Upsala J Med Sci 99: 1–38, 1994

# Hodgkin's Disease in Young and Elderly Patients. Clinical and Pathological Studies

*Minireview based on a doctoral thesis*

Gunilla Enblad

*Department of Oncology, University Hospital, Uppsala, Sweden*

## INTRODUCTION

Hodgkin´s disease (HD) is a malignant lymphoma with an annual incidence of approximately 200 in Sweden. HD is characterized by an unusual bimodal age distribution with one incidence peak in young adults and one above the age of 60 (Kaplan,1980). The malignant cells, the Hodgkin and Reed-Sternberg cells (HRS), comprise only a minority of the cells in the tumours and the greater part of the tumour cell volume is composed of "reactive cells" mainly lymphocytes, eosinophils, neutrophils, fibroblasts, plasma cells, epitheloid cells and histiocytes (Kaplan,1980).

There are few other malignant disorders where advances in therapy have been so outstanding. From being an inevitably fatal disease, the majority of the patients are now cured. The estimated five-year survival for young patients is 70-80%, but treatment-related morbidity is present, also including secondary malignancies (Rosenberg,1991, Swerdlow,1993). For patients above the age of 60, the outcome is much worse with a five-year survival of 20-30% (Vaughan Hudson, 1983, Wedelin, 1984, Walker 1990). The reason for this difference is not known but several explanations have been proposed, eg. another disease entity (MacMahon,1966), a reduced tolerance to therapy (Peterson, 1982, Austin-Seymor, 1984) and a more pronounced immunological deficiency in elderly patients (Björkholm, 1990). The knowledge about treatment effects in elderly patients is rather limited, and usually based on hospital series where patient selection may be substantial, with only a small proportion of the total number of elderly patients being reported.

Two main issues in the management of HD are, thus, to improve the prognosis for elderly patients and to individualize treatment in the young by identifying poor-risk patients who may benefit from intensified treatment and patients with a favourable prognosis in whom reduced, less toxic treatments can be delivered. In order to accomplish these goals is important to study the clinical and tumour-biological features of HD in all ages, preferably in unselected patient material, where all patients with HD have been identified.

## LITERATURE REVIEW

### HISTORY

In 1832, Thomas Hodgkin described seven patients with enlargements of lymph nodes and spleen (Hodgkin, 1832). In some of these patients, the diagnosis of HD has been confirmed by

subsequent investigations (Fox, 1926, Dawson, 1968, Kaplan,1980). The disease was named by Sir Samuel Wilks, in 1865 (Wilks, 1865) and in 1878, the first histopathological description was published by Greenfield (Greenfield, 1878). In 1898, Carl Sternberg described the giant cells characteristic of HD (Sternberg, 1898) and Dorothy Reed the prominent nucleoli (Reed, 1902), and thereafter the cells are designated Reed-Sternberg cells. Reed also described an association with tuberculosis, which was identified in so many cases that many pathologists considered HD to be a variant of tubeculosis. The presence of Reed-Sternberg cells and their mononuclear variant, the Hodgkin cell, is required for a correct diagnosis. The first histologic sub-classification was outlined in 1936 by Rosenthal, who divided HD into four types, depending on the degree of lymphocytic infiltration (Rosenthal, 1936). He could also demonstrate a relation to prognosis. In a later classification Jackson and Parker divided HD into paragranuloma, granuloma and sarcoma (Jackson, 1947). This was modified by Smetana and Cohen who recognized a special form of granuloma with marked fibrosis and a significantly better prognosis (Smetana, 1956). In 1966, Lukes et al published a classification that combined all previous attempts to relate the microscopical features of the disease to prognosis and HD was divided into six groups. This was subsequently simplified to four, which formed the Rye classification (Lukes, 1966). This classification has gained general acceptance and is today used world-wide. In the Rye classification, HD is divided into the lymphocytic predominance (LP), nodular sclerosis (NS), mixed cellularity (MC) and lymphocytic depletion (LP) subtypes.

## EPIDEMIOLOGY

The incidence of HD varies in different parts of the world, with a high incidence in Western Europe and the United States and a low one in for example Japan and India (Correa, 1971). Characterisitic of Hodgkin´s disease is a bimodal age distribution. Correa and O´Conor have described three different epidemiological patterns: **I.** In developing countries a first incidence peak is seen in children with a predominance for males and a second peak above the age of 50. **III.** In developed urban countries there occurs a first peak in the third decade, then a decline in incidence and a afterwards a rising trend above the age of 40 with a second peak in the seventh decade **II.** An intermediate pattern is seen in for example Eastern Europe and rural Norway. This findings are supported by alterations in trends as regards deaths from HD in the USA (Correa, 1971) and Scotland (Boyle, 1988) were decreases in death rates in children with HD semm to be associated withsocio-economic inprovements. Furthermore, incidence rates in Cuba have changed and a shift towards a bimodal pattern has taken place, as the country has developed from an agricultural ecomomy to an industrialized one (Merk, 1990). Higher HD incidence rates have been noted in urban areas, compared with rural ones (Franssila, 1977) and in persons with higher socioeconomic levels (Gutensohn, 1982, Glaser, 1987). The incidence rates as regards elderly patients are reported to have remained essentially unaltered, regardless of geography (MacMahon,1966, Correa and O´Conor, 1971). No study has, however, dealt specifically with the incidence of HD in the elderly. The proportions of the histopathological subgroups also differ from country to country and between age groups. The prognostically unfavourable subgroups MC and LD, are more common in developing countries, the rural areas of developed countries and in the elderly. NS is more common in the young adult (Correa and O'Conor, 1971, Franssila, 1977) and Mac Mahon has proposed three different disease entites: 1. a disease of children, 2. a disease of young adulthood and 3. a disease of old age, with a male predominance in the first and last groups (MacMahon, 1966).

## ETIOLOGY

The etiology of HD is unknown. A number of factors have been associated with an increased risk of developing HD. HD is more common in male patients in all studied populations (Kaplan, 1980). High social class and associated factors such as low number of siblings and high maternal education have been shown to increase the risk of HD in the young. In contrast, these factors are associated with a lower risk in elderly patients (Guthenson 1982, Mueller, 1987, 1991, Serraino 1991). Relatives of HD patients also have a higer risk of HD (Kerzin-Storrar,1983, Kaplan,1980) and case clusters have been described (Vianna, 1973) although the methodology applied has been criticized (Kaplan, 1980).

Due to the close association between HD and tuberculosis, an infectious cause has been suspected. Epidemiological data indicate that HD may develop as a rare consequence of a common childhood infection (Mueller, 1987, 1991). The association between HD and tuberculosis has, however, also been attributed to the immune-deficiency in HD patients (Jackson, 1947, Björkholm,1984).

