma

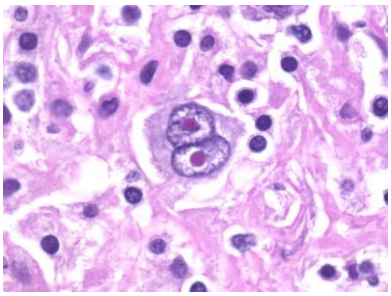


Figure 5. Classic bilobed Reed-Sternberg cell (From http://www.webpathology.com /image.asp?case=388&n=5).

CHL accounts for the vast majority (95%) of all HL in the Western world. It has a bimodal age curve, with typical peaks at 15-35 years of age and another peak late in life. About 55% of patients present with stage I/II disease and 40% have B symptoms at the time of diagnosis. The neoplastic Reed-Sternberg cell only represents a minority of the tumour mass, or 0.1-10%. It is 20-60 µm in diameter and with a large rim of cytoplasm. It is bilobed or multilobulated and has at least two nucleoli that cover more than 50% of the nucleus (Figure 5). A mononuclear variant is called a Hodgkin cell. The presence of a Reed-Sternberg cell is not sufficient to make a diagnosis of a HL because similar cells can be seen in reactive lesions and other neoplastic lesions (7, 9). The classical phenotype in CHL is CD30+/CD15+/CD20- (specifics of the antigens will be discussed later). The microenvironment differs between histological subtypes but the major players are small lymphocytes, eosinophils, neutrophils, histiocytes/macrophages, plasma cells, fibroblasts and collagen fibres. With modern treatment prognosis is similar for all subclasses of CHL when other prognostic factors are adjusted for (5, 7).

1.2.2.1 Nodular sclerosis classical Hodgkin lymphoma

In order to diagnose NS, HL with collagen bands surrounding at least one nodule has to be found. With this fibrosing process the capsule is often thickened. In NS a cytoplasmic retraction is often seen around the Reed-Sternberg cells and they are then termed lacunar cells. These neoplastic cells are found in a reactive infiltrate mostly made of eosinophils, neutrophils and lymphocytes but histiocytes and plasma cells are sparse (5, 7). It is the most common subtype in the Western world and accounts for 2/3 of all HL. It is equally common in men and women and is the only HL type that does not have a male predominance. Most patients present with stage II disease, 40% have B symptoms and mediastinal involvement is seen in 80% of patients (7).

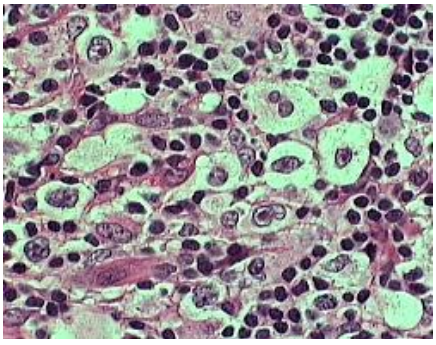


Figure 6. Lacunar cells in reactive infiltrate (From http://www.med-ed.virginia.edu/courses/path/innes/wcd/hodgkin.cfm).

1.2.2.2 Mixed cellularity classical Hodgkin lymphoma

The WHO definition of MC subtype is that of scattered classical Reed-Sternberg cells in a diffuse or vaguely nodular background with mixed inflammatory cells without nodular sclerosing fibrosis. The capsule is not thickened. The background is composed of small lymphocytes, eosinophils,

neutrophils, histiocytes and plasma cells. It is variable which background cell is most prominent. In the Western world it is the second most common subtype and accounts for about 30% of HL. It is more common in the developing countries and is often associated with Epstein-Barr virus (EBV) and Human immunodeficiency virus (HIV) infection. It shows male predominance and patients often present with stage III or IV disease and B symptoms (5, 7).

1.2.2.3 Lymphocyte depleted classical Hodgkin lymphoma

LD morphology usually contains numerous classical Reed-Sternberg cells in a lymphocyte sparse background. It can resemble MC but with more Reed-Sternberg cells. It can be hard to differentiate LD from anaplastic forms of large-cell non-Hodgkin lymphoma (non-HL). The immunophenotype is the same as for other CHL. LD is the rarest variant of HL and accounts for 1-5% of HL patients. It is more common in the developing countries and is often associated with HIV infection. The male to female ratio is 3:1. Patients often present with advanced stage and B symptoms, but show a relative sparing of peripheral lymph nodes (5, 7).

1.2.2.4 Lymphocyte rich classical Hodgkin lymphoma

The typical morphology of LR is that of scattered Reed-Sternberg cells in a nodular or diffuse background. As the name implies lymphocytes are common in the microenvironment. It usually has numerous histiocytes but eosinophils and neutrophils are rare. The Reed-Sternberg cells can resemble L&H cells of LP so one needs to establish a classical HL phenotype for diagnosis which is the same as for other forms of CHL. LR comprises approximately 5% of all HL. It has a higher median age than other HL and 70% of patients are male. Most present with stage I or II disease, B symptoms are rare and the clinical features are similar to LP (5, 7).

1.2.3 Microenvironment

It has been clear for a long time that the microenvironment is important in HL. The malignant cells, the classical Reed-Sternberg cell or the L&H cell, only represent a minority of the tumour mass. The main component of the tumour is that of non-neoplastic B- and T-cells, neutrophils, plasma cell, eosinophils, histiocytes/macrophages and fibroblasts. These cells are recruited by and/or induced to proliferate by tumour cells and produce variable molecules involved in tumour cell growth and survival (24). Several studies have been published on whether specific factors in the microenvironments affect survival. Of those, some have linked high proportion of cytotoxic T-cells in the microenvironment to worse prognosis (25, 26). In addition has low expression of macrophages, in early stage disease, been linked to 100% disease free survival and overexpression of macrophages has been linked to failure of first-line treatment and high-dose chemotherapy (HDCT) with autologous stem cell transplantation (ASCT) (27). The concept of targeting the microenvironment has provided alternative treatment strategies although these are still in an experimental stage.

1.3 Pathogenesis and etiology

The origin of the classical Reed-Sternberg and the L&H cells has been vigorously investigated over the years. The classical Reed-Sternberg cell only rarely expresses B lineage specific markers such as CD20, CD79a or the B-cell receptor while the converse is true for L&H cells suggesting a B-cell origin for the L&H cells (7). In 1994 the cellular origin of the classical Reed-Sternberg cell and its malignant character was determined by Küppers and colleagues (28). They established that it was a B-cell by amplifying rearranged immunoglobulin genes from a single Reed-Sternberg cell, thus proving its clonality. A few years later Braeuninger and colleagues also established the B-cell clonality and the immunoglobulin rearrangement for the L&H cell (29).

When B-cells encounter antigen (presented by T-cells) in lymph nodes they activate and enter primary follicles. There they form germinal centers where proliferation and somatic hypermutation takes place. Somatic hypermutation involves mutation of the variable region of the immunoglobulin genes and normally only B-cells that acquire high affinity antibodies for the presented antigen survive in the germinal center whereas B-cells which develop unfavourable mutation get eliminated by apoptosis (30).

The pattern of expression of the variable chain of the immunoglobulin indicates that the classical Reed-Sternberg cell and L&H cell are of germinal center or post-germinal center origin (Figure 7) (29, 30). As previously mentioned classical Reed-Sternberg cells do not express the B-cell receptor. In about 25% of cases this is because of "crippling" mutations in the rearrangement of the immunoglobulin gene, others do have functional immunoglobulin genes but have lost their immunoglobulin gene transcription ability (31). The main effect is that immunoglobulin gene transcription is damaged in the Reed-Sternberg cell but how it escapes apoptosis is unknown.

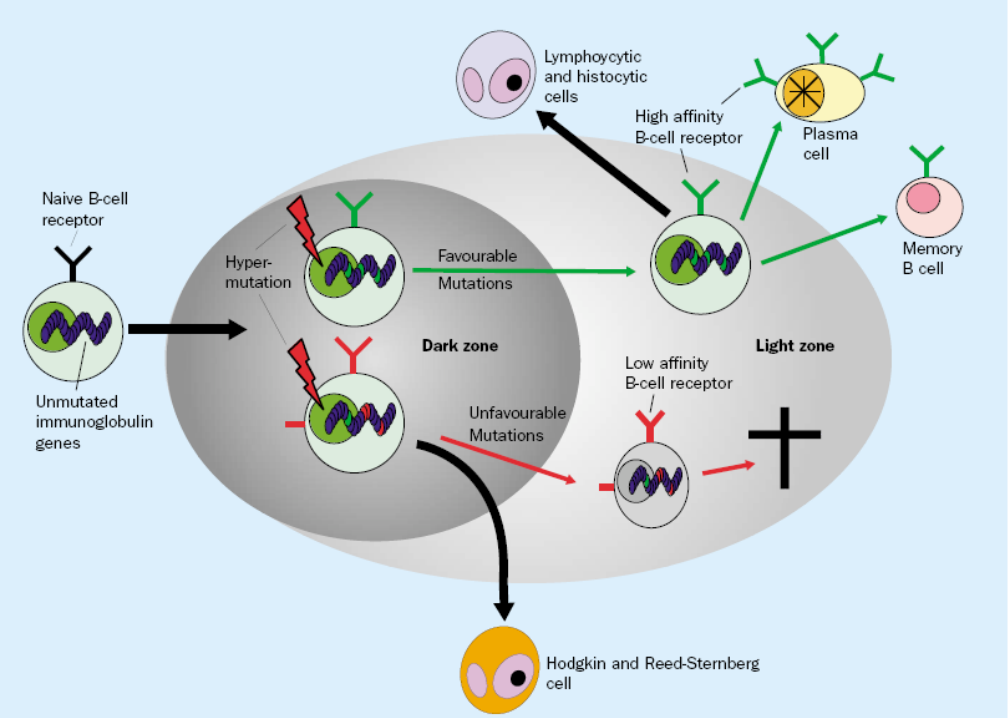


Figure 7. B-cell differentiation process in the germinal center and possible scenario for the generation of the classical Reed-Sternberg and L&H cells. Hypermutation occurs in the dark zone of the germinal center, thus increasing antibody affinity. Negative selection takes place in the light zone and the B-cells that get unfavourable mutations such as low affinity B-cell receptor undergo apoptosis. Reed-Sternberg are thought to come from pre-apoptotic germinal center B-cells that escape negative selection and L&H from germinal center B-cells with antigen specificity, they do have B-cell receptor with ongoing mutations (32).

It was debated for years whether HL was a cancer or infection. Then the clonality of the Reed-Sternberg cell and thus its malignant nature was recognized (28). The etiology however remains unknown. EBV is a human herpes virus with a sero-positivity of 90% in the worldwide population. EBV protein expression is detected in Reed-Sternberg cells in 40% of CHL patients and is most commonly associated with the MC and LD subtypes and is more common in children and older adults (33). Also it is more often associated with HL in the developing countries and is found in Reed-Sternberg cells of nearly 100% of HIV infected patients with HL (33, 34). Its effect on survival depends on age and subtype with a positive effect in children (0-14 years) and possibly young adults (15-44 years) and a negative effect in older (>45 years) individuals with NS (35). The fact that some patients are sero-negative for EBV indicates that HL can develop without the presence of EBV (36).

HL is more common amongst HIV positive than HIV negative individuals although it is not defined as an acquired immunodeficiency syndrome (AIDS) related malignancy. After the introduction of anti-retroviral treatment the frequency of the AIDS related malignancies, non-HL and Kaposi sarcoma, decreased considerably. On the other hand the frequency of HIV related HL has not changed (37). HIV related HL is more commonly associated with MC and LD

subclasses and is nearly always EBV related. Patients more commonly present at later stages and have B symptoms and bone marrow and extranodal involvement (38).

A role of the immune system in the etiology of HL has been postulated and a large population based case-control study from Sweden and Denmark showed a connection between rheumatoid arthritis and systemic lupus erythematosus and HL, which suggests a possible role of immune dysregulation or inflammation in the pathogenesis of HL (39). The same study showed a personal and familial association between HL and sarcoidosis, which suggests a shared susceptibility. Patients with HL may have an underlying immune abnormality as 15 years prior to diagnosis of HL they were more commonly treated for infection by their general practitioner compared to a control group (40).

Genetic factors play a potential role in the etiology of HL as several investigators have demonstrated a familial aggregation of HL as well as an association with other malignancies. Among them is an Icelandic study that showed that the relative risk of developing HL and non-HL was 3.27 and 2.56, respectively, in first degree relatives of HL patients. In addition second degree relatives had an increased risk of developing multiple myeloma as well as liver, lip, kidney and stomach cancer. Increased risk of developing cancer was even seen in fourth degree relatives of patients with HL whereas spouses of HL patients did not have an elevated cancer risk (41). Another Swedish/Danish population based case-control study revealed a relative risk of 3.11 of developing HL if a first degree relative had been diagnosed with HL. The risk was greater for siblings than for parent/offspring. The absolute lifetime risk of developing HL in this context increases however only from 0.24% to 0.69% (42).

1.4 Transcription factors and antigens

Various antigens and transcription factors have been used in elucidating the pathogenesis of HL. Although their roles are not always known many researchers have tried to analyze their clinical relevance. Special focus has been on finding molecules that could either lead to more targeted and less toxic treatment or to finding patients who are at high risk of having resistant disease prompting early aggressive treatment.

1.4.1 NFκB

Various transcription factors have been shown to be constantly activated in HL, one of which is NFκB thought to be a key factor involved in the pathogenesis of HL. It mediates expression of many genes involved in different function such as inflammation, inhibition of apoptosis and cell proliferation and most of the chemokines and cytokines expressed in Reed-Sternberg cells are thought to be regulated by NFκB. Activation of TNFα, CD30, CD40 (CD30 and CD40 are overexpressed by Reed-Sternberg cells) in Reed-Sternberg cells leads to the activation of NFκB. In EBV positive cases activation of NFκB can also be via the latent membrane protein 1 (LMP1). Additionally mutations in NFκB inhibitor (IκB) have been described in Reed-Sternberg cells. The constitutive activation leads to survival and proliferation of the Reed-Sternberg cell (44).

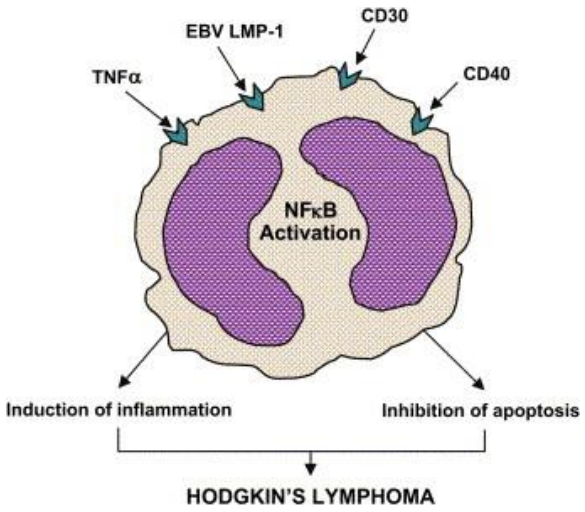


Figure 8. Proposed role of NFκB transcription factor in Hodgkin lymphoma (43).

1.4.2 Oct2 and BOB.1

Oct2 is a B-cell transcription factor involved in the transcription of immunoglobulin genes and BOB.1 is its cofactor. Normally those factors are not expressed in classical Reed-Sternberg cells which might lead to the lack of transcription of immunoglobulin genes and thus functional immunoglobulins (5, 7).

1.4.3 CD30

CD30 is a cell surface receptor that is a part of the tumour necrosis factor (TNF) superfamily (45). It was first described on classical Reed-Sternberg cells (46). It is almost always expressed on Reed-Sternberg cells in CHL and is also seen in non-malignant activated T- and B-cells as well as anaplastic large-cell lymphoma (7, 47). Its activation can lead to cell proliferation under certain circumstances but under other circumstances it can lead to cell death (48, 49). In Reed-Sternberg cells its overexpression leads to activation of NFκB (50). Currently immunotherapy against CD30 is being investigated but development has been slow (51).

1.4.4 CD15

CD15 is a cell surface complex and the first antigen to be associated with the Reed-Sternberg cell. It is expressed in 75-85% of CHL cases (7). It is also found in healthy tissues such as in various epithelial cells and late cells of granulopoeisis and can be seen in EBV transformed B- and T-cells. The function of CD15 is unknown (45). Lack of CD15 expression in CHL has been

shown to be a negative prognostic factor in some studies, while others have shown that CD15 has no correlation with prognosis (52, 53).

1.4.5 CD20

CD20 is a B-cell surface molecule that is involved in B-cell activation (54). It is expressed in CHL in 40% of the cases and in the majority of LP cases, as well as being expressed in non-HL (7). The clinical relevance of CD20 in CHL is not clear as studies give conflicting results. Most have found it to be a negative prognostic factor while others have found its presence insignificant (53, 55-57). Rituximab, a monoclonal antibody against CD20 has been shown to be effective against LP and even in CD20 negative CHL, at least in the palliation of B symptoms (51, 58, 59). The effect of rituximab in CHL is possibly through elimination of CD20+ B-cells in the environment of the Reed-Sternberg cells and by their elimination the Reed-Sternberg cell would be deprived of survival signals (60).

1.4.6 Epstein-Barr virus

The association of EBV with HL is not fully understood. In EBV-infected Reed-Sternberg cells three EBV proteins are expressed, the EBV nuclear antigen (EBNA), LMP1 and LMP2a. EBNA is the virus genome maintenance protein and is essential for DNA viral replication in infected cells but its role in the pathogenesis of HL has not been well established (61). LMP1 is a known viral oncogene as LMP1 transgenic mice develop B-cell lymphomas (62). In HL patients, LMP1 functions as an activated TNF receptor by imitating the CD40 receptor and thereby activating NFκB (63). LMP2a on the other hand can rescue B-cells with non-functional immunoglobulin genes and protect them from apoptosis by providing survival signals and by mimicking the B-cell receptor (64, 65). LMP1 is a marker of EBV positivity and its clinical significance has been extensively investigated but results are conflicting (66). Recent population based studies have indicated that there is an age dependent association between EBV status and prognosis with EBV expression being an adverse prognostic marker in elderly patients and potentially a protective factor in children younger than 15 years (35, 66-69).

1.4.7 MUM1

Multiple Myeloma-1/Interferon Regulatory Factor-4 (MUM1) is a transcriptional factor expressed in B-cells and activated T-cells. It is required for lymphocyte activation and the generation of immunoglobulin secreting plasma cells (70). MUM1 might have a role in the pathogenesis of HL, as MUM1 expression is induced through CD40 activation via NFκB (71). That might lead to survival and proliferation of the Reed-Sternberg cell. Other have shown that in CHL, CD40L T-cells form rosettes around the Reed-Sternberg cells and thus could affect them as Reed-Sternberg cells are dependent on survival signals from their microenvironment (24). MUM1 is expressed in most cases of CHL but lack of expression of MUM1 has been shown in two studies to be a potential adverse prognostic factor in CHL (72, 73).

1.4.8 Bcl-2

Bcl-2 is an anti-apoptotic protein and is found within many cell types. It is expressed on Reed-Sternberg cells in 20-60% of HL patients (various cut-off points for positivity) but none of those studies were done in an unselected material (74-77). The clinical significance of bcl-2 expression in HL has been intensely investigated. Most investigators have found its expression to be an adverse prognostic factor, but a possible role for bcl-2 is to prevent apoptosis of the Reed-Sternberg cell (76-78).

1.4.9 Bcl-6

Bcl-6 is a transcriptional repressor that affects genes involved in activation, inflammation and terminal differentiation of B-cells (79). It is rarely positive in CHL but almost always in LP (80). Bcl-6 has not been shown to have clinical significance in CHL but its significance in LP HL has not been tested (73).

1.4.10 CD45

CD45, also known as leukocyte common antigen, is expressed on virtually all hematopoietic cells. It is involved in cell-to-cell interaction and intracellular signalling and augments signaling through B- and T-cell antigen receptors (54). It is normally positive in B- and T-cell non-HL and lymphoid leukemias. CD45 is positive in about 2/3 of LP HL patients and in less than 5% of CHL patients (54, 81). Its clinical significance in HL has not been tested.

1.4.11 CD3 and CD5

CD3 and CD5 are T-cell markers and normally not expressed in HL. If they are expressed it can be an indication of a possible T-cell variant of HL. Other T-cell markers are CD2, CD4 and CD8. When T-cell markers are expressed in HL it may cause diagnostic difficulties with peripheral T-cell lymphomas such as anaplastic large-cell lymphoma (82). CD5 is also a typical marker of mantle cell lymphoma and chronic lymphocytic leukemia (83). True T-cell origin by clonal T-cell receptor rearrangement has only rarely been established (82).

1.5 Tissue microarray

In 1987 Wan and colleagues first described tissue microarray which is a high throughput way of analysing tissue samples (84). The area of interest in the tissue sample is identified, punched out of the paraffin block and transferred to an empty paraffin block (recipient block). In this way tissue samples from numerous patients can be fitted on the same slide (Figure 9).

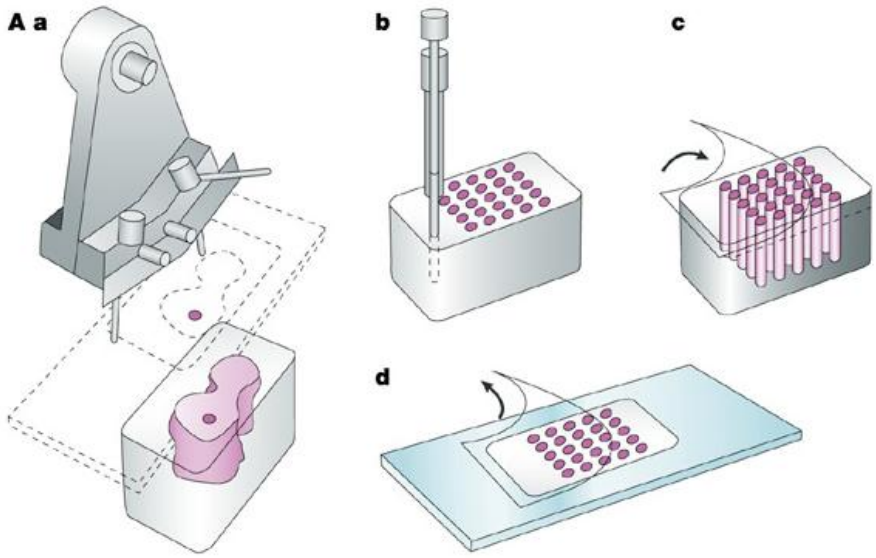


Figure 9. Making of a tissue microarray. Area of interest is located on the donor sample tissue, cylinder cores are punched out with a tissue microarrayer and moved to pre-made holes in an empty recipient block (b). The use of adhesive coated slide system facilitates the making of the tissue microarray block (c). Thin slides are cut and put on a microscopic glass slide (d) (picture adapted from http://www.nature.com/nrd/journal/v2/n12/fig_tab/nrd1254_F2.html).

The main disadvantage of tissue microarray, especially in a heterogenous tumour like HL, is the possibility that no Reed-Sternberg cells will be present in the punch biopsy (84). This has been addressed by several investigators who have found relatively good correlation between whole tissue sections and microarray studies (74, 85, 86).

1.6 Clinical features

A typical presentation of HL is a young adult who notices enlarging lymph nodes in the neck which are usually painless and rubbery. Sometimes HL is also diagnosed accidentally when an enlarged lymph node is palpated or seen on a diagnostic image. Constitutional symptoms may include fatigue, weight loss, fever, pruritus, night sweats and pain in the affected lymph node after alcohol ingestion. Drenching night sweats or fever more than 38°C for the past month or weight loss of more than 10% of baseline bodyweight for the last six months are referred to as B symptoms and confer a less favourable prognosis. Fever is usually low grade and irregular but sometimes the classic Pel-Epstein fever is seen which is felt to be virtually diagnostic. It constitutes a cyclic pattern of high fever for 1-2 weeks with alternating similarly long afebrile periods. When the tumour is in the thorax the patient can present with chest pain, dyspnea or a cough and when it is in the abdomen it can cause symptoms from the digestive system. The disease typically starts in one lymph node area and spreads contiguously to adjacent lymph nodes and then to other organs such as the liver, spleen and the bone marrow. The tumour growth can also extend directly from a lymph node to adjacent organs such as from hilar lymph nodes to the lungs but this type of spread holds a less grave prognosis than when the tumour is disseminated.

The most common anatomic sites at presentation are as follows: cervical/supraclavicular nodes 50-70%, mediastinal nodes 50-60%, paraaortic nodes 30-40%, axillary nodes 25-35%, spleen 30-35%, hilar nodes 15-35% and iliac nodes 15-20%. Less common sites include the inguinal nodes, extranodal sites, liver, mesenteric nodes and the Waldeyer ring. The histological subtypes have different clinical courses as LP usually presents with a localized peripheral disease. NS often presents with cervical or supraclavicular lymphadenopathy and in 80% of cases there is a mediastinal involvement. In MC the peripheral lymph nodes are often involved but the mediastinum only rarely. LD is often diagnosed in the spleen and the liver and retroperitoneal lymph nodes and bone marrow but is seldom found in the peripheral lymph nodes, whereas in LR the peripheral lymph nodes are typically involved (9, 12). Isolated infradiaphragmatic disease is seen in 5-13% of HL patients and is more often associated with higher age, male sex, more than three involved areas, B symptoms and MC or LP subtype. It was considered to be an adverse prognostic factor but when other prognostic factors are taken into account, prognosis is similar to supradiaphragmatic disease (87, 88).

Patient with HL are staged according to the Ann Arbor classification (Table 2) (16). Stage I is classified as a disease in one lymph node region/lymphoid structure, stage II is classified as a disease in two or more lymph node regions on either side of the diaphragm, stage III is in two or more lymph nodes regions on both sides of the diaphragm and stage IV refers to a disease that is in extranodal areas (not a as direct growth from lymph nodes). In addition to the history and physical examination, work-up includes complete blood count and differential, erythrocyte sedimentation rate, lactate dehydrogenase, alkaline phosphatase, albumin, liver function tests and virology. Computerized tomography of the chest, abdomen and pelvis and a bone marrow aspirate and a biopsy (can be omitted in clinical stage I/II disease) are performed. Gallium scans are recommended before and after treatment to evaluate the response of the disease. Gallium-67 uptake indicates the presence of viable lymphoma. Fluorine-18-fluoro-2-deoxy-d-glucose (FDG) positron emission tomography (PET) scan has proven useful in the staging and evaluation of treatment response in HL and is especially effective to detect disease in structures without any morphologic abnormalities (12, 89).

A commonly used prognostic score for advanced disease is the International Prognostic Factor Project (IPS) published in 1998. It includes seven risk factors, each of which reduces the prognosis of freedom from progression by 8%. These factors are: age ≥45 years, male gender, stage IV disease, hemoglobin <105 g/L, serum albumin <40 g/L, leukocytosis ≥15x10^9 and lymphocytopenia <0,6x10^9/L or <8% of white blood cell count (90). Although still used the value of the IPS score has decreased with improved treatment modalities (91).

1.7 Epidemiology

HL is a rare cancer and in Iceland it only accounts for 0.5 per 100 diagnosed malignancies (92). The incidence in Iceland, as in other developing countries, has been fairly constant over the last 50 years, see Figure 10 (92-94).

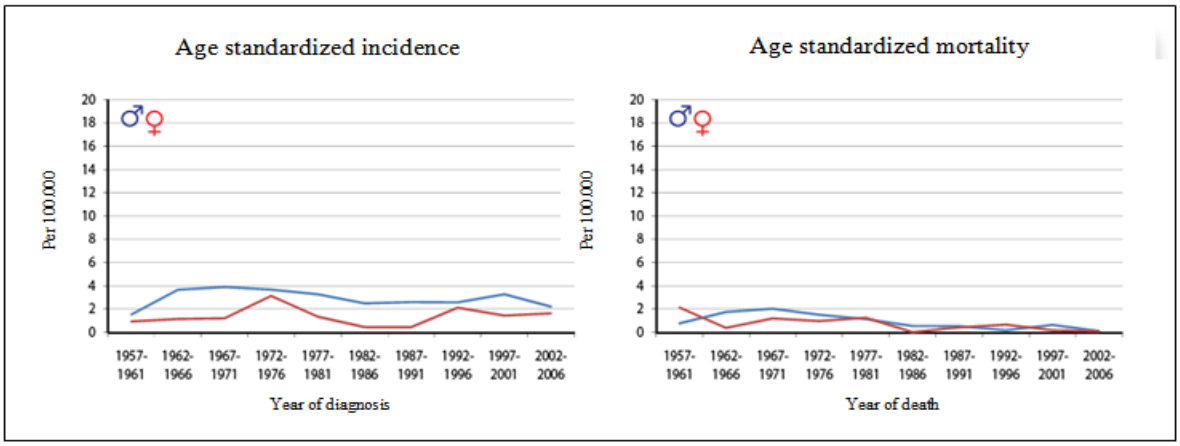


Figure 10. Age standardized incidence (left) and mortality (right) of HL in Iceland (92).