### Epstein-Barr virus

Epstein-Barr virus (EBV) is the primary candidate for implication as the the "common childhood virus" for several reasons. Persons with a history of infectious mononucleosis, which is the more serious form of EBV infection and affects primarily young adults, have an increased risk of developing HD (Mueller, 1987, 1991). HD patients also have elevated titers of antibodies reactive with several EBV antigens (Johansson, 1970, Mueller, 1987) at the time of diagnosis. Elevated titers to EBV antigens have also been demonstrated prior to the diagnosis of HD (Mueller, 1989). The main hypothesis is that EBV is involved in the pathogenesis of HD in the younger age group whereas the risk factors for developing the elderly form of HD more resemble those in NHL patients (Mueller, 1991).

With modern techniques such as in situ hybridisation and the polymerase chain reaction (PCR) it has been possible to demonstrate the presence of EBV DNA in the HRS in a substantial number of HD patients (15-80%) (Herbst, 1992). The number of EBV- positive patients has been reported to vary with geographical region, with a higher proportion of positive cases in developing countries (Zhou, 1993, Ambinder,1993, Chang,1993). A higher proportion of EBV-positive patients has also been described in children and in patients above 50 years (Jarret, 1991) and in HD affecting HIV-infected patients (Audouin,1992). Furthermore, several authors have found more EBV positive cases within the MC subtype of HD (Vestlev, 1992). Thus, most available data contradict the hypothesis that EBV is the causative agent in young adults with NS histology and clearly more research is needed to elucidate the role played by EBV in HD.

### Immunodeficiency

Many HD patients have an impaired cell-mediated immunity. Circumstantial evidence indicates that the immunodeficiency is present prior to the HD diagnosis (Björkholm,1984, Merk 1990). A similar cell-mediated immune-deficiency is present in a substantial proportion of first-degree relatives of HD patients (Cimino 1988, Merk 1990) and patients off successful therapy for a long time have a persistent immune defect (Cimino, 1988, Liberati,1987). The immmunodeficiency has been reported to be more pronounced in elderly patients and in patients with advanced stages, systemic symptoms and unfavourable histologies. It is also related to a

poor prognosis (van Rijswijk 1986, Tullgren 1991). The immunodeficiency does not appear to be a prerequisite for the development of HD, since HD patients with immune parameters entirely comparable with those of normal controls exist (Tullgren, 1991).

The immunodeficiency is characterized by a reduced, delayed skin hypersensitivity and by a reduced number of lymphocytes in peripheral blood: this applies especially CD 4+ T-cells (helper cells) whereas the number of CD 8+ T-cells (suppressor cells) is normal or only slightly depressed. The peripheral lymphocytes of HD patients also respond poorly to mitogens in vitro and have an increased spontaneous DNA synthesis. B-lymphocyte functions are generally well-preserved, except in patients with advanced stages, and Ig production after vaccination for Hemophilus influenzae type B and Streptococcus pneumoniae has been reported to be essentially normal (Romagnani, 1985, Bergmann, 1987, Tullgren 1991).

The mechanism behind the immunodeficiency is not entirely understood. Factors, such as an inherent lymphocyte-dysfunction, a reduced IL-2 production (Bergmann, 1987, Mantovani, 1987), an overwhelming stimulation of the immune system (Romagnani, 1985), and trapping of CD4+ T-lymphocytes in tumour-involved organs, have been proposed (Grimfors, 1990). The cause could be inherited or transmittable, since family members, especially twins and first-degree relatives exhibit the same type of immunodeficiency as patients (Cimino 1988, Merk 1990).

## HISTOPATHOLOGY

### The Hodgkin and Reed-Sternberg cells

The origin of the HRS is not known and most cells in the hematopoietic and lymphoid systems have been proposed as the normal counterpart (Knowles,1992, Wolf,1994). Most authors now agree on an origin from an early lymphocyte of 0-, B- and/or T-type based on studies of antigen expression and studies of rearrangement of the T-cell receptor and immunoglobulin gene (Greisser 1988, Poppema,1992). A special case is the nodular paragranuloma form of LP believed to be of B-cell origin (Pinkus 1985, Hansmann, 1989, Jaffe,1994). The true malignant properties of HD have been debated, due to the histological appearance and the clinical presentation which, in some respects, mimic an infectious disease (fever, chills and nightly sweating) (MacMahon 1966, Wolf 1994). Recently, the monoclonal origin of the HRS has been demonstrated by the detection of monoclonal EBV and immunoglobulin gene rearrangements in HRS cells (Brinker 1987, Weiss 1987, Anagnostopoulos,1989). The HRS often exhibits karyotype abnormalities but no specific chromosome abberation has been found, although a frequent involvement of chromosome 14 has been noted (Cabanillas, 1988, Poppema, 1993).

The establishment of cell-lines from HD-derived tissue has been extremely difficult and only a few cell-lines have been described to date. Most of the cell-lines are derived from patients with advanced disease and with unusual manifestations, such as pleural effusions or leukemic HRS (Drexler, 1987, Tesch, 1992). The representativity of these cell-lines for all cases of HD is, thus, doubtful but, nevertheless, much knowledge about the properties of the HRS has been derived from them.

### The surrounding cells

The biological background to the cellular admixture surrounding the HRS is thought to be the result of a complex interplay of cytokines produced by the HRS and the bystander cells

(Poppema, 1992). However, also an antigen-triggering response underlying the gathering of lymphocytes in the lymph nodes has also been proposed (Wolf, 1994). The HRS expresses a number of antigens, eg. B-and T-cell related, the Ki1-antigen (CD 30) originally, thought to be HD specific, the LeuM1 (CD 15) a monocyte/macrophage related antigen and HLA-DR (Knowles, 1992). The HRS also express cytokine-receptors eg. the interleukin-2 receptor (CD 25), the IL-6R and cell-adhesion molecules eg ICAM-1 (CD 54), LFA-3 (CD 58) and the transferrin receptor (CD 71). The HRS produces a number of cytokines, eg. IL-1, IL-5, IL-6, IL-9, GM-CSF and TGFß (Tesch, 1992, Poppema, 1992, Paietta, 1992.). Most interesting is the production of both IL-6 and the IL-6 receptor, which might represent an autocrine loop (Jücker,1991), the production of IL-2 by the surrounding T-lymphocytes and the expression of IL-2 receptor by the HRS and the production of IL-9 by the HRS (Gruss, 1992). IL-9 is a cytokine detected only in HD and the closely related anaplastic large cell lymphoma (ALC) and its role in vivo is presently unknown. Mice injected with autonomously growing T-cells that secrete IL-9 have developed malignant lymphomas with features resembling those of HD and ALC (Tesch, 1992).