The incidence is variable in different parts of the world. In a report from The International Agency for Research on Cancer (IARC) the age standardized ratio for HL in 2002 was 1.2 per 100.000 for men and 0.8 per 100.000 for women in the whole world. In North-America the age standardized ratio was 3.2, in Europe 2.3-2.5 and in China 0.2 per 100.000 of the population (95).

HL is most common among young adults and accounts for 15% of all cancer in the 15-24 age group. The median age of diagnosis is 38 years. Younger patients have better prognosis with age adjusted mortality rate 0.3 per 100.000 for patients younger than 65 and 2.1 for those older than 65 in USA (94).

1.8 Treatment

Treatment today is based on chemotherapy with or without radiation. The mainstay of chemotherapy is the ABVD regimen. The cure rate for HL has been high for the last two decades. Thus most new clinical trials have focused on reducing late toxic effect, while maintaining good clinical response. The latest trends are to minimize radiation therapy as well as the intensity of chemotherapy, mostly by reducing doses of alkylating agents and anthracyclins, for patients with good prognosis.

1.8.1 Chemotherapy regimens

1.8.1.1 MOPP

The MOPP chemotherapy regimen has four drugs: nitrogen mustard, vincristine, procarbazine and prednisone. Nitrogen mustard is an alkylating drug that hinders DNA replication and like all

alkylating agents can depress bone marrow function and causes gastrointestinal symptoms. It is leukemogenic and can thus cause secondary acute leukemia often preceded by myelodysplastic syndrome. The risk of secondary leukemia, usually acute myeloid leukemia, starts to increase two years after the start of chemotherapy and peaks in five years. Ovarian dysfunction is common in women older than 30 years and oligospermia is seen in 90% of males receiving six or more cycles of therapy. Vincristine is a relatively nontoxic vinca alkaloid that hinders cellular division through its effect on preventing spindle formation in cells during mitosis. It has mild myelosuppressive activity but frequently causes paraesthesia and neuromuscular abnormalities. Procarbazine is an alkylating agent. Like other alkylating agents it is associated with infertility, but other unwanted effects include disulfiram-like action with alcohol, hypertension when given with sympathomimetic drugs and exacerbation of the effect of central nervous system depressants (19, 96, 97).

1.8.1.2 ABVD

The ABVD regiment contains doxorubicin, bleomycin, vinblastine and dacarbazine. Doxorubicin is an anthracycline whose main cytotoxic action is mediated through topoisomerase II which is very active in proliferating cells. Its major toxic effect is dose related and it can lead to cardiac damage, arrythmia and heart failure. Other side effects include nausea, vomiting, myelosuppression and hair loss. Bleomycin is also an anthracycline whose main cytotoxic effect is mediated through damaging DNA and preventing its repair. It causes little myelosuppression but causes pulmonary fibrosis in 10% of patients which is potentially lethal in 1% of patients. Other unwanted side effects are fever, allergies and mucocutaneous reaction, mostly in the palms. Vinblastine is a vinca alkaloid that can cause leukopenia. Dacarbazine is a pro-drug, when cleaved in target cells it releases an alkylating derivative which forms covalent bond with DNA and impends DNA replication. Its main adverse effects are myelosuppression and nausea and vomiting (97).

1.8.1.3 Other regimens

Other combination chemotherapies have been developed especially for advanced and/or aggressive disease. These include Stanford V and BEACOPP escalated regimens.

Stanford V contains doxorubicin, vinblastine, nitrogen mustard, bleomycin, vincristine, etoposide and prednisone. The regimen includes radiation therapy on initially bulky sites more than or equal to 5 cm. It has less bleomycin and doxorubicin than ABVD and no procarbazine and was designed with focus on reducing pulmonary and cardiac toxicity and to decrease sterility and secondary leukemias. It has been shown to be effective in advanced HL with bulky disease, with a ten year overall survival of 96%. Not all studies confirm these results and a clinical trial comparing Stanford V to ABVD has not yet been published (98). The Stanford V regimen is now also sometimes used for treatment of localized disease (99).

BEACOPP and BEACOPP escalated regimens were designed by the German Hodgkin Study Group for advanced HL and contains bleomycin, etoposide, doxorubicin, cyclophosphamide,

vincristine, procarbazine and prednisone. BEACOPP escalated has higher doses of cyclophosphamide, etoposide and doxorubicin than BEACOPP. The BEACOPP escalated regimen was shown in a large prospective trial to be very effective for treatment of advanced HL with a five and ten year survival of 92% and 86% respectively. On the other hand BEACOPP escalated has more risk of developing secondary leukemia/myelodysplastic syndrome and is associated with a very high infertility rate (90%) for males, thus limiting its use (100).

1.8.2 Salvage chemotherapy

Patients with resistant or relapsed disease receive second line or salvage therapy. Which type of second line therapy is given depends on what first line therapy the patient received as well as how long after finishing the first treatment the relapse occurs. Second line chemotherapy regimens include 1) MIME (methyl-GAG, ifosfamide, methotrexate and etoposide) 2) DexaBEAM (dexamethasone, carmustine, etoposide, cytarabine and melphalan) 3) DHAP (dexamethasone, cytarabin and cisplatin) and 4) ICE (ifosphamide, carboplatin and etoposide) (101).

HDCT often includes the same drugs as conventional salvage treatment but in higher myeloablative doses and is followed with ASCT. Before HDCT, induction treatment is given. In the recovery phase of the induction regimen stem cells are collected, most commonly peripherally. Then HDCT is given followed with ASCT in order to rescue bone marrow function.

1.8.3 Treatment according to stage

1.8.3.1 Early stage favourable

Early stage favourable disease is usually defined as non-bulky, stage I or II disease without risk factors (such as advanced age, high erythrocyte sedimentation rate, multiple sites on the same side of the diaphragm, etc.). Most patients (90-98%) with early stage favourable disease can be cured. Before the 1990s this patient group was mostly treated with radiation therapy alone. However due to the late toxic effects of radiation therapy, mostly increased risk of secondary solid tumours and cardiovascular disease, interest rose in reducing radiation therapy. Currently these patients are treated with combined modality treatment or chemotherapy only (102-104). Combined therapy accounts for chemotherapy followed by radiation therapy, usually in the involved field. There has been ongoing discussion whether radiation therapy should be omitted altogether in treating this group. Most trials have not been able to show a survival difference when patients are treated with chemotherapy only vs. combinations therapy. However a recent meta-analysis that included both favourable and unfavourable early stage HL patients did show an improved survival for patients treated with combined modality treatment (105). Current guidelines (2009 and 2010) from the European Society for Medical Oncology (ESMO) and The National Comprehensive Cancer Network (NCCN) recommend combined therapy, either ABVD 2-4 cycles or Stanford V (NCCN) with radiation therapy. Chemotherapy (ABVD) without radiation therapy is however given as an alternative option for young patients in whom the toxic effects of radiation needs to be minimized (99, 106).

1.8.3.2 Early stage unfavourable

Early stage unfavourable is usually defined as stage I or II disease with risk factors such as a bulky disease, advanced age (above 40 or 50 years), high erythrocyte sedimentation rate and multiple sites (on the same side of the diaphragm). Several trials have addressed the application of chemotherapy only but with inconclusive results (107, 108). However a new meta-analysis showed better survival for patients treated with combined modality treatment (105). Current guidelines from ESMO and NCCN recommend 4-6 cycles of ABVD or Stanford V (NCCN) with radiation therapy (99, 106).

1.8.3.3 Advanced stage

Advanced disease is generally defined as stage III and IV disease though some include those with B symptoms at early stage who have other adverse risk factors. With modern treatment, patients with advanced disease have an 80% chance of cure. Chemotherapy (ABVD, Stanford V, BEACOPP escalated) is used for advanced stage with or without radiation therapy. The role of consolidative radiation therapy in advanced HL has long been debated and probably is not superior to chemotherapy only although subgroups with a bulky disease and those with partial remission seem to benefit (109). NCCN guidelines recommends 6-8 cycles ABVD or Stanford V with radiation on bulky sites (more than 10 cm ABVD and more than 5 cm Stanford V). For patients with adverse prognostic factors they recommend BEACOPP escalated with consolidative radiation therapy for initially bulky sites more than 5 cm and residual PET positive sites (106). ESMO guidelines recommend eight cycles ABVD or BEACOPP escalated (for patients younger than 60 years) with involved field radiation therapy for residual sites larger than 1.5 cm (99).

1.8.3.4 Lymphocyte-predominant

For localised disease without risk factors (IA or IIA) involved field radiation therapy is the standard of care, but for a more advanced disease treatment is same as for CHL (99, 106).

1.8.3.5 Resistant disease and relapsed disease

As previously mentioned the type of salvage therapy given to patients with relapsed or resistant disease, depends on which first line treatment the patients received.

Patients whose first line therapy was radiation therapy only have a relapse rate of 30%. Generally they can be salvaged with conventional chemotherapy and ten year survival for those patients that are treated with ABVD is 80-90% (101).

Patients who relapse after more than 12 months after initial chemotherapy, can possibly be salvaged with second line chemotherapy or HDCT with ASCT. Which treatment is used depends on time to relapse, which first line therapy was given, stage at relapse, performance status of the patient as well as other prognostic factors. For patients with an early relapse (less than 12 months) or a resistant disease HDCT with ASCT has been the standard of care for the last 20 years. Whether patients respond well to the induction chemotherapy is an important prognostic

factor. A study by Sirohi and colleagues showed 79% five year survival for those who had complete remission prior to HDCT compared to 17% for those who had induction failure. The longest follow up for patients with relapsed or resistant disease receiving HDCT with ASCT showed a median survival of nine years, 55% five year survival and 49% ten year survival (110).

Data on radiation therapy as a salvage treatment is limited but there is a possible role for it in patients with favourable stage I/II disease who relapse only in the original site, after treatment with chemotherapy only. However radiation therapy as a salvage treatment for isolated relapse in LP is well established (111).

Special consideration should also be given to those with relapsed LP as treatment with rituximab shows very promising results with 94% achieving complete remission (112).

1.8.4 Complications of therapy

Therapy of HL has various complications. Even more than 25 years after therapy an increased mortality from other causes than HL is seen.

The gravest complications of therapy are secondary malignancies which are both due to radiation therapy and treatment with alkylating agents. Radiation therapy causes a two to fivefold increase of secondary malignancies. That risk becomes apparent about ten years after finishing treatment. The most commonly affected organs are the lungs, breasts, thyroid gland and stomach, and the risk is greatest when patients are treated at a young age. Radiation therapy does not cause increased risk of developing secondary leukemia. Alkylating agents used in MOPP, Stanford V (low dose), BEACOPP and HDCT do increase the risk of secondary leukemia significantly. The risk starts to increase two years after treatment and peaks in five years. These same drugs also increase the risk of lung cancer as well as having an additive effect of radiation therapy and smoking on lung cancer risk (96, 113).

Both radiation therapy and chemotherapy affect the cardiovascular system. Radiation therapy increases the risk of coronary artery disease through direct damage to the endothelium, causes myocardial fibrosis, damage to the cardiac valves and can lead to electrical abnormalities. Anthracyclins, like doxorubicin (in ABVD) are also directly cardiotoxic, the damage being on the myocardium and the effect dose dependent. The effects of anthracyclins and radiation therapy on the heart are additive with the risk being greatest in young people (114).

Infertility is another major issue when treating HL. If the gonads are included in the radiation field radiation can affect spermatogenesis and cause ovarian dysfunction. It can also damage the uterus leading to miscarriage and an increased risk of preterm deliveries. The effect of radiation therapy is dose dependent. The older the patients are the greater the risk is of becoming infertile. Procarbazine (in MOPP and BEACOPP) and other alkylating agents can also lead to infertility in men and women in a dose dependent manner.

Pulmonary toxicity after bleomycin (in ABVD, BEACOPP escalated and Stanford V) is another possible serious complication of HL therapy. Hypothyroidism after radiation therapy to the neck is quite common (113).

2 Aim of the study

The aim of this study is twofold:

1. To study the epidemiology of HL in Iceland during 1990-2005 and to investigate what therapy was given.
2. To perform immunohistochemical staining on lymph node tissue from patients in order to investigate the phenotype of HL in Iceland as well as to correlate antigen expression with prognosis.

3 Methods and materials

3.1 Basis of the study

This study can be divided in two parts. One part is a retrospective descriptive analysis on classical aspects of HL in Iceland from 1990-2005. The other part is an immunohistochemical study of pathology specimens with results linked to clinical information.

This study is unique in being based on information from the total population in Iceland, as all cancer cases, including HL are registered in the Icelandic Cancer Registry. All tumour specimens in Iceland are stored in tumour banks and lymph node tissue was available for study from all the patients in the study.

3.2 Clinical Data

Clinical data was obtained from hospital charts. The following clinical information was obtained: date of birth, gender, date of diagnosis, stage at presentation (according to the Ann Arbor classification), clinical presentation, clinical and/or pathological staging, the initial therapy and therapeutic response, resistant disease, relapse and subsequent therapies and follow-up, and date and cause of death. The day of last follow-up was 31st of December 2007. Treatment outcome was measured using five year overall survival, five year disease specific survival and overall survival at the end of study period (31st of December 2007). Survival time was measured from the date of diagnosis to date of death from any cause for overall survival, and to the date of death from HL or because of treatment for disease specific survival. Patients alive at the end of the follow-up time were censored at that time. For disease specific survival, patients dying from other causes than HL or because of treatment were censored at the time of death. Complete remission was defined as no indication of the disease one month after completing therapy. Equivocal medical cases regarding clinical information were reviewed in special meetings with Friðbjörn Sigurðsson medical oncologist and Brynjar Viðarsson hematologist. All data was registered in SPSS version 11.

3.3 Pathology data

3.3.1 Tissue slides

Microscopic slides of all cases stained with hemotoxylin and eosin (H&E) were retrieved from the three pathology laboratories in Iceland (Landspitali University Hospital, the Hospital in Akureyri and the Pathology Laboratory Álfheimum 74, Reykjavík). The slides were reviewed and compared with previous pathology classifications. Equivocal cases were brought to Bjarni A. Agnarsson (BAA), pathologist, for consultation. BAA reviewed all tissue slides that he had not previously evaluated.