The majority of the infiltrating lymphocytes are CD4+ T-cells (helper cells) (Fomi, 1985) and an accumulation of these cells in HD-involved tissue has been associated with the relative depletion from peripheral blood (Grimfors, 1990). A possible explanation can be an over-expression of the intercellular adhesion molecule-1 (ICAM-1) in HD and an accumulation of CD4+ T-cells in the HD-involved tissue, since CD4+ T-cells express the ligand to ICAM-1, the leucocyte function related antigen (LFA-1) (Pizzolo, 1993).

The number of eosinophils infiltrating HD-involved lymph nodes varies. Eosinophils are normally stimulated by IL-5 and HRS have been shown to produce IL-5 in cases of tissue eosinophilia (Samoszouk,1990). A correlation between eosinophil infiltration and histopathological subgroup has been noted by some authors, with more eosinophils in NS cases (Fuggle,1984). Eosinophils have been shown to be the major source of TGFß which can stimulate fibroblasts and could be responsible for the sclerosis in NS (Kadin, 1992).

**Histopathological classification**

The diagnosis of HD is based on the identification of the HRS in the appropriate cellular background. HD is divided into four main groups according to the Rye classification (Lukes,1966). The distinction between the subgroups is entirely morphological and is based on the type of neoplastic cell and the composition of the cellular background. **Lymphocytic predominance (LP)** is subdivided into nodular (LPn) and diffuse form (LPd). LPn is characterized by a nodular pattern of small lymphocytes with scattered HRS of a special type, the L&H cells. LPd shows a similar but diffuse background of small lymphocytes with infrequent HRS. **Nodular sclerosis (NS)** has three main features; nodularity, banded sclerosis and HRS of the lacunar type. The HRS are present in the nodules and the microscopic appearance of the nodules can mimic the LP, MC or LD patterns. NS has been subdivided into NS I and NS II on the basis of the microscopic features within the nodules (Bennet, 1983). For practical purposes, the main difference between NS I and NS II is that the number of HRS is greater in the latter group. **Mixed cellularity (MC)** is characterized by a background of lymphocytes, eosinophils, histiocytes, fibroblasts and plasma cells. There is a moderate number of classical HRS scattered throughout the lymph node. **Lymphocytic depletion (LD)** is characterized by a depletion of lymphocytes, which are replaced either by numerous HRS or diffuse fibrosis.

The prognostic importance of the Rye classification was originally considerable but its value has diminished with the arrival of modern forms of therapy. MC and LD histology are still associated with a poorer prognosis, but the increased risk is small (Löffler, 1992). The poor prognostic value of the Rye classification led to a division of NS into type 1 and 2 and the authors could show a poorer prognosis for type 2. The sub-classification is, however, debated and both supporting (Wijlhuizen, 1989) and contradicting (Bernhards,1992) studies have been published. In the original description of the Rye classification, there was 17% LP, 40% NS, 26% MC and 18% LD, whereas the frequency of NS is nowadays usually 60-80% in large materials. The change in classification is most likely due to gliding criteria since re-examinations of large materials (Bernhards, 1992, Butler,1992) assign more cases to the NS group, when compared with the original classification. The proportion of LP and LD diminish, partly by being classified as NS and partly as NHL. New entities of NHL have recently been recognized such as anaplastic large cell lymphoma (ALC) and angioimmunoblastic lymphadenopathia (AILD), both of which were formerly often classified as HD. In re-examinations of large materials, the number of erroneous diagnoses is often 10-25%, but even higher figures have been reported (Silverman, 1977, Franssila, 1977, Bernhards, 1992). Since NHL is more frequent in the elderly, the number of erroneous HD diagnoses is largest in that age group. The distinction between HD and NHL can sometimes be difficult and many authors now agree that there is a continuous spectrum of malignant disorders from NHL to HD with a proportion of borderline cases impossible to definitely assign to either group (Bernhards, 1992).

## PATTERNS OF SPREAD, STAGE AND STAGING

Most patients with HD present with an enlarged lymph node often within the cervical region. Two theories for the mode of spread of HD have been proposed: the susceptibility (Smithers,1970) and contiguity (Rosenberg & Kaplan, 1966) theories. The susceptibility theory claims that HD is a disease of the entire lymphatic system, possibly polyclonal, and that the susceptibility of different lymph-nodes to be involved with HD varies. Following involvement of one lymph node, the probability of involvement of the remaining ones is equal. The contiguity theory claims that HD spreads from one lymph node to the adjacent via lymphatic channels. There is now overwhelming evidence in favour of the contiguity theory by extensive studie of patterns of invovlement of HD (Diehl, 1991, Mauch, 1993). The only remnant of the susceptibility theory is the fact that HD involves certain lymph nodes more often than others. For example, the cervical lymph nodes are often involved, whereas involvement of the tonsils is extremely rare. The disease is often confined to the supradiaphragmatic region and exclusive infradiaphragmatic disease is rare (Kaplan,1980). Stage is defined according to the Ann Arbor classification (Carbone, 1971), modified in Cotswolds, (Lister, 1989).

### Staging procedures

After the establishment of the diagnosis, the extent of the disease is carefully assessed. Staging procedures include a detailed medical history with special regard to B-symptoms, routine blood tests and a careful clinical examination in order to assess enlarged peripheral lymph nodes. The extent of the disease in the chest and abdomen is usually investigated by a chest x-ray, but computed tomography of the chest and abdomen is also recommended in the Cotswold classification. Alternatively, ultrasonic investigation or lymphangiography can be performed. To further delineate the disease in the abdomen and especially the spleen, the staging laparotomy was

developed (Glatstein, 1970). It should include splenectomy, liver biopsy and biopsies from the paraaortic, iliac, hilar, portal and mesenterial lymph nodes should be performed. The issue of whether a staging laparotomy should be performed in patients with HD has been debated. The risk of abdominal involvement that cannot be detected by non-invasive methods in CS I+II has been reported to be around 30% (Kaplan, 1980, Mauch, 1992) with a higher risk in patients with B-symptoms and two or more involved sites. Staging laparotomy is recommended, if the result of it can alter the planned therapy.

**Table 1.** The Ann Arbor Staging Classification of Hodgkin's disease (modified in Cotswold).

| | |
|---|---|
| **Stage I:** | Involvement of a single lymph-node region or lymphoid structure |
| **Stage II:** | Involvement of two or more lymph-node regions on the same side of the diaphragm. The mediastinum is considered as a single site, whereas hilar lymph nodes are considered bilateral. The number of anatomical sites should be indicated by a subscript (e.g. $II_4$). |
| **Stage III:** | Involvement of lymph-node regions or structures on both side of the diaphragm |
| **$III_1$** | With or without involvement of splenic, hilar, celiac or portal nodes |
| **$III_2$** | With involvement of the paraaortic, iliac, and mesenteric nodes. |
| **Stage IV:** | Involvement of one or more extranodal sites in addition to a site for which the designation "E" has been used (see below) |
| **Designations applicable to any disease stage** | |
| **A** | No symptoms |
| **B** | If any of the following symptoms are present:<br>1.Unexplained weight loss of >10% of the body weight in the preceding 6 months.<br>2. Unexplained fever, with temperature above 38 C.<br>3. Night sweats. |
| **X** | Bulky disease -a peripheral lymph node or region greater than 10 cm or a mediastinal mass larger than 1/3 of the thorax at the level of carina on a chest x-ray. |
| **CS** | Clinical stage |
| **PS** | Pathological stage (as determined by staging laparotomy) |

## TREATMENT AND TREATMENT RESULTS

### Primary therapy

HD is sensitive to both radiotherapy and chemotherapy and the choice of treatment depends largely on the stage of the disease. Therapy traditions vary throughout the world but, generally speaking patients in low stages (I+II) are treated with radiotherapy and patients with advanced stages (III+IV) are treated with chemotherapy. The modern principles of radiotherapy were delineated by Peters (Peters, 1958) and by Kaplan (Kaplan, 1966). In patients with stages I or II supradiaphragmatic disease, all lymph node regions above the diaphragm are irradiated with the "mantle" technique. Most centres have recommended that a staging laparotomy is performed if the patients is to be treated with radiotherapy, alone. The delivered dose is usually 40 Gy in 4 weeks. Patients with bulky disease and patients with stage IIB have a poorer prognosis with radiotherapy alone and it is generally agreed that they should be treated with combinations of radiotherapy and chemotherapy (deVita,1993, Urba,1992). In patients with infradiaphragmatic disease, CS+PS IA and PS IIA, the lymph node regions are irradiated with the "inverted Y" technique. The results of radiotherapy are excellent, with most studies presenting survival rates of 90% (Urba, 1992).

The first chemotherapy regimen, MOPP, was introduced in 1964 (DeVita, 1967). It consists of four drugs, each with proven activity as single drugs (Table 2). Of the first 198 treated patients, of which the majority had stage IV disease, 58% were alive with no evidence of disease after 10 years (De Vita, 1980). Later, it was noted that doxorubicin and bleomycin were effective in the treatment of HD and a "second line" regimen ABVD (Table x) was introduced. ABVD could induce durable responses in patients who had failed on MOPP and was considered "non-cross resistant". MOPP and ABVD were compared in a randomized way and found equally effective (Bonadonna,1975). Due to the equal efficacy of both regimens, the concept that patients who failed one were sometimes cured by the other and the possibility of reducing toxicity produced by either regimen, alternating MOPP/ABVD was introduced. Numerous attempts to design new regimens to further improve treatment results have been made, but so far no regimen has proved to be better than MOPP/ABVD. Recently, a development of MOPP/ABVD has been described, the MOPP/ABV hybrid (Table 2). The rationale for the regimen was to introduce seven effective drugs during the first month and by excluding dacarbazine (probably of limited value) to increase the doxorubicin dose. There is a great controversy in literature as to whether seven- or eight-drug regimens are superior to four-drug regimens. In a recently reported trial, patients with advanced disease were randomized to MOPP, ABVD or MOPP/ABVD. The CR rates were 62%, 82% and 83%, respectively and the relapse-free survival was 48%, 64% and 64%, respectively. This study claims that MOPP/ABVD and ABVD are equally effective and superior to MOPP (Canellos, 1992). Serious criticism has, however, been levelled against the study, since the dose-intensity in the MOPP arm was comparatively low (DeVita, 1993). Another criticism was that, in the initial studies, MOPP and ABVD proved to be equally effective.

**Table 2.** MOPP, ABVD, MOPP/ABVD and MOPP/ABV hybrid regimens

| Regimen | Recommended dose $mg/m^2$ | Route | Cycle days cycle length 28 days |
|---|---|---|---|
| MOPP | | | |
| Mechlorethamine | 6 | intravenous (i.v.) | 1 and 8 |
| Vincristine | 1.4 | i.v. | 1 and 8 |
| Procarbazine | 100 | oral | 1-14 |
| Prednisone | 40 | oral | 1-14 |
| ABVD | | | |
| Doxorubicin | 25 | i.v. | 1 and 15 |
| Bleomycin | 10 | i.v. | 1 and 15 |
| Vinblastine | 6 | i.v. | 1 and 15 |
| Dacarbazine | 375 | i.v. | 1 and 15 |
| MOPP/ABVD | Alternating monthly cycles of MOPP and ABVD | | |
| MOPP/ABV hybrid | | | |
| Mechlorethamine | 6 | i.v. | 1 |
| Vincristine | 1.4 (max 2.0) | i.v. | 1 |
| Procarbazine | 100 | oral | 1-7 |
| Prednisone | 40 | oral | 1-14 |
| Doxorubicin | 25 | i.v. | 8 |
| Bleomycin | 10 | i.v. | 8 |
| Vinblastine | 6 | i.v. | 8 |

There is only one study that compares MOPP/ABVD with the MOPP/ABV hybrid, and it has demonstrated equal results (Connors, 1992). In conclusion there is no evidence in literature of the superiority as regards overall survival of seven or eight drug regimens over MOPP *per se* provided that MOPP is delivered without dose reductions. However, in clinical practice, this seems to be the major problem. Yet, several authors prefer to give seven- or eight-drug regimens in order to spread out the toxicity associated with each regimen and to keep the dose intensity "high" ( Urba, 1992, DeVita, 1993).