3.3.2 Tissue microarray construction

Tissue microarray procedures were done according to standard operating procedures in the Pathology Department at the Landspítali University Hospital. The tissue microarray and immunohistochemical staining was done by Kristrún Ólafsdóttir, chief histotechnologist, with Hallgerður Lind Kristjánsdóttir (HLK) observing the procedures. HLK identified the area on the H&E slides to be punched out and interpreted the results of the immunohistochemical staining. Difficulties in interpretation were resolved with BAA utilizing a double-headed microscope.

An H&E-stained slide from each patient was used to define the representative tumour region, with emphasis on regions with large numbers of Reed-Sternberg cells. The lymph node specimen had to be at least 1 mm thick in order to proceed. Two tissue cores with a diameter of 1 mm were punched out manually from the tumour areas of each lymph node and brought into a recipient paraffin block using a Beecher MTA III tissue microarray instrument with x-y guide, creating two 98-spot tissue microarray blocks. The tissue microarray block was stored in a heat chamber at 37°C for 10-15 min to ease further processing. A microscopic glass slide was laid on the surface and equal pressure applied to press any protruding tissue cylinders into the tissue microarray block which was subsequently cut. Adhesive-coated tape sectioning aid system was used to cut the tissue microarray block into 3 µm slices and put on microscopic glass slides (Waldemar Knittel) for immunochemical staining.

3.3.2.1 Immunohistochemistry

Immunohistochemical staining of tumour was performed for CD30, CD15, CD20, CD45, CD5, CD3, LMP1, bcl-2, bcl-6 and MUM1.

For deparaffinization freshly cut tissue microarray slides where dried, heated in 60°C heat chamber for 30-60 min, transferred first to a xylol bath, then to an alcohol bath and then rinsed in water. To open up binding sites microwave antigen retrieval was used. The tissue microarray slide was first laid in a TE buffer pH 9.0, then heated in a commercial microwave (Siemens) 800W for 10 min and 360W for 15 min and subsequently dried at room temperature, rinsed in water and bathed in TRIS buffer with pH 7.6.

EnVision^{TM} Dual Link System- HRP (Dako) was used for immunohistochemical staining which is a two step antibody polymer staining technique, see Figure 11. First the tissue is stained with primary antibody directed against specific antigens. Then a secondary antibody, which binds to all tissue-bound mouse and rabbit antibodies, is applied. This complex is colourless and to transform it into a coloured end-product an enzyme substrate reaction is used. Chromogens or electron donors are utilized which upon oxidation become coloured products (115). In our study chromogen 3.3 diaminobenzidinetrahydrochlorine (DAB) was used, which gives a brown colour.

Tissue microarray slides where bathed in H_2O_2 (peroxidase) blocking solution (Dako), rinsed and incubated with the primary antibody for 30 min. Specifics of the monoclonal antibodies are described in Table 3. The samples were then stained with a secondary or bridging enzyme

(Dako) and rinsed in buffer and water and subsequently stained with DAB for ten minutes and then finally rinsed. Nuclear staining was done with H&E.

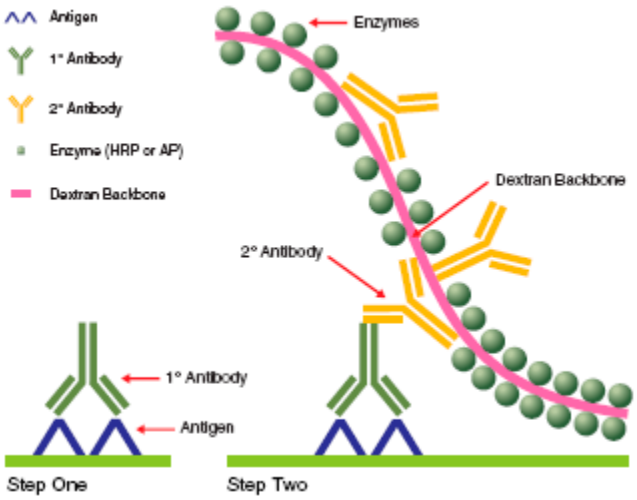


Figure 11. Two step antibody polymer staining technique (115).

Tissue microarray was successfully performed on pathology specimens for 98 patients. For those patients, antibodies for CD30, CD15, CD20, LMP1, MUM1, bcl-2, bcl-6, CD3, CD5 and CD45 were applied.

For seven patients tissue microarray could not be made as their samples were too small to use. For those patients phenotypic information from their work-up at diagnosis was used. If that information was not available, immunohistochemistry on whole tissue sections was performed for CD30, CD15, CD20, LMP1, MUM1, and bcl-2. Staining in these cases for bcl-6, CD3, CD5 and CD45 was omitted.

Table 3. Monoclonal antibodies used in the immunohistochemistical staining for HL.

Antibody	Manufacturer	Clone	Dilution
CD30	Dako	Ber-H2	1:10
CD15	Becton Dickinson	MMA	1:10
CD20	Dako	L26	1:20
CD45	Dako	2B11+PD7/26	1:500
CD5	Novocastra	4C7	1:50
CD3	Dako	Polyclonal	1:500
LMP1	Dako	CS1-4	1:100
MUM1	Dako	MUM1p	1:100
Bcl-2	Dako	124	1:100
Bcl-6	Dako	PG-B6p	1:10

3.4 Statistics

Statistical analysis was done in SPSS version 11 and R version 2.9.2. Age standardized incidence was calculated according to World standardized million. Nominal and ordinal data were compared with the Chi-squared test. When the Chi-squared test could not be used because of few observations the Fisher's-Exact Test was applied. Comparison of the means of continuous variables was done by using the 2-tailed Student's t-test for independent samples. Survival analysis was done using Kaplan-Meier analysis and groups compared with the log-rank test. Multivariate analysis was done in R (another analysis was done in SPSS) using stratified Cox proportional hazard regression analysis. Only CHL cases were entered into the Cox proportional hazard model. The following variables were entered: gender, age (as a continuous variable), histological subclassification (NS, MC, "other" CHL), stage I/II vs. III/IV disease, B symptoms, bulky disease, CD30, CD15, CD20, LMP1, MUM1, and bcl-2 expression. Each variable was tested to see if it violated the assumption of the proportion hazard before being entered into the model. Gender did violate that assumption and was thus used to divide the data into strata. Forward selection method was used and a variable was kept in the model if $p<0.05$. Results were considered significant if $p<0.05$.

3.5 Permits

The study was approved by the National Bioethics Committee and The Data Protection Authority in Iceland. Special permission was obtained to access data from The Icelandic Cancer Registry as well as from medical directors of each hospital and health clinics to access hospital charts.

4 Results

According to the Icelandic Cancer Registry 106 patients were diagnosed with HL in Iceland during the study period, 1990-2005. After reviewing the tissue samples one patient was excluded due to erroneous diagnosis of HL thus 105 patients were included for further analysis in the study.

4.1 Incidence and age distribution

The age adjusted (World Standardized Million) annual incidence per 100.000 population was 2.05 and varied from 0.36-3.70 from the years 1990-2005. When dividing the entire period into two eight year periods, 1990-1997 and 1998-2005, there were 48 patients in the former period and 57 patients in the latter period. Figure 12 depicts the number of cases divided by age intervals and Figure 13 shows the mean annual age-specific incidence in the years 1990-2005.

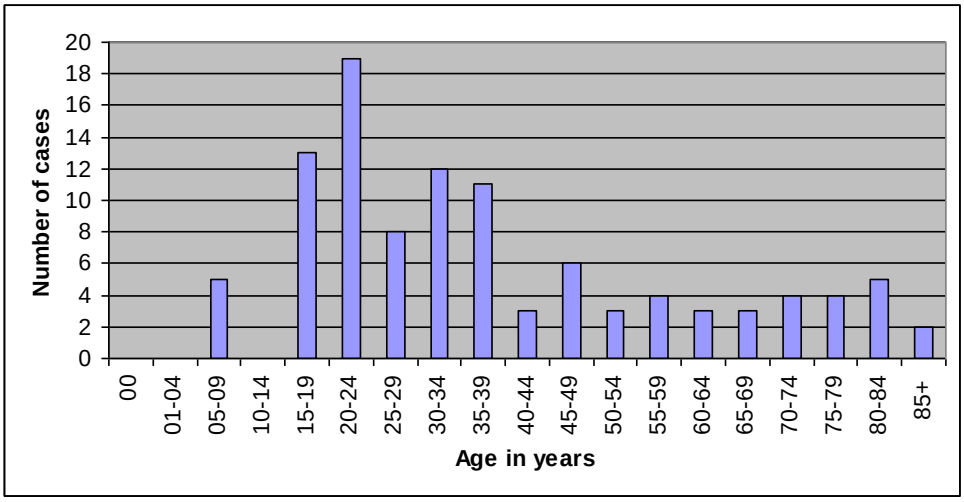


Figure 12. Number of HL cases (n=105) diagnosed in Iceland in the years 1990-2005 divided by five year age groups.

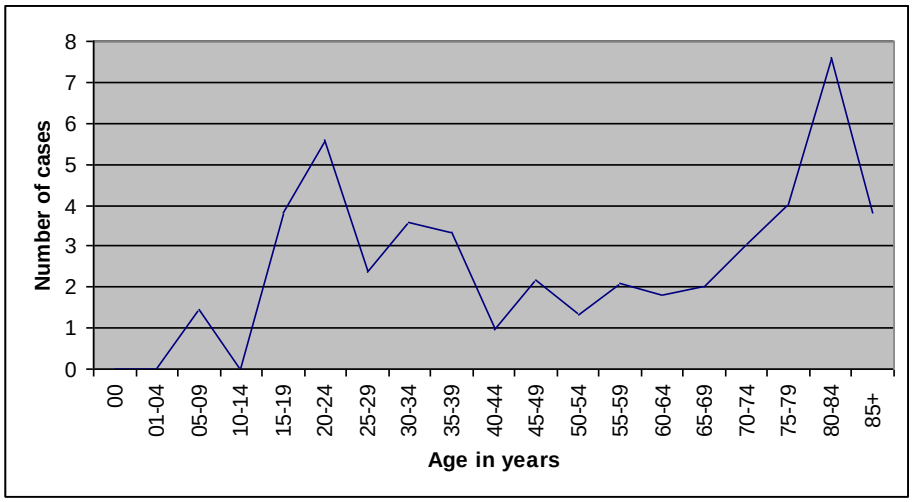


Figure 13. Mean annual age-specific incidence of HL diagnosed in Iceland in the years 1990-2005 (n=105).

4.2 Staging and localisation

The clinical features and age distribution can be seen in Table 4. The disease was more common in men and the male to female ratio was 3:2. The mean age at diagnosis for the whole group was 38 years and was not significantly different between male (36 years) and female (42 years) patients (p=0.16). At diagnosis 64% of the patients had stage II disease and 44% had B symptoms. The frequency of B symptoms varied between stages of the disease and was 21%, 48%, 33% and 58% for stage I-IV disease, respectively. Isolated extranodal disease was seen in three cases, all involving the lungs. Isolated infradiaphragmatic disease was seen in 10% of the patients.

Table 4. Clinical data on the 105 patients with HL.

Characteristics		No. of patients	%
Gender	Male	64	61
	Female	41	39
Age	Median	32 years	
	IQR	21-53 years	
	Range	5-88 years	
	Mean	38 years	
Stage	I	14	13
	II	67	64
	III	12	11
	IV	12	11
B symptoms	Yes	46	44
	No	59	56
Bulky disease	Yes	15	14
	No	90	86

IQR interquartile range

Post-mortem diagnosis was made in three patients. Work-up performed at diagnosis was computerized tomography for all except two patients, one of whom was diagnosed at autopsy while the other one died shortly after diagnosis. No patient had a lymphangiogram, two patients underwent pathological staging with explorative laparotomy and two were splenectomized. Gallium scan was performed in 18% of cases before treatment and was always positive. Bone marrow biopsy was done in 89% of the cases, of which 3% were insufficient for diagnosis. It was positive in three patients (were all at stage IV, regardless of the bone marrow infiltration). Bone marrow biopsy was omitted in 13 patients, two of whom were diagnosed at autopsy and one who died shortly after diagnosis. The other ten patients that did not have a bone marrow biopsy were all with stage I-II disease. Localization of the disease at diagnosis can be seen in Table 5.

Table 5. Localization of HL in the 105 patients with HL.

	Patients (n)	%
Lymph nodes organs		
Cervical/supraclavicular nodes	69	66
Mediastinal nodes	64	61
Axillary nodes	11	11
Infradiaphragmic nodes	19	18
Inguinal/femoral nodes	6	6
Spleen	6	6
Bone marrow	3	3
Epitrochlear nodes	1	1
Waldayer ring	1	1
Extranodal areas		
Lung	4	4
Liver	5	5
Duodenum	1	1
Sacroiliac joint	1	1
Diaphragm	1	1
Esophagus	1	1
Epigastrium	1	1

4.3 Subclassification and immunohistochemistry

Histological subclassification, according to the WHO classification, of the 105 patients included in the study is shown in Table 6.

Table 6. Histological subtypes in the 105 patients with HL.

Subtype	Patients (n)	%
NS	73	70
MC	17	16
LD	4	4
LR	1	1
CHL unclassifiable	6	6
LP	4	4

Expression of CD30 is virtually universal in CHL or 95%, CD15 was expressed in 52%, CD20 in 15%, LMP1 in 23%, MUM1 in 87%, and bcl-2 in 36% of the CHL patients. Detailed analysis of the immunohistochemical staining for different subclasses of CHL and LP can be seen in Table 7.

Staining of bcl-6, CD45, CD3 and CD5 was only done on tissue samples from 98 patients (that were in the tissue microarray blocks). It is therefore missing for seven patients. Bcl-6 was positive in five patients with CHL (three patients with NS and two patients with "other" CHL) and no patient with LP. The bcl-6 staining was generally weak. Two patients had T-cell differentiation. Their phenotypic expressions were CD30+/CD15+/CD20-/CD3+/CD5+/CD45+ and CD30+/CD15+/CD20-/CD3-/CD5+/CD45-. CD45 was positive in two other patients (one patient with NS and another patient with LP).

Table 7. Immunohistochemical staining in the 105 patients with HL.