### Treatment of recurrent HD

Patients who relapse after initial radiotherapy have complete remission rates and a long-term survival of 50-80%, i.e. equal to those of patients in the same stages primarily treated with chemotherapy (Urba, 1992). Patients, who relapse after chemotherapy more than 12 months after achieving a complete remission, have a complete remission rate of 95%, if the same combination of drugs is used but only 25% become long-time survivors (Urba,1992 DeVita,1993). In contrast, relapses after shorter complete remission durations and in patients who fail to reach a complete remission on standard chemotherapy, the prognosis is poor and several salvage regimens have been developed, e.g. CEP (CCNU, etoposide, prednimustine) (Santoro,1986) and MIME (metyl-gag, ifosfamide, methotrexate, etoposide) (Hagemeister,1987). High-dose chemotherapy with autologous bone-marrow or stem-cell transplantation is currently recommended for these patients. The results of ABMT differ due to selection criteria and many centres require chemotherapy-responsive patients in order to perform ABMT, thereby selecting the group of failing patients with the best prognosis. There is no published randomized study which compares ABMT with ordinary salvage treatment. Usually, the 5-year survival rates range from 20 to 40% (Urba,1992) but no plateau in the survival curves has been reached, indicating that late relapses and subsequent deaths occur. The results are better in patients with chemo-responsive disease and a small tumour burden at the time of transplantation (Anderson, 1993 Rapoport, 1993).

## PROGNOSTIC FACTORS

The rationale for studying prognostic factors in a malignant disease such as HD is, to identify patients who run an increased risk of relapse and subsequent death from the disease and could benefit from more intensive treatment. Another, also important, motive is to identify patients with little risk of treatment failure in order to minimize acute and late toxicity (see below). A third reason is to gain further insight into the biological properties of the disease. There are, however, several problems connected with interpreting the extensive literature available due to many reasons. Most studies are hospital-based and highly selected, including only a minority of patients with HD in the studied population. Due to the composition of the patient materials, the relative importance of factors can vary. Age, for example, is an important prognostic factor in many studies but, if the majority of included patients is young, the relative importance tends to diminish. The value of a prognostic factor can decresae as soon as it is identified, eg. bulky disease. In a recent study by the Swedish National Health Care Program for HD (see below), patients with bulky disease did not have a poorer outcome (Glimelius, 1994), probably because the increased risk associated with bulky disease had been identified and the patients were treated accordingly, with a combined modality treatment. The choice of treatment and the staging

procedures applied could, thus, obviously influence the prognostic factors identified in each study. Many prognostic factors are also related to each other, so that multi-variate analyses must be performed to identify the relative importance of each factor. With this reservations in mind, the prognostic factors in HD could be divided into the following somewhat artificial groups:

**Host-related prognostic factors**

**Age** has been the most important factor in many studies. The decline in treatment results starts in patients above 40 years of age. Age as a prognostic factor and HD in elderly patients are both discussed in detail below.

**Sex**. In many studies, male patients have a poorer prognosis and a higher risk of abdominal involvement at staging laparotomy (Kaplan, 1980). The reason for this difference is not known.

**Immunodeficiency.** Impaired cell-mediated immunity has been discussed in detail above. In summary, patients with immune- deficiency have a poorer prognosis but they also have a more advanced disease and a higher age. In multi-variate analyses immune-deficiency seems to be an independent, prognostic factor (Tullgren, 1991).

A poorer prognosis related to different HLA-phenotypes has also been described (Kaplan, 1980).

**Prognostic factors related to the extent of the disease**

The extent of disease, as defined by the Ann Arbor classification, is the most important prognostic variable identified. Although treatment is governed by stage and more advanced stages are more aggressively treated, stage remains a prognostic factor in most materials. Closely related to the extent of the disease are the B-symptoms. In each given stage, patients with B-symptoms have a poorer prognosis (Löffler, 1992). The cause of the B-symptoms is not known but they are associated with increased levels of most serum factors studied (see below). B-symptoms are not entirely related to the total tumour volume, since patients with large mediastinal bulks are often asymptomatic. If the B-symptoms are related to properties in the tumourcells themselves, i.e. an increased cytokine production, or cellular proliferation or to host-related features, is not known.

Several attempts have been made to increase the prognostic accuracy of stage. Stage represents first and foremost the spread of the disease and, to a lesser extent, the total tumour volume. There are many examples of patients with higher tumour volumes having a poorer prognosis, in each stage. Patients with bulky disease have already been mentioned. Patients in stage II with more than two involved sites have a poorer prognosis (Tubiana, 1985), as do patients with more than four noduli in the spleen (Hoppe, 1980). Specht et al have carefully delineated the importance of total tumour burden in stages I, II and III and found it to be the most important prognostic factor (Specht,1988,1990).

**Prognostic factors related to clinical management**

Careful staging procedures are essential since patients who are understaged receive inefficient treatment with a high risk of relapsing. Subsequent salvage therapy is also more likely to be hampered by bone-marrow toxicity. Dose intensity is another important subject, which is not always accounted for. Animal experimental studies of curable malignancies show that the remission rates are not always reduced by reduced dose intensity, but relapses are more frequent and fewer individuals are actually cured. In HD there are data to support this hypothesis (DeVita, 1993). Dose intensity can be estimated by a method described by Hryniuk, in which the amount of administred therapy is expressed in mg/m$^2$/week (Hryniuk, 1987).

### Prognostic factors related to properties inherent to the HRS

The histopathological subgroup and the attempts to improve the prognostic value by dividing NS into type 1 and 2 have already been described (Bennet, 1983). Based on the concept of tumour burden, Specht et al have estimated the tumour cell concentration by counting the HRS and found a poorer prognosis in patients with high tumour cell concentration (Specht, 1990). High numbers of eosinophils and plasma cells in tumour tissue have been correlated to a poor prognosis but the method by which the numbers have been estimated was not stated (Tóth, 1977).

### Blood and serum markers

Many routine laboratory tests, as well as more tumour-specific tests, are of prognostic importance in HD. In a large study, Vaughan Hudson et al found alterations in ESR, Hb, lymphocyte count or S-albumin in 88% of the patients, indicating that the majority of HD patients have "systemic disturbances", and that the systemic disturbances increased with increasing tumour malignancy as defined by histopathology and stage. The cause of these alterations is not known but is thought to be related to interactions between the host and the tumour. The expressions of "systemic disturbance" were also closely related to each other and to prognosis and it was concluded that B-symptoms and ESR were the most valuable parameters (Vaughan Hudson, 1987). Many other authors have also identified the prognostic value of routine tests. ESR, in particular is closely associated with prognosis (Henry-Amar, 1991). In addition, the serum levels of lactic dehydrogenase (Schilling, 1982), alkaline phosphatase (Thyss, 1985), ferritin (Bezwoda, 1985), lymphocyte count (Tullgren, 1991), and B-Hb are of prognostic value. The B-Hb and lymphocyte count are included in a prognostic index developed by Proctor et al currently in use for diversifying therapy (Proctor, 1992). New factors should therefore be examined in relation to routine tests.

Hypothetically, the most valuable factors would be those related to the tumour cells themselves, either by relation to the total tumour burden, the proliferation rate or representing the malignant potential of the HRS. The serum levels of soluble interleukin-2 receptor (S-sIL-2R) have recently been reported to be associated with stage and prognosis in HD (Pizzolo, 1987, Gause, 1991, 1992, Ambrosetti, 1993, Pui, 1993). The theoretical background behind the measuring of S-sIL-2R is that IL-2R is expressed on the HRS and released from the cell-lines derived from HRS, when cultured in vitro. Soluble IL-2R has retained its ability to bind circulating IL-2, which is a potent activator of T-cells in vivo. Since HD patients have an impaired cell-mediated immunity, the serum levels of sIL-2R could be of particular interest in HD and reflect both the total tumour burden and the ability to bind circulating IL-2, thereby further enhancing the immunodeificiency.

Serum levels of soluble CD 30 have also been reported to be related to stage and prognosis in a few studies (Pizzolo, 1990, Gause, 1992). S-sCD 30 would be of particular interest since it is closely related to the HRS and the HRS is most likely the major source of sCD 30 in vivo. Soluble CD 30 could therefore be an indirect measurement of the total tumour burden in HD patients and also useful in monitoring therapy effects.

## HD IN ELDERLY PATIENTS

The proportion of elderly patients varies substantially from study to study with a lesser

proportion in hospital-based materials mainly due to the referral of the youngest and fittest or to age limits in treatment protocols (Austin-Seymour, 1984, Peterson, 1982). In register studies, the proportion of elderly patients is larger but a number of cases can be erroneously included cases of NHL, misinterpreted as HD if the material is not re-examined by an experienced hematopathologist. In re-examined register-based studies, the proportion of patients 50 years or older is between 35-50%, and 15-25% are over 60 years of age (Nordentoft, 1980, Norberg, 1991).

Most studies of HD have identified age as an important prognostic factor. In many large series, the decline in prognosis starts at about the age of 40 (Nordentoft, 1980) and, with advancing age, survival becomes progressively poorer. There are only a few studies that have specifically dealt with HD in the elderly. In a large study from the British National Lymphoma Investigation (BNLI) of 1,500 HD patients (23% > 50 years), the decline in survival started at the age of 50 and was present in both sexes, all histological subtypes and all stages. Fewer elderly patients achieved a complete remission but, once a remission was obtained, the recurrence-free survival was identical in patients below and above the age of 50 (Vaughan Hudson, 1983). Similar results were obtained in a Dutch study (Erdkamp, 1992). Walker et al used register data to compare patients 15-34 years with patients above 50 years of age (32%).

Elderly patients more often have an advanced stage, B-symptoms, and MC and LD histology at presentation. The poor outcome in elderly HD patients was, however, present also after correction for these factors (Walker, 1990). In a large study by Peterson et al of patients in stages III and IV, elderly patients (19% >60) were less intensively treated, had a lower response rate and a higher risk of recurrence. However, patients receiving $\geq$ 90% of the projected drug doses also had a lower complete remission rate, a shorter median time to recurrence and a poorer survival (Peterson, 1982). Similar results were obtained from a study byAustin-Seymour et al, Stanford who examined 52 patients above 60 years (4% of all patients). Elderly patients in PS IIIB-IV, who were adequately staged and treated, had a 5-year survival of 32% but, in adequately staged and treated patients in PS I-IIIA, the 5-year survival was 86% (Austin-Seymour, 1984). Wedelin et al examined a material from Stockholm and found a five-year survival rate in patients above 50 years (43% of all patients) of 28% compared with 74% in the younger age group. There was no difference in stage distribution but elderly patients more often had MC and LD histology. Age was the most important prognostic factor in a multivariate analysis and prognosis in elderly patients was not related to stage and B-symptoms to the same extent as was the case in younger patients (Wedelin, 1984). In a Danish material, Specht et al examined age as a prognostic factor in 506 patients, of whom 176 (34%) were above 50 years of age. MC histology and abdominal disease were more often present in the elderly patients. Age had no prognostic significance for DFS but for HD specific survival in that study, but patient not achieving CR were not included in the calculations of DFS and patients dead from treatment related complications were omitted from the survival calculations, complictaing comparisons with other materials where those patients were included in the survival estimates (Specht, 1989).

There is considerable controversy in literature as to whether age is an independent prognostic factor or merely associated with other unfavourable factors. Many different explanations for the poor outcome in the elderly have been proposed:

### Another disease

Epidemiological data indicate that HD in the elderly is another disease. This is supported by the finding by certain authors of a higher proportion of EBV in the tumours in the elderly (Jarret, 1991). Elderly patients also have a more advanced stage, more often B-symptoms and MC and LD histology, all of which might contribute to the poor outcome. In the BNLI study mentioned above, the poor prognosis in the elderly was present in all stages, all histologies and irrespective of whether the patient had A-or B-symptoms.

### Inadequate staging or treatment

Elderly patients are often inadequately staged and treated. However, even when patients are staged and treated with the same intensity as the young, they have a poorer outcome in the Stanford study and in the study by Peterson et al.

### Diminished tolerance to therapy

There are many reasons for the dimineshed tolerance to aggressive therapy in elderly patients. Age has been repeoted to be a risk factor as regards anthracycline cardiotoxicity and bleomycin-induced pulmonary fibrosis (Sweetenham, 1987) and elderly cancer patients more often suffer from cumulative hematological toxicity. The cause may be a decreased renal and hepatic clearance of drugs described in elderly patients (Sweetenham, 1987). Elderly HD patients have a much higher proportion of treatment-related deaths and severe and life-threatening toxicity (Peterson, 1982, Norberg, 1991, Austin-Seymour, 1984). Furthermore, they also have a more pronounced immuneodeficiency when compared with younger patients, which might contribute to the treatment-related morbidity and mortality (Björkholm, 1990).

## LATE EFFECTS OF THERAPY

Apart from the acute toxictiy of treatment with radio- and chemotherapy which is mainly attributable to serious infections due to the depressive effects of chemotherapy on the bone-marrow, several serious late effects of therapy have been described. Patients who are splenectomized have an increased risk of pneumococcal septicemia, which is lethal in a substantial number of patients. This can partly be overcome by pneumococcal vaccination (Grimfors, 1990).

Sterility can be induced by chemo- and radiotherapy. Radiotherapy can induce sterility by direct effects on the ovaries and testicles. All chemotherapy regimens are not equal in inducing infertility. Almost all men who have received two cycles or more of MOPP are permanently sterile, but only half of the patients treated with ABVD (Viviani, 1985). In women, the situation is better but the risk of permanent amenorrhoea increases with increasing age at therapy and with increasing amounts of chemotherapy given. After the age of 30, 60% of female patients become amenorrhoic (Viviani, 1985).