			Antigens											
			CD30		CD15		CD20		LMP1		MUM1		Bcl-2	
			n	%	n	%	n	%	n	%	n	%	n	%
Subclasses of HL	All CHL	P	96	95	52	52	15	15	23	23	88	87	36	36
		N	1	1	47	47	83	82	75	74	11	11	63	62
		U	4	4	2	2	3	3	3	3	2	2	2	2
	NS	P	69	95	44	60	11	15	8	11	69	95	26	36
		N	0	0	27	37	59	80	62	85	3	4	45	62
		U	4	6	2	3	3	4	3	4	1	1	2	3
	MC	P	17	100	6	35	2	12	11	65	14	82	5	29
		N	0	0	11	65	15	88	6	35	3	18	12	71
		U	0	0	0	0	0	0	0	0	0	0	0	0
	Other CHL	P	10	91	2	18	2	18	4	36	5	46	5	45
		N	1	10	9	82	9	82	7	63	5	46	6	55
		U	0	0	0	0	0	0	0	0	1	9	0	0
	LP	P	1	25	0	0	3	75	1	25	2	50	0	0
		N	3	75	4	100	1	25	3	75	2	50	4	100
		U	0	0	0	0	0	0	0	0	0	0	0	0

All CHL: 73 patients with NS, 17 with MC, four with LD, one with LR and six with CHL unclassifiable; Other CHL includes four patients with LD, one with LR and six with CHL unclassifiable; P positive; N negative; U uncertain staining or technical flaws

LMP1 was positive in 23% of patients with CHL and when the group was analyzed further (Table 8.) it could be seen that patients lacking LMP1 expression had more often stage I/II disease and the NS subtype. No difference in LMP1 expression was seen according to age 0-15 years, 16-59 years and 60 years and older.

Table 8. Characteristics of the 101 patients with CHL according to LMP1 expression.

Characteristics	LMP1 positive n (%)	LMP1 negative/U n (%)	p value
Median age	46 (IQR 21-65)	31 (IQR 20-45)	
0-15 years	2 (8)	3 (5)	0.19
16-59 years	14 (63)	62 (79)	
≥60 years	7 (29)	13 (17)	
Male	17 (74)	45 (58)	0.16
Female	6 (26)	33 (42)	
NS	8 (35)	65 (83)	**<0.0001**
MC	11 (48)	6 (8)	
Other	4 (17)	7 (9)	
Stage I/II	13 (57)	65 (83)	**0.007**
Stage III/IV	10 (43)	13 (17)	

U uncertain staining or technical flaws, IQR interquartile range

4.4 Treatment

Out of the 105 HL cases in this study, 100 were eligible for treatment since three patients were diagnosed at autopsy and additional two died shortly after diagnosis. Those five patients are therefore not included in the analysis of treatment modalities.

The most common treatment modality was chemotherapy only, given to 54% of the patients, 38% received combination therapy (chemotherapy and radiation therapy) and 8% received radiation therapy only. The type of therapy given according to the stage of the disease is given in Table 9.

Table 9. Therapy given according to stage of the disease for the 100 patients that were treated for HL.

	Stage I/II disease non-bulky n (%)	Stage I/II disease bulky n (%)	Stage III/IV disease n (%)
Chemotherapy	35 (51)	2 (17)	17 (81)
Combination therapy	24 (35)	10 (83)	4 (19)
Radiation therapy	8 (13)	0	0

The type of chemotherapy changed when comparing the time periods 1990-1997 and 1998-2005 (Table 10). Chemotherapy with the MOPP-ABVD regimen was the most common in the former period, whereas treatment with the ABVD regimen was dominant in the latter period.

Table 10. Type of first line chemotherapy given to 92 patients with HL. A comparison between time periods.

	Patients that had chemotherapy, n	ABVD n (%)	MOPP n (%)	MOPP-ABVD n (%)	Other n (%)
1990-1997	39	10 (26)	3 (8)	25 (64)	1 (3)
1998-2005	53	50 (94)	1*(2)	0	2 (4)

*Chlorambucil instead of nitrogen mustard; Other includes 1) BEACOPP 2) prednisone, etoposide, and vincristine and 3) methotrexate, cytarabine, hydrocortisone, cyclophosphamide, doxorubicin and vincristine.

The frequency of patients receiving radiation therapy was similar between the two time periods (Table 11). The use of extensive field radiation therapy decreased in the latter time period, as well as the use of radiation therapy as a single treatment modality. The radiation dose was lower in the latter time period or on average 31Gy (Confidence interval (CI) 29.4-33.1) vs. 36Gy (CI 33.7-39.1) in the former time period (p=0.003)**.** Of the patients receiving extensive field radiation therapy nine had mantle zone radiation and one had inverted Y.

Table 11. Type of radiation therapy given to 46 patients with HL. A comparison between time periods.

	Patients that had radiation therapy, n	Extended field n (%)	Involved field n (%)	Radiation alone n (%)
1990-1997	24	9 (38)	15 (63)	7 (15)
1998-2005	22	1 (5)	21 (95)	1 (2)

4.5 Survival analysis

Overall five year survival was 81% and disease specific five year survival was 83%. If untreated patients (diagnosed at autopsy or died very shortly after diagnosis and thus were not treated, n=5) were excluded the overall five year survival was 85%. The median follow-up time was 6.7 years (IQR 3.8-12.2) and overall survival on 31st of December 2007 was 76%.

Five year survival was significantly better amongst males than females (Figure 14), but for patients younger than 60 years no difference was seen in survival between the genders (Figure 15). Five year survival was the same between the two time periods 1990-1997 and 1998-2005 (81%). Survival according to age 0-59 years and 60 years and older is seen in Figure 16 and was significantly worse for patients 60 years and older. The five year survival for HL patients 60 years and older, which was 29%, is much worse than for individuals 60 years and older in the general population (95% five year survival, p<0.0001) (116). When dividing the group into four age categories 0-19, 20-39, 40-59 and 60 years and older the five year survival was 87%, 100%, 88% and 29%, respectively.

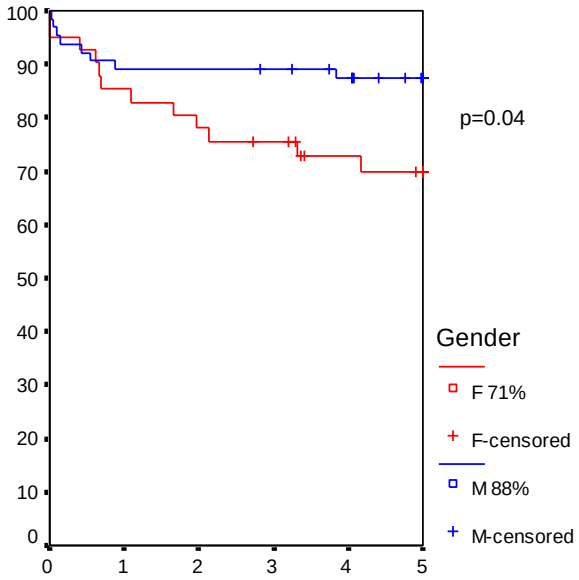


Figure 14. Kaplan-Meier curve for five year survival according to gender (includes all HL patients, n=105).

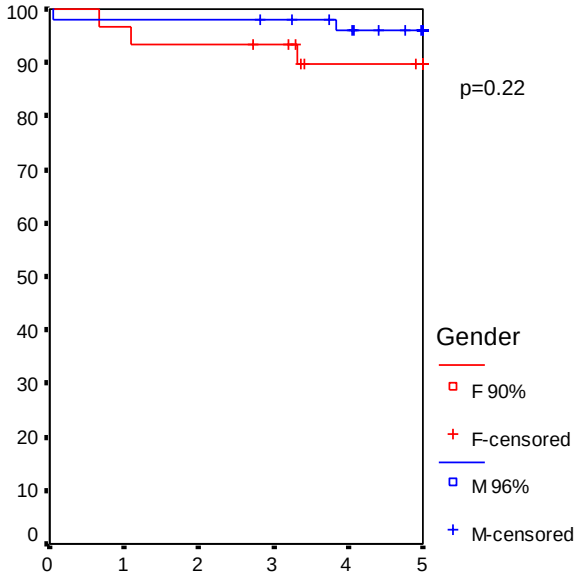


Figure 15. Kaplan-Meier curve for five year survival for patients 0-59 years according to gender (includes HL patients, n=84).

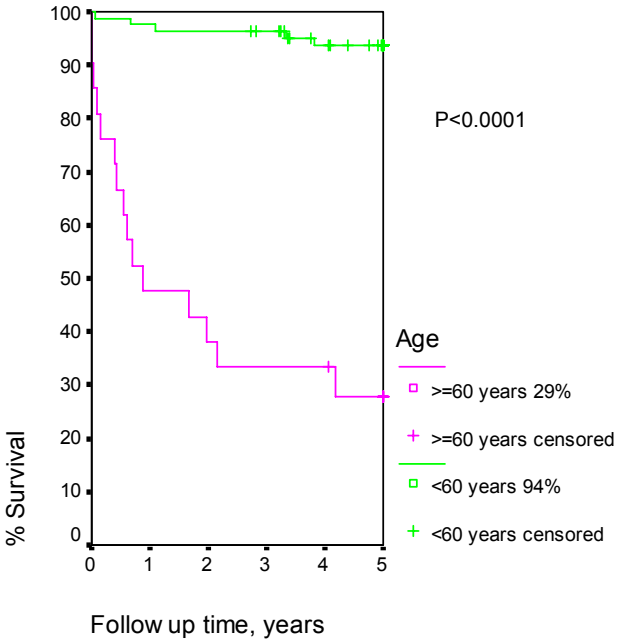


Figure 16.Kaplan-Meier curve for five year survival for patients 0-59 years and 60 years and older (includes all patients with HL, n=105).

Table 12. Five year survival for all subtypes of HL (Kaplan-Meier analysis).

Subtypes (n)	Five year survival
CHL (101)	81%
NS (73)	89%
MC (17)	78%
LD (4)	25%
LR (1)	100%
CHL unclassifiable (6)	33%
LP (4)	75%

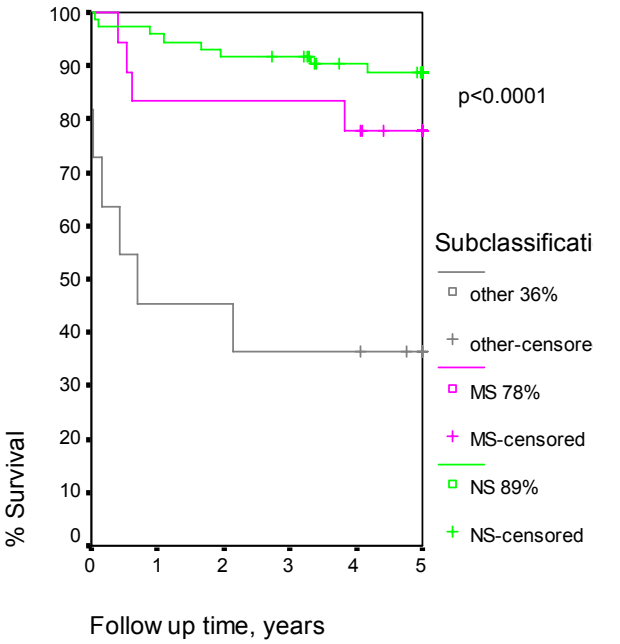


Figure 17. Kaplan-Meier curve for five year survival according to histological subclassification

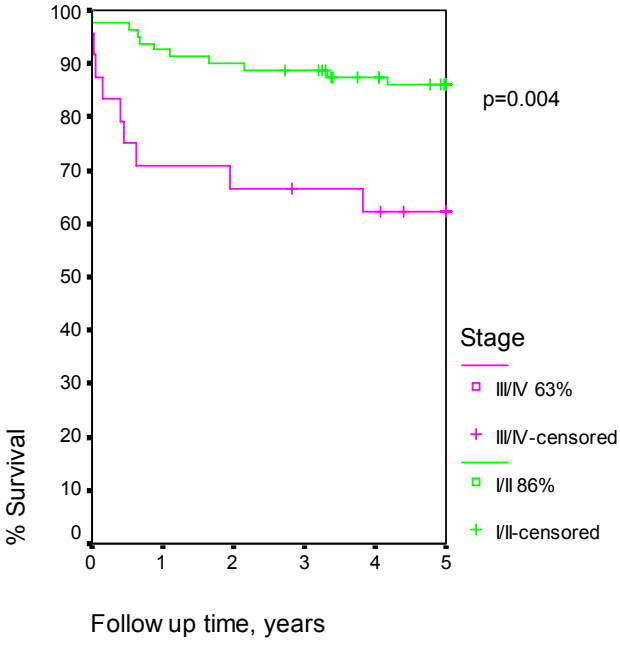


Figure 18. Kaplan-Meier curve for five year survival according to stage of the disease (includes all HL patients, n=105).

Overall survival according to histological subclassification for CHL is shown in Figure 17 and Table 12. Due to small number of cases LD, LR and unclassifiable CHL are grouped together as "other" in Figure 17. The subclassification affected survival showing better survival for NS and MC than for "other" subtypes.

The stage of the disease affected survival with the higher stages (stage III or IV disease) leading to worse outcome (Figure 18). The location of the disease affected survival. The worst survival was for patients with infradiaphragmatic disease or 40% but it was 92% for patients with supradiaphragmatic disease and 66% for patients with disease on both sides of the diaphragm ($p<0.0001$). The presence of B symptoms ($p=0.26$) and bulky disease ($p=0.41$) did not affect survival.

Survival according to therapy was analyzed further. Figure 19 shows five year survival for patients with non-bulky stage I/II disease, which was the same for all treatment modalities. Therapy for bulky stage I/II disease and stage III/IV disease was not analyzed because of low number of patients in one of the treatment groups.