Even more serious is the development of secondary malignancies, mainly acute non-lymphocytic leukemia (ANLL), NHL or solid cancers. An incresaed risk of secondary malignancies have beern noted by many authors (Pedersen-Bjergaard, 1987, Tucker, 1988, Lavey, 1990, Kaldor, 1990). The risk of secondary cancers has recently been reviewed in two large studies (Henry-Amar, 1992, Swerdlow, 1993) who summarized findings in 12,411 patients included in the international database on HD (IDHD) and 2,846 patients, included in the BNLI,

respectively. The risk of developing ANLL was great in both studies, with a cumulative incidence of ANLL of 2,2% (Henry-Amar, 1992) at 15 years. A high risk was associated with chemotherapy and this was even higher in patients treated with combined modality treatment. In the BNLI study, the risk of ANLL was higher, the younger the patient was at diagnosis. In contrast, patients with higher age, had a higher risk in the IDHD study. Secondary ANLL usually develops within the first 10 years after the primary treatment and the incidence levelled out after 17 years.

The cumulative incidence of NHL in patients treated for HD was 1.8% at 15 years. The risk varied with the histological subtype of HD with a higher risk in patients with LP histology. There are, however, authors who claim that LPn is gradually transforming into a B-cell NHL (Hansmann, 1989) and part of the increased risk might represent the natural course of the disease.

The cumulative incidence of solid cancers was 7.5% (Henry-Amar, 1992). The risk was reported to be higher the younger the patient was at diagnosis (Swerdlow, 1993) and also higher in older patients (Henry-Amar, 1992) . The risk was associated with extended field radiotherapy, but also with combined modality treatment, and continuously increasing 17 years after therapy.

In conclusion, secondary malignancies are a major contribution to the mortality involved in HD. When analysing the causes of death in the IDHD, 73% of all deaths were related to HD or to treatment, 14% to intercurrent deaths and10% to secondary cancers.

## PATIENTS

All patients with HD diagnosed between 1979 and 1988 in three Swedish counties were identified from the Swedish Cancer Registry, the National Health Care Program for HD (1984) (see below) and from the files of the local hospitals. Additionally, all patients with HD diagnosed between 1985 and 1988 in the Swedish Health Care region of Uppsala/Örebro were identified from the National Health Care Program and from the Regional Cancer Registry. In papers IV and V all patients with HD diagnosed between 1979 and 1991 who had frozen serum samples available were included.

Patients older then 60 were defined as elderly. The reason for this age limit is the shape of the age distribution curve (Figure 1) and our preliminary finding that the decline in prognosis started at 60 years in the National Health Care Program (unpublished data). The staging and treatment recommendations of the National Health Care Program were generally followed in all papers, with the exception that, in some cases, no chemotherapy was given prior to radiotherapy in bulky disease and that before 1982 only MOPP was given.

In Sweden a **National Health Care Program for HD** in patients above the age of 16 years has been in use in five of six health care regions since 1985. The completeness of the registration is continously cross-checked with the Regional Cancer Registries, resulting in virtually all patients with HD being registred. The program provides tailored principles for the staging, treatment and follow-up of all patients.

## STAGING

Briefly, staging involves a clinical history, a physical examination, an ENT-examination with biopsies in cases of suspicious findings, blood laboratory investigations and a bone-marrow

biopsy. A chest x-ray is performed in every patient and a computed tomography (CT) of the thorax in cases of known mediastinal involvement, infradiaphragmatic disease only or NS histology. Radiological investigation of the abdomen includes ultrasonography examination, CT and/or bipedal lymphogram. Staging laparotomy with splenectomy is recommended below the age of 60 with supra-diaphragmatic stages IB, IIA and "limited" stage IIB but not for stage IA, provided at least two x-ray examinations of the abdomen show no abnormality. Stage at diagnosis was defined according to the Ann Arbour classification (Carbone, 1971). Bulky disease was defined as a peripheral lymph node or region larger than 10 cm or a mediastinal mass larger than 1/3 of the thorax at the level of the carina on an AP chest x-ray.

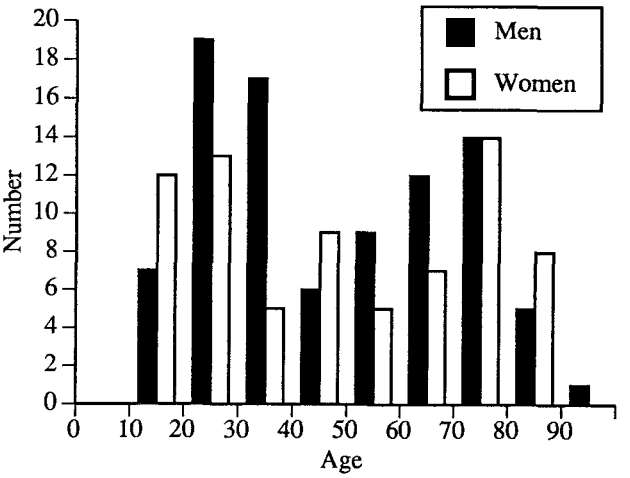


Figure 1. Distribution of patients according to age and sex at diagnosis

## TREATMENT

The treatment recommendations are: Patients in CS and PS IA and PS IIA are treated with radiotherapy alone (mantle field or inverted Y-field, 40Gy). Patients with CS IIA and more advanced stages are treated with 6-8 courses of chemotherapy, mainly MOPP/ABVD. Patients with bulky disease in stages CS and PS IA and PS IIA and patients with PS IIB receive 2-4 cycles of chemotherapy before radiotherapy (mantle field or inverted Y-field, 40Gy) and patients with advanced stages with bulky disease receive additional involved-field radiotherapy. In patients older than 60, the treatment recommendations are the same as those for the younger adults. Patients younger than16 years are not included in the program and are mainly treated with chemotherapy, regardless of stage.

## EVALUATION OF STAGING AND TREATMENT INTENSITY

Staging was classified as adequate, acceptable or inadequate by comparing the staging procedures of each patient with those recommended by the National Health Care Program for HD. **Adequate** was defined as all recommended investigations performed and **acceptable** as all investigations except ENT-status or no CT of the thorax in the cases specified above. Otherwise, the staging procedure was considered **inadequate.** The patients were divided into three groups

according to the amount of treatment given and to the treatment intensity. Due to the retrospective nature of the study, it was not possible to obtain exact data of all the patients as regards the dates and doses of drugs. It was therefore not possible to, for example, use the dose-intensity model of Hryniuk (Hryniuk, 1987). Instead, a simplified grouping was performed. **Group A** had received 80% or more of the total intended dose of chemotherapy with, at the most, little delay (at most 20% prolongation of the total treatment time) or full irradiation volume, **group B** had received 40-80% of a planned total dose of chemotherapy, more than 20% prolongation or radiotherapy to reduced volumes (involved field and adjacent lymph nodes) and **group C** had recieved less than 40% of cytostatics or radiotherapy to involved field only.