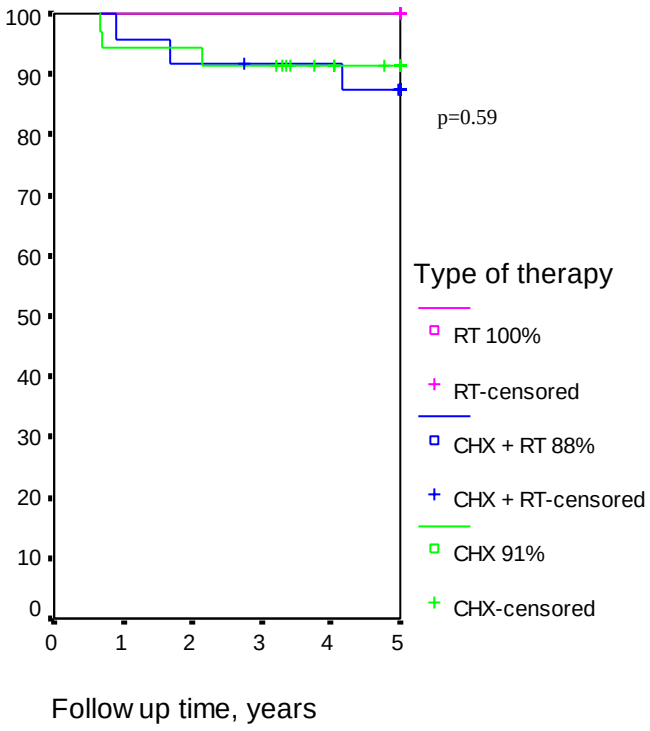


Figure 19. Kaplan-Meier curve for five year survival for patients with non-bulky stage I/II disease according to treatment modalities (n=67) (RT radiation therapy, CHX chemotherapy).

4.5.1 Resistant and relapsed disease

There were eight patients with a disease that was resistant to primary chemotherapy. Their clinical characteristics, histological subclassification, treatment and status in five years can be seen in Table 13. Three were cured of the disease, two of whom had HDCT with ASCT. The third patient (patients 6) had salvage therapy with ICE regimen and stem cell collection was done. He responded well to the ICE regimen but in addition he received radiation therapy and rituximab. HDCT was not given. Those three patients were alive in complete remission at the end of the follow-up period.

Five patients with resistant disease died. One of those patients (patient 1) was in complete remission after HDCT and ASCT but had secondary acute leukemia two and a half years later that led to his death. Another patient (patient 3) had highly resistant disease and died one year after diagnosis. Three were not treated with curative intent (patient 2, 5, and 7) and died shortly after diagnosis. Five year survival for patients with resistant disease was 38%.

Table 13. HL resistant to primary chemotherapy. Clinical characteristics, histological subclassification, first treatment, salvage therapy and survival for eight patients.

Pat.	G	Age	YD	Histo	Stage	Location	First treatment	Salvage therapy	ASCT	5 YS
1.	M	17	1992	MC	IVA	SID	MOPP ABVD & IF	MIME, whole body radiation	Y	Dead
2.	F	75	2002	MC	IVA	SID	MOPP ABVD	IF (palliative)	N	Dead
3.	F	16	1994	NS	IIBX	SD	ABVD & EF	MIME, cyclosporine, VAD, IF	N	Dead
4.	M	23	1995	NS	IVB	SID	MOPP ABVD	BEAM	Y	Alive-CR
5.	M	70	2001	NS	IIB	ID	ABVD	IF (palliative)	N	Dead
6.	M	38	2003	LR	IIA	ID	ABVD	ICE, rituximab, IF	N	Alive-CR
7.	F	81	2004	CHL	IIB	ID	ABVD	Chlorambucil (palliative)	N	Dead
8.	M	29	2005	LP	IVB	SID	ABVD	BEACOPP, ICE, rituximab, BEAM	Y	Alive-CR

Pat patients; G gender; M male; F female; YD year of diagnosis; Histo histological subclassification; CHL unclassifiable CHL; SID supra- and infradiaphragmatic disease; SD supradiaphragmatic disease; ID infradiaphragmatic disease; IF involved field radiation therapy; VAD vincristine, doxorubicin, dexamethason; EF extended field radiation therapy; ASCT autologous stem cell transplantation; Y yes, N no; 5 YS status at five years, CR complete remission

Sixteen patients had a relapse of their disease, ten after chemotherapy and six after radiation therapy. Their clinical characteristics, histological subclassification, treatment and status at the end of the follow-up period can be seen in Table 14 and Table 15.

For patients relapsing after chemotherapy the median follow-up time was 5.12 years (IQR 2.9-13.4) and the median time to relapse was 1.55 years. The relapse rate was 11%. Of the ten patients relapsing after chemotherapy, four had HDCT with ASCT, three of which were alive in complete remission at the end of the follow-up period. All four patients who had HDCT with ASCT went into complete remission. The one who died had a second relapse one and a half years after diagnosis (patient 4). Four were salvaged without HDCT, all of whom relapsed more than 12 months after the first diagnosis. Of those four, three had conventional chemotherapy with radiation and one was salvaged with radiation therapy only. Those four patients salvaged with conventional chemotherapy and radiation therapy were all in complete remission at the end of the study period. Two patients (patients 1 and 8) were not treated with curative intent and died shortly after the relapse. Two patients relapsed within 12 months and one had a very late relapse after ten years. Survival after five years and at the end of the follow-up period was the same or 70% for patients who relapsed after chemotherapy.

Table 14. Relapse after chemotherapy for HL. Clinical characteristics, histological subclassification, first line therapy and salvage therapy and survival for ten patients.

Pat	G	Age	YD	Histo	Stage	Loc-ation	First therapy	D-R years	Salvage therapy	ASCT	Status 2007‡
1.	F	64	1990	CHL	IIA	SD	MOPP	0.51	IF (pall)	N	Dead
2.	M	55	1990	NS	IIA	SD	MOPP ABVD	3.45	MOPP-ABVD IF	N	Alive-CR
3.	M	54	1992	NS	IIIA	SID	ABVD	3.12	EF	N	Alive-CR
4.	F	14	1995	NS	IIBX	SD	MOPP ABVD & IF	1.52	BEAM, carmustin, ifosfamide etoposide	Y	Dead*
5.	M	18	1995	NS	IVA	SID	MOPP ABVD & IF	1.58	Dexa-BEAM BEAM	Y	Alive-CR
6.	M	19	1996	MC	IIB	SD	MOPP ABVD	10.64	ABVD, IF	N	Alive-CR
7.	F	18	2001	NS	IIA	SD	ABVD	1.31	ABVD, IF	N	Alive-CR
8.	F	76	2002	NS	IIIA	SID	ABVD	0.84	AVD	N	Dead
9.	M	67	2003	CHL	IIB	SD	ABVD	2.95	ICE, BEAM,	Y	Alive-CR
10.	F	21	2004	NS	IIA	SD	ABVD	1.45	ICE, BEAM,	Y	Alive-CR

Pat patients; G gender; M male; F female; YD year of diagnosis; Histo. histological subclassification; CHL unclassifiable CHL; SD supradiaphragmatic disease; SID supra- and infradiaphragmatic disease; EF extended field radiation therapy; IF involved field radiation therapy; pall palliative therapy; D-R time from diagnosis to relapse in years; Y yes, N no; ‡status on 31st of December 2007, CR complete remission, *second relapse in year 1997

Relapse rate for patients treated with radiation therapy only was much higher or 80%. Median follow up time was 14.7 years (IQR 10.6-15.2). Three relapsed within 12 months, one after two years and two after 11 years. Seven of the eight patients relapsing after radiation therapy went into complete remission after conventional chemotherapy and one needed an additional second line therapy. One patient, a smoker who had received mantle zone radiation died five years later

of lung cancer (patient 1). Five year survival for patients who relapsed after radiation therapy was 100%, and survival at the end of the follow-up (31st of December) period was 83%.

Table 15. Relapse after radiation therapy for HL. Clinical characteristics, histological subclassification, radiation field, salvage therapy and survival for six patients.

Pat.	G	Age	YD	Histo	Stage	Location	RT	D-R years	Salvage therapy	Status 2007‡
1.	M	60	1990	CHL	IIA	SD	EF	0.99	ABVD	Dead*
2.	M	20	1992	NS	IIA	SD	EF	1.08	MOPP-ABVD, MIME	Alive-CR
3.	M	16	1992	NS	IIA	SD	EF	0.60	MOPP-ABVD	Alive-CR
4.	M	31	1993	NS	IIA	SD	IF	1.95	MOPP-ABVD	Alive-CR
5.	F	19	1993	NS	IIA	SD	EF	11.98	MOPP-ABVD	Alive-CR
6.	M	22	1995	NS	IIA	SD	EF	11.08	ABVD	Alive-CR

G gender; M male; F female; YD year of diagnosis; Histo. histological subclassification; CHL unclassifiable CHL; SD supradiaphragmatic disease; RT radiation therapy; extended field radiation therapy; IF involved field radiation therapy; D-R time from diagnosis to relapse in years; ‡status on 31st of December 2007, *patient went into remission after ABVD, died from lung cancer five years later, CR complete remission

Table 16 shows the clinical characteristics, treatment and follow-up time for all patients who died during the follow-up period. Patients over 60 years accounted for 75% of all deaths and most died within one year from diagnosis.

Table 16. Patients with HL who died during the follow-up period. Their clinical characteristics, histological subclassification, therapy, follow-up time and cause of death.

Pat	G	Age	Hist	Stage	First line therapy	Res/ rel	Salvage therapy	Follow-up time	Cause of death
1.	F	14	NS	IIBX	MOPP ABVD & IF	Rel	HDCT ASCT	3,32	HL/treat
2.	F	16	NS	IIBX	ABVD &EF	Res	Conv. Salvage	1,1	HL/treat
3.	M	17	MC	IVA	MOPP ABVD & IF	Res	WBR ASCT	3,83	HL/treat
4.	M	31	NS	IIA	MOPP & IF	No	-	10.55	Other
5.	F	47	LP	IA	MOPP ABVD	No	-	0,66	Other
6.	M	50	NS	IVAX	-	-	-	0,05	HL/treat*
7.	M	60	CHL	IIA	EF	Rel	ABVD	5.52	Other
8.	F	64	CHL	IIA	MOPP	Rel	IF (pall)	0,69	HL/treat
9.	M	65	MC	IVB	ABVD	No	-	8.79	Other
10.	F	68	NS	IIB	MOPP ABVD & IF	No	-	4,18	Other
11.	M	70	NS	IIB	ABVD	Res	IF(pall)	0,88	HL/treat
12.	M	72	CHL	IIIA	MOPP	No	-	0,44	HL/treat
13.	M	73	MC	IBX	ABVD	No	-	0,54	HL/treat
14.	M	73	LD	IVB	ABVD	No	-	0,02	HL/treat
15.	F	75	MC	IVA	MOPP ABVD	Res	IF (pall)	0,39	HL/treat
16.	F	76	LD	IIIB	MOPP	No	-	6.36	Other
17.	F	76	NS	IIIA	ABVD	Rel	AVD	1,96	HL/treat
18.	F	76	MC	IIIA	ABVD	-	-	0,62	HL/treat
19.	F	80	NS	IB	MOPP ABVD & IF	No	-	1,66	HL/treat
20.	F	81	CHL	IIB	ABVD	Res	Chlor-ambucil (pall)	2,14	HL/treat
21.	F	81	LD	IVB	-	-	-	0	HL/treat**
22.	M	82	NS	IA	IF	No	-	7.82	Other
23.	F	82	CHL	IB	-	-	-	0	HL/treat**
24.	M	84	LD	IVA	-	-	-	0,15	HL/treat
25.	M	88	NS	IIB	-	-	-	0	HL/treat**

G gender; F female; M male; Hist histological subclassification; CHL unclassifiable CHL; X bulky disease; res resistant disease; rel relapsed disease; conv conventional; WBR whole body radiation; pall palliative; *patient died of surgical complications after laparoscopic biopsy; ** patients diagnosed at autopsy

Overall five year survival according to immunohistochemical results of the 101 CHL patients is shown in Table 17. The survival was significantly better for patients who were MUM1 positive, but expression of other antigens did not have a significant effect on survival. Survival analysis for the age groups 16-59 years and 60 years and older, according to LMP1 expression was performed. In patients 16-59 years the five year survival was 93% for LMP1 positive patients and 97% for LMP1 negative patients (p=0.6). In patients 60 years and older the five year survival was 43% for LMP1 positive patients and 21% for LMP1 negative patients (p=0.26). Survival analysis for age 0-15 years according to LMP1 expression was not done because of few patients (one patient died). Because of five or less positive samples for bcl-6, CD3, CD5 or CD45 survival differences were not calculated.

Table 17. Five year survival for 101 CHL patients according to immunohistochemistry results (Kaplan-Meier analysis).

Antigen (% positive patients)	Positive	Negative	p value
CD15 (52%)	88	73	0.055
CD20 (15%)	80	81	0.94
LMP1 (23%)	78	82	0.64
MUM1 (87%)	85	54	**0.003**
Bcl-2 (36%)	89	77	0.15

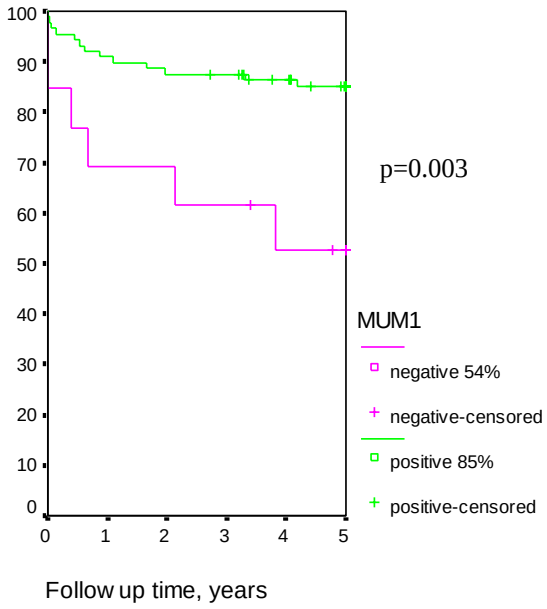


Figure 20. Kaplan-Meier curve for five year survival according to MUM1 expression (includes CHL patients only, n=101).

Table 18. Clinical characteristics of the 101 CHL patients according to MUM1 expression.

Characteristics	MUM1 positive n (%)	MUM1 negative/U n (%)	p value
Median age	31 (IQR 20-48)	45 (IQR 24-78)	
0-59 years	74 (84)	7 (54)	**0.011**
≥60 years	14 (16)	6 (46)	
Male	55 (63)	7 (54)	0.55
Female	33 (38)	6 (46)	
NS	69 (78)	4 (31)	**<0.0001**
MC	14 (16)	3 (23)	
Other	5 (6)	6 (46)	
Stage I/II	69 (78)	9 (69)	0.46
Stage III/IV	19 (22)	4 (31)	

U uncertain staining or technical flaw, IQR interquartile range

4.5.2 Multivariate survival analysis and comparison between groups

The following variables were entered into the Cox proportional hazard model: gender (stratified as it violated the proportional hazard test), age (as a continuous variable), B symptoms (yes/no), bulky disease (yes/no), stage I/II vs. III/IV, CD30, CD15, CD20, LMP1, MUM1 and bcl-2 expression. The results for the 101 patients with CHL are shown in Table 19.

Table 19. Cox proportion hazard model for five year survival (for the 101 patients with CHL, stratified for gender).

	Hazard ratio (HR)	95% CI for HR	p value
Age (continuous)	1.41 for every 5 years	1.25-1.60	<0.0001
Non-bulky	1		
Bulky disease	4.85	1.34-17.43	0.016
CD15-	1		
CD15+	0.35	0.12-0.99	0.049

Bulky disease was not a significant prognostic factor in the univariate analysis whereas gender, histological subclassification, stage and MUM1 expression were. For further analysis comparison of these factors was done, see Table 20-22. In patients younger than 60 years, five year survival was worse in patients with bulky disease (Figure 21). There was only one patient over 60 years who had a bulky disease.

Table 20. Clinical characteristics and histological subclassification for patients 0-59 years and 60 years and older.

	0-59 years n (%)	≥60 years n (%)	p value
Non-bulky	70 (83)	20 (95)	0.29
Bulky	14 (17)	1 (5)	
Stage I/II	69 (82)	12 (57)	**0.015**
Stage III/IV	15 (18)	9 (43)	
A	50 (60)	9 (43)	0.17
B sympt.	34 (41)	12 (57)	
NS	67 (80)	6 (29)	**<0.0001**
MC	13 (16)	4 (19)	
Other	1 (1)	10 (48)	
LP	3 (4)	1 (5)	

Table 21. Clinical characteristics and histological subclassification according to bulky disease.

	Bulky n (%)	Non-bulky n (%)	p value
Male	9 (60)	55 (61)	1
Female	6 (40)	35 (39)	
Stage I/II	12 (80)	69 (77)	1
Stage III/IV	3 (20)	21 (23)	
A	6 (40)	53 (59)	0.17
B sympt.	9 (60)	37 (41)	
NS	12 (80)	61 (68)	0.46
MC	2 (13)	15 (17)	
Other	0	11 (12)	
LP	1 (7)	3 (3)	

Table 22. Clinical characteristics and histological subclassification according to gender.

	Male n (%)	Female n (%)	p value
0-59 years	54 (84)	30 (73)	0.16
≥60 years	10 (16)	11 (27)	
Stage I/II	45 (70)	36 (88)	0.04
Stage III/IV	19 (30)	5 (12)	
A	37 (58)	22 (54)	0.68
B sympt.	27 (42)	19 (46)	
Non-bulky	55 (86)	35 (85)	0.94
Bulky	9 (14)	6 (15)	
NS	43 (67)	30 (73)	0.5
MC	13 (20)	4 (10)	
Other	6 (9)	5 (12)	
LP	2 (3)	2 (5)	

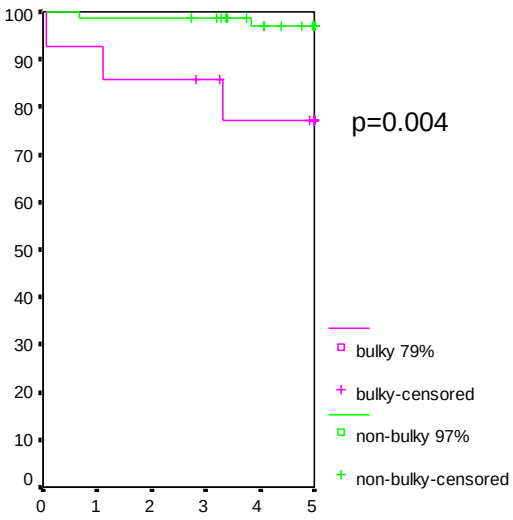


Figure 21. Kaplan–Meier curve for five year survival for patients younger than 60 years according to bulky disease (n=84).

4.5.3 Secondary malignancies after Hodgkin lymphoma

Six patients developed secondary malignancies after HL. They were diagnosed with myelodysplastic syndrome, mycosis fungoides, acute myeloid leukemia and colon and lung cancer (two patients). Because of the small number of cases no attempt was made to correlate the risk to the general risk in the population. Details on patients with secondary malignancies are given in Table 23.

Hodgkin lymphoma in relation to other lymphoma pathology

Two patients had Richter's transformation, six months and two years after a diagnosis of chronic lymphocytic leukemia (CLL), both were in complete remission from CLL, one of whom had been treated with fludarabine. The other patient was LMP1 positive. Both patients died six months after the diagnosis of HL. One patient had a previous history of non-HL and had been treated with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) and rituximab and was considered to be in complete remission. Five years later he developed HL in a different location with no signs of non-HL. One patient was diagnosed with a rare variant of composite lymphoma made of NS and follicular lymphoma involving paraduodenal nodes and the mesenterium.

Table 23. Secondary malignancies after HL. Clinical characteristics, histological subclassification, treatment and time to secondary cancer of six patients.

Pat	G	Age	Histo	Stage	Location	Treatment	TTSC-years	Secondary cancer
1.	F	80	NS	IB	Inguinal/ femoral	MOPP-ABVD & IF 36Gy	0.81	Myelo-dysplastic syndrome
2.	F	21	NS	IIA	Cervical/ mediastinal	ABVD/ICE/ BEAM/ASCT	3.21	Mycosis fungoides
3.	M	82	NS	IA	Axillary	IF 40Gy	6.17	Colon cancer
4.	M	65	MC	IVB	SID-spleen, liver	ABVD	7.96	Lung cancer
5.	M	60	CHL	IIA	Cervical/ mediastinal	ABVD/ mantle RT 40Gy	4.93	Lung cancer
6.	M	17	MC	IVA	SID-spleen, liver	MOPP-ABVD/IF 30Gy/MIMEx2/ whole body radiation/ASCT	2.47	Acute myeloid leukemia

G gender; F female; M male; histological subtype, SID supra- and infradiaphragmatic disease; ASCT autologus stem cell transplantation; IF involved field radiation therapy; TTSC-y Time to secondary cancer in years, calculated from the time of diagnosis of the HL to the time of diagnosis of the secondary malignancy; *status on 31st of December 2007.

5 Discussion

While the incidence of non-HL has been increasing in the world, the incidence of HL has been relatively stable in Western countries (117). The same probably holds true for Iceland, with an age standardized incidence of 2.05 per 100.000 in our study. The age standardized incidence in Iceland however fluctuates between years because of our low numbers of patients. Figure 22 shows trends in the incidence of HL for men and women in the Nordic countries from 1963-2003 published by Storm and colleagues and the great variation in incidence in Iceland (yellow) (118).

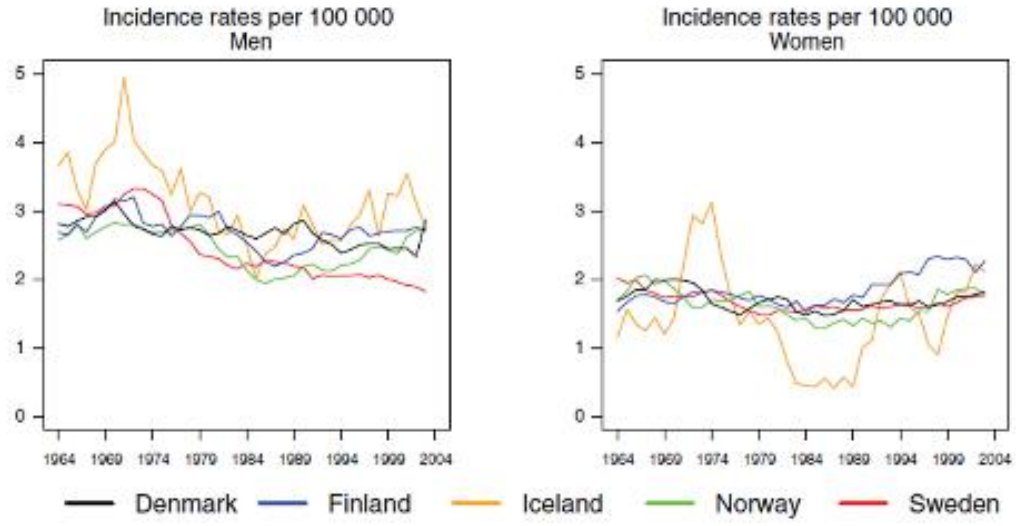


Figure 22. Trends in incidence in the Nordic countries from 1964-2003 (118).

Our study shows that the age standardized incidence in Iceland is bimodal, with one peak in young adults and the other peak after the age of 70 years. This bimodal incidence for HL is well known in other Western countries but is not found in developing countries (117). Iceland has a similar male to female ratio (3:2) as is found in Scandinavia although the gender ratio is more equal in some other Western countries (119-121).

Most patients in our study were diagnosed with stage II disease and only 22% were diagnosed with stage III/IV disease. Other studies have shown a higher incidence of stage III/IV disease or in approximately 40% of cases (35, 68, 122). One study, which included over 8000 patients (based on the SEER database, USA), additionally showed that stage of the disease was dependent on the histological subclassification and age at diagnosis. Younger people tended to be diagnosed at an earlier stage, have less B symptoms and have more often the NS subtype. As our study shows similar division of histological subtypes and age as most other studies it is difficult to explain the small number of patients diagnosed at stage III/IV. However this could be due to chance as the number of patients are few or possibly due to inaccurate staging. There were 44% of patients with B symptoms in our study which correlates well with other studies. Patients with stage I disease had B symptoms in 21% of cases whereas patients with stage II, III

and IV disease experienced B symptoms in 48%, 33% and 58% of cases, respectively. The fact that fewer patients with stage III than stage II disease had B symptoms and relatively many patients with stage I disease had B symptoms, can probably be explained, in part, by few patients and inaccurate documentation in patient's charts. Isolated infradiaphragmatic disease was seen in 10% of cases which is in concordance with other studies (104). Bulky disease and bone marrow infiltration was present in 14% and 3% of the patients respectively, which is also similar to other studies (12).

The use of tissue microarray for immunohistochemical staining has rapidly increased in recent years. This technique makes the analysis of large number of samples less time consuming as well as conserving tissue. In our study CD30 was positive in nearly all cases of CHL which is consistent with previous reports (7). In the literature there is however a big discrepancy in CD15 positivity (38-100%) (53). This difference can, at least in part, be explained by different fixation and tissue processing as well as different patient material and different cut-off points for positivity. The largest study done so far included 1286 patients who had participated in clinical trials conducted by the German Hodgkin Study Group which showed CD15 positivity in 85% of the CHL patients (cut-off point for positive staining >20%) (53). On the other hand Enblad and colleagues in a study of an unselected Swedish population (154 patients) found CD15 to be positive in only 57% of cases (cut-off point for positive staining >20%) which is very comparable to our result of 52% (any staining on Reed-Sternberg cell considered positive) (123). Staining for CD15 was generally strong but only unequivocally positive samples were listed as such. Staining in CHL for CD20 is also highly variable with different studies showing CD20 positivity in 5-80% of the cases and there does not appear to be any obvious explanations for this disparity (other than the ones previously mentioned for CD15) (53). In our study CD20 was positive in 15% of CHL patients compared to 5% in the German Hodgkin Study Group study and 26% in the Enblad study (LP cases included) (53, 123).

The number of patients reported positive for EBV also varies between studies and ranges from 17-68% depending heavily on the study population (66). EBV positive HL is more common in the MC subtype and in children and older adults (35). Our result of 23% LMP1 positivity is however quite similar to those found in other population based studies which include all age groups (17-27%) (67, 68). The LMP1 negative patients in our study were more often diagnosed with the NS subtype and more often diagnosed with stage I/II disease. No difference in expression was seen according to age 0-15 years, 16-59 years or 60 years and older in our study. MUM1 has only recently been investigated in relation to HL and is found to be positive in most cases of HL which agrees with our study (87% of CHL patients MUM1+) (124). MUM1 was positive in most of the NS cases (95%) and more frequent expression was seen in younger patients (younger than 60 years). However, only 13 patients were MUM1 negative so comparison between these groups is difficult. Nonetheless these results seem to correlate well with the study of Valsami and colleagues which is the only previous study addressing this point (73). Expression of bcl-2 was seen in 36% of our CHL patients which correlates well with other studies that show

that 10-60% of cases are positive for bcl-2 (cut-off points for positivity from 10-50%) (74-77). Two possible HL cases of T-cell origin were in the cohort, with phenotypes CD30+/CD15+/CD20-/CD3+/CD5+/CD45+ and CD30+/CD15+/CD20-/CD3-/CD5+/CD45-. It has been shown that in 5% of cases the Reed-Sternberg cell may express T-cell antigens, but only in a handful of cases has T-cell receptor gene rearrangement been established and thus true T-cellular origin been confirmed (82, 125). In the literature, CD45 positivity is seen in the majority of LP patients but in less than 5% of CHL patients (81). In our study only three samples were CD45 positive (one in LP) and staining for bcl-6 was generally weak (possibly due to technical reasons) and not seen in LP cases as would be expected (80).

Our study shows that the treatment of HL in Iceland has in general followed what could be considered to be the standard of care elsewhere (104, 108, 109). Patients were treated with chemotherapy only in 54% of cases, with combination therapy (chemotherapy and radiation therapy) in 38% of cases and radiation therapy only in 8% of cases. MOPP-ABVD hybrid was the most commonly used chemotherapy combination in the former period (64%), while ABVD was the most popular regimen in the latter period (94%). The use of radiation therapy after chemotherapy for stage I/II disease has been debated but in our study 35% of the patients with non-bulky stage I/II disease and 83% of the patients with bulky stage I/II disease received combination therapy. Few large clinical trials have addressed the use of chemotherapy only in HL and guidelines from NCCN and ESMO recommend, as a first choice, combined treatment for both favourable and unfavourable limited disease. However, in both these guidelines treatment with chemotherapy only for young patients without risk factors is an option (99, 106). The risk associated with radiation therapy, i.e. increased risk of secondary malignancies and heart disease, is largely dose dependent and thus much less apparent with the use of low dose involved field radiation which usually follows chemotherapy (126). In the latter time period less extensive radiation fields and smaller doses were used, as well as the use of radiation as a single treatment modality was hardly practiced (one patient). All eight patients treated with radiation as the only therapy had non-bulky stage IA or IIA disease

Overall five year survival in this study was 81% or 85% when only those patients who received treatment are included. That is comparable to the general survival rate in the developed world. In the Nordic countries, Europe and USA the current five year overall survival is 78% (Europe) and relative survival is 83-85% (Nordic countries, Europe and USA) (118, 122, 127). Cancer registry databases usually calculate relative survival which means that survival is calculated relative to the general population. However in this study overall survival was used, as do most clinical studies. Whether relative or overall survival is used affects survival calculation, especially for older populations, in which differences between these two methods of calculation can be significant (127). As the patient population in this study is relatively young, differences in overall and relative survival would be expected to be minimal.

In our study, in contrast to most other studies, there is an indication that five year survival is better for male patients (88%) than female patients (71%) ($p=0.04$) (118, 128). This possible

survival advantage for the males was not present in patients younger than 60 years but in spite of this no significant difference was seen in either the mean age for males and females or the ratio of male and female older than 60 years. It is possible that males had more often stage III/IV disease (p=0.04), but no difference was seen between the genders regarding bulky disease, B symptoms or histological subclassification. The multivariate analysis had to be stratified for gender as it violated the proportional hazard assumption.

The survival rate was similar for the two time periods though treatment had changed. A recent study has shown that the survival for HL in the Nordic countries has been steadily improving from 1964 to 2003, and that advantage is most striking in older patients (118). For example in Sweden in the years 1964-2003 the five year relative survival went from 65% to 95% for patients 0-29 years, from 39% to 99% for patients 40-49 years and from 10% to 45% for patients 70-89 years, respectively. Iceland was not included in this analysis because of our few patients. This study clearly demonstrates the increase in survival rate with the introduction of the multidrug chemotherapy in the 1960s, and how HL has gone from a universally fatal disease to a highly curable one. Over the last 10-20 years there has only been minimal improvement in survival as the survival is already quite good. Thus the main focus has been on decreasing early and late toxic effects of therapy. When only looking at the time period presented in our study (1990-2005), the survival in Sweden (1989-2003) went from 78% to 84% for men and women (all age).

Many studies have shown a steep decline in survival in HL after 45 years (122, 129). Our study on the other hand showed very comparable survival for all age groups up to 60 years (0-19, 20-39 and 40-59), but after that survival declined rapidly. In our study the five year overall survival for patients 40-59 years was 88%, as compared to results from Europe of 80% for patients 45-54 years and 67% for those 54-64 years (127). In the multivariate analysis increasing age was a strong negative prognostic factor. Five year survival for patients 60 years and older was 29% but 94% for those who were younger than 60 years. Patients older than 60 years accounted for 22% of the patients in our study whereas 75% of the deaths were in this group. Patients older than 60 years were more often diagnosed with stage III/IV disease and were more often diagnosed with the “other” CHL subtype which included LD, LR and CHL unclassifiable. HL seems to follow a more aggressive path in the elderly and higher stage disease and B symptoms are more common in older patients. In addition B symptoms have a more adverse prognostic effect in older patients but age by itself is also an independent adverse prognostic factor (122). Various reasons probably account for worse survival in elderly HL patients. Elderly patients are more likely to have co-morbid conditions that can influence therapeutic options available as well as being more prone to toxic effects of treatment. However some studies indicate that this group might be undertreated (130). In our study all but one patient 60 years or older were treated with standard regimens. The one who was not treated died shortly after diagnosis. If autopsy cases are excluded for purpose of analysis, 18 patients were diagnosed 60 years and older, of which nine died within one year or 50%. Those patients all died because of HL, treatment related toxicities or infections.

In the univariate analysis better survival was seen for patients with the NS and MC subtypes than for "other" subtypes of CHL. This difference was abolished in the multivariate analysis. This can probably be explained by the fact that diagnosis of histological subtypes differed between age groups, as patients 60 years and older were most often diagnosed with “other“ subtype while patients younger than 60 years were most often diagnosed with the NS subtype ($p<0.0001$). Historically MC and LD had a worse prognosis than NS but after the introduction of modern day treatment this difference has largely been abolished (7).

In our study being diagnosed at a higher stage was a negative prognostic factor in the univariate analysis. It was however rendered insignificant in the multivariate analysis. That could be due to the fact that younger patients (0-59 years) were diagnosed at an earlier stage than those who were older, which is in agreement with other studies (122). Our study showed inferior survival for patients with infradiaphragmatic vs. supradiaphragmatic disease, being even worse than for patients with stage III/IV disease. This data is however based on very few events, and studies that have analyzed large numbers of patients where other prognostic factors are taken into account do not show difference in survival (87). In the univariate analysis bulky disease was not associated with worse survival whereas in the multivariate analysis it was found to be a negative prognostic factor. This can possibly be explained by an association between age and bulky disease as patients with bulky disease were all but one younger than 60 years. When analysing only patients younger than 60 years, the five year survival for patients with non-bulky disease (97%) was significantly better than for patients with bulky disease (79%). However, it should be noted that there were only five deaths in both of these groups, two in patients with non-bulky disease (n=70) and three in patients with bulky disease (n=14). Thus our results can only be interpreted as an indication that bulky disease is a negative prognostic factor in HL, which is however in agreement with the literature.

Prognosis for early stage favourable disease is very good with current treatment with five year survival reported up to 98% (103). Decreasing early and late toxic effect of treatment without compromising good survival is thus what most clinical trials are currently focusing on. Whether radiation therapy should be omitted in patients with localized non-bulky disease has been debated for the past 10-15 years. Most large clinical trials that have been published, have been based on treating those patients with combination therapy (102, 104). In our study patients with localized non-bulky disease received combination therapy in 35% of cases and chemotherapy only in 51% of cases. Five year survival was the same irrespective of treatment modalities for this group. Few studies have however been able to show survival differences for stage I/II disease according to treatment modalities as survival is in general good and thus a large number of patients is needed to show a significant difference. However a recent meta-analysis done on all published randomized clinical trials in favourable and unfavourable early stage HL did show an improved survival when combined modality treatment was used instead of chemotherapy only (105).

Patients with resistant disease had dismal survival in our study as in other studies with a five year survival of 38% (131). This is the group on which much focus has been on recently regarding finding new prognostic factors and treatment modalities, as early aggressive therapy

might improve survival. Out of eight patients with resistant disease three were treated with HDCT with ASCT, two of which were alive after five years. Three patients 60 years and older had resistant disease, all of whom died shortly after diagnosis, but it has been shown to be virtually impossible to salvage patients older than 60 years who do not respond to conventional chemotherapy (130).

On the other hand did patients with relapsed disease show the same survival as those who did not relapse, regardless of what primary treatment they had been given, highlighting the efficacy of salvage therapy. Relapse rate was 11% for those treated with chemotherapy with or without radiation which is similar to other reports (121). It has been shown by others that time to relapse (less than 12 months) affects survival, as well as stage III/IV disease and anemia (132). In our study two patients relapsed within a year, both of whom died of HL.

Seven patients received HDCT with ASCT (three for resistant disease and four after relapse) and long term survival was 71% (31st of December 2007) and although only few patients were treated in this manner survival was comparable to other studies (110).

The relapse rate for patients given radiation therapy only, as first line treatment was 80% and is much higher than expected. All patients treated with radiation therapy only were at stage IA or IIA. It is known that patients treated with radiation therapy more commonly relapse (30-40%) than others and have better survival at relapse than patients treated with other treatment modalities (132). That is in accordance with our study which showed 83% survival in the end of the study period (31st of December 2007). Seven of the eight patients who relapsed after radiation therapy were salvaged with conventional chemotherapy and one needed additional second-line chemotherapy. Studies have shown that short time to relapse is not an adverse prognostic factor when occurring after treatment with radiation therapy only, as opposed to relapse after chemotherapy (111). The two patients in our study who relapsed within a year after radiation therapy went into complete remission after conventional chemotherapy. The high relapse rate in our study can not readily be explained but could be due to chance due to the low number of patients.

Studies linking immunohistochemical staining to prognosis are often conflicting (53). That is probably due to the fact that patients are often few, events are few, the study populations may be selected, the cut-off points for positivity for the antigens are often different and the tissue processing can be dissimilar. Different statistical analysis might also be applied and different end-points used (overall survival, failure free survival, disease specific survival) making it hard to draw any generalized conclusion from these studies (133). Our results should therefore, not least because of the limited number of patients in this study, be interpreted as a mere indication of possible prognostic factors and as with all prognostic factors should be validated in a large cohort, preferably in a prospective manner.

CD30 cannot be used as a prognostic factor as it is positive in nearly all the patients. CD15 on the other hand has been studied as a possible prognostic factor and most studies have shown CD15 positivity to be related to better survival or to be insignificant (52, 53, 134, 135). This is in concordance with our study which showed that CD15 positivity was a possible positive prognostic factor in both the

univariate and the multivariate analysis. CD20 has also been investigated as a possible prognostic factor but the results have been inconclusive although most investigators have found it to be an adverse prognostic factor or insignificant (53, 56, 57, 136). In our study CD20 did not affect survival, but again the small number of patients may preclude meaningful results.

The association between EBV and survival in HL is not clear though it may be age dependent (61). Population based studies have shown worse survival for older adults and a possible protective effect in children and young adults (35, 66). In our study no survival difference related to EBV expression was seen irrespective of age (analysis not done for patients 0-15 years) but a trend was actually seen towards better survival in patients 60 years and older who were LMP1 positive.

Despite the fact that MUM1 is positive in the majority of CHL patients its possible prognostic value is being studied. In our study expression of MUM1 (87% positive) was linked with a favourable outcome in the univariate analysis. It was however insignificant in the multivariate analysis probably because MUM1 was more often expressed in patients younger than 60 years. Two studies have been published on MUM1 expression in HL in relation to prognosis, both showing a relationship to better prognosis for those who were MUM1 positive (72, 73). One of these studies presented a molecular risk score (including 11 genes involved in apoptosis, cell cycle and MUM1) that identified patients with an extremely poor outcome (25% five year failure free survival) (72). A better established prognostic factor is bcl-2 which in most studies has been linked with a worse outcome (76-78). In our study however it did not affect survival. Again these results need to be interpreted with caution due to the small number of cases. No attempt was made to correlate immunohistochemical results for bcl-6, CD3, CD5 or CD45 to survival because of few positive samples.

In this study six secondary malignancies occurred in the HL patients. Three were hematological (myelodysplastic syndrome, mycosis fungoides and acute myeloid leukemia), one was a colon cancer and there were two lung cancers. The relationship between HL and secondary or treatment related acute leukemia is well known (137). The risk is related to the dose of alkylating agents given and peaks in five years after treatment and holds grave prognosis. The ABVD regimen does not have an alkylating agent, compared to previously used MOPP regimen, but newer chemotherapies such as BEACOPP do. Both the patient with acute myeloid leukemia and the one with myelodysplastic syndrome had been treated with alkylating agents and died within a year from diagnosis from the secondary malignancies. Mycosis fungoides is a cutaneous T-cell lymphoma and compromises 2% of all lymphomas. An association seems to be present between B-cell malignancies and mycosis fungoides, as a simultaneous diagnosis of mycosis fungoides and B-cell malignancies is more common than one would expect if occurring only by chance (138, 139). A relationship between HL and mycosis fungoides has been reported, though more commonly with mycosis fungoides as the primary malignancy (138-140). It as been postulated that the risk of getting secondary non-HL is related to treatment with alkylating agents or immune deficiency secondary to HL treatment which is interesting as the patient with mycosis fungoides was treated with three kinds of alkylating agents (ifosfamid, melphalan and carmustin) and

recieved HDCT (141). Radiation therapy on the other hand has been associated with an increased risk of solid tumours, especially lung and breast cancer in a dose-responsive manner (137). The risk of radiation therapy related lung cancer is also highly affected by smoking and treatment with alkylating agents. The elevated risk of lung cancer associated with radiation therapy is commonly not seen until ten years after therapy but earlier when associated with alkylating agents (142). In our study there were two patients who developed lung cancer, neither however where treated with alkylating agents but one had been treated with mantle zone radiation therapy and was a smoker. Modern day treatment has maintained or improved survival using less toxic agents and thus the risk of getting secondary malignancies has decreased.

In our study two patients had Richter's transformation from CLL into HL (1.9%). A recent population based study from Tyrol in Austria showed a similar frequency of Richter's transformed HL (1.3%) (121). The HL variant of the Richter's transformation is seen in about 1% of CLL patients and has been associated with fludarabine treatment and EBV infection (143, 144). One of our patients with Richter's transformation was treated with fludarabine and the other one was LMP1 positive. Patients who develop HL by Richter's transformation generally have a worse prognosis than patients with primary HL and this is born out by the survival of the two patients which was only six months (144). One patient had a previous history of follicular lymphoma and was in complete remission. As previously mentioned an elevated risk of developing HL after being diagnosed with non-HL has been documented most likely related to the treatment of the non-HL (137, 145). Of interest in our study is a composite lymphoma of a follicular lymphoma and NS in the same lymph node. Only four cases of such composite lymphoma occurring in the same lymph node have been reported previously in the literature (146-149).

5.1 Limitations and strength

This is a retrospective study and as such is limited to the fact that information is often not well registered in charts and also not always in a standardized manner. For example staging was sometimes documented incorrectly in the charts, i.e. liver involvement was classified as stage III disease but not stage IV disease. Staging was corrected for the purpose of the study but errors like that were possibly missed for some patients and thus may effect how staging relates to overall survival. It was also hard to get a complete overview of all the therapies some patients received and accurate timing of when complete remission was achieved was not systematically recorded in patients chart. The study has of necessity relatively few patients and few events and therefore survival analysis for different subgroups may have too few end-points to be significant.

The main strength of this study is that it is population based and therefore has no selection bias. Many clinical studies today are based on clinical trials or single institution reports and thus deal with a selected group of patients. Often survival is better in patients participating in clinical trials than in the general patient population, especially for older patients (150). Thus a population based study would be expected to yield more accurate results regarding treatment and prognosis, although there is some evidence that the results of clinical trials regarding HL may reflect the outcome in the general population better than for other malignancies (151).

6 Conclusions

This study shows that the epidemiology of HL in Iceland in the years 1990-2005 is similar as in other Western countries and that the therapy given following what has been regarded the standard of care. Histological subclassification and the phenotype of HL, is similar as in other Western countries. Relapse rate was unusually high or 80% for patients treated with radiation therapy with no apparent explanation. Survival for those patients was however the same as for non-relapsed patients. Overall five year survival was 81% for all patients and 85% excluding patients who did not receive any therapy. Increasing age was a very strong negative prognostic factor and half of the patients older than 60 years died within a year from the diagnosis of HL. Bulky disease was a possible negative prognostic factor. Few patients and more importantly few events were the limiting factor in finding prognostic factors.

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