## MORPHOLOGICAL EXAMINATION AND IMMUNOHISTO-CHEMICAL TECHNIQUE

All diagnostic specimens pertinent to patients included were re-examined by an experienced hematopathologist using the Rye-classification with a subdivision of NS into type 1 and 2 (Bennet, 1983). After revision, the HD diagnosis was confirmed in 78% of the patientsdiagnosed between 1979-1988 and in 87% of the patients diagnosed between 1985-1988.

The following monoclonal antibodies were used: LN1, MB2 and L26 (CD 20) UCHL1 (CD 45 RO), Ber H2 (CD 30), Leu M1 (CD 15) and the polyclonal CD 3 antibody. LN1, MB2 and L26 react primarily with B-cells and UCHL1 and CD3 with T-cells. LeuM1 stains primarily monocytes and a majority of the HRS. Ber H2 identifies the Ki1 antigen, which was originally thought to be HD- specific but is expressed on a minority of activated lymphocytes and some unusual forms of NHL. The tissue specimens were routinely fixed in neutral-buffered formalin, paraffin-embedded and sectioned in 3 μm thick sections and deparaffinized. When staining with LN1, UCHL1 Ber H2, CD 3 MB2 and L26, the APAAP (Cordell, 1984) technique was used and, in the case of LeuM1, the PAP (Sternberger, 1970) technique.

## SERUM MARKERS

All routine blood and serum tests were obtained from the patients records. S-sIL-2R and S-sCD 30 were measured with commercially available sandwich enzyme immunoassay kits. Briefly, the methods utilize microwells precoated with a monoclonal antibody reactive with the examined substance. The serum samples are added together with an enzyme-conjugated monoclonal antibody also reactive with the examined substance. The substance binds to the coated antibody and the enzyme-linked antibody binds to a second, distinct epitope of the substance, completing the sandwich. Unreactived components are removed by washing. A chromogen solution is added to the wells forming a colored end product that is proportional to the amount of substance present in the sample. Absorbance is measured and a standard curve constructed. Values of the serum samples are determined from the standard curve.

## COUNTING OF CELLS

Eosinophils and HRS were counted. The cells were counted in hematoxylin-eosin stained sections (thickness 3 μm) from each biopsy specimen without knowledge of stage, clinical course and treatment. Ten randomly selected high power (x500) vision fields (VF) were examined. To facilitate counting, an eye-piece equipped with a lattice square net was used and only cells falling within the lattice framework were counted (area /VF was 0.063 $mm^2$). In

nodular sclerosis cases, cellular regions were counted. The absolute number of eosinophils was counted in each VF and the numbers in 10 VF were added. Since the eosinophilic infiltration was in most cases patchy, the absolute number of eosinophils counted was expected to vary between examinations, fro this reason the cases were divided into three groups. **Group 0**: 0-10 , **group 1**: 11-200 and **group 2**: >200 eosinophils/10 VF. These limits were chosen to describe the three patterns observed with almost no eosinophils in one group, heavy infiltration in one group and an intermediate group. HRS were counted in a similar manner. To compare the results with those prevoisly presented by Specht et al, the ratio between the area of a high-power vision-field x 400 (the magnification used by them) and the area of the lattice square net (x500) was determined. The ratio was 3.1 and the group limits used by Specht et al were divided by 3.1 to give the following group limits: **group 1**: 0-2 tumour cells/ vision field, **group 2**: 2-8 tumour cells/ vision field and **group 3**: > 8 tumour cells/ vision field, in order to create corresponding groups.

## TUMOUR BURDEN

The total tumour volume in patients in stages I and II was also estimated by methods described by Specht et al (Specht, 1990). Briefly, the number of sites and the sizes of all tumours are scored and summed. The patients are divided into four groups, with increasing scores. A combination of tumour volume and HRS-concentration was also used, designated combined tumour burden. The tumour burden was not estimated in patients with stages II, infradiaphragmatic disease and stages III-IV. In stages II and III, this was due to the fact that most abdominal investigations were perfored with an ultrasonic technique and exact measurements of the size and number of involved lymph nodes were not given. In addition, in stage IV, the tumour volume of for example, diffuse bone-marrow or liver involvement, was considered too uncertain to provide accurate information.

## STATISTICAL METHODS

Chi-square analyses were performed to compare differences in proportions between groups. The Mann-Whitney U-test was used to calculate differences in distributions of serum factors between groups (Stat View 4.0). Correlations between serum factors were calculated using Spearman Rank Correlation (Stat View 4.0). Linear regression and paired t-test were used to examine differences between observers when counting eosinophils. Life-table survival analysis with the Log rank significance test was performed according to Peto (Peto, 1975). The cause-specific survival and the disease-free survival (DFS) were analyzed. In the cause-specific survival calculations, patients dying of other reasons than HD were excluded from the population at risk after their death, whereas patients with known HD were considered as dying of HD irrespective of the actual cause of death. DFS was defined as no evidence of disease at the time of follow-up and no known recurrences. Patients who never reached a CR thus had a DFS of zero months. To further exclude any bias from excess mortality in the older age group, the relative survival (RS) was analyzed. RS was calculated as the ratio between the observed survival (OS) from all causes of death and the expected survival rate (ES). The ES was the survival of a group of the general population similar to the study group with respect to age, sex and calendar time. A computer program package was used for these calculations (Hakulinen, 1985). Uni- and multivariate analyses with the Cox proportional hazard model were performed with Statistica 3.0b software.

## RESULTS AND DISCUSSION

### STAGING TREATMENT AND TREATMENT INTENSITY

Staging, treatment and treatment intensity were evaluated in a population-based material where all patients with HD diagnosed between 1979 and 1988 in three Swedish counties had been identified. Sixty-one (37%) patients were above the age of 60. This is the highest figure reported in literature and reflects the low degree of selection and also the long life-expectancy in the Swedish population. The age-standardized incidence was 2.3/100,000 for men and 2.0/100,000 for women; comparable with the incidence reported in literature (Kaplan, 1980). The poor prognosis in the elderly was confirmed and the decline in survival appeared to start at 60 years of age (figure 2).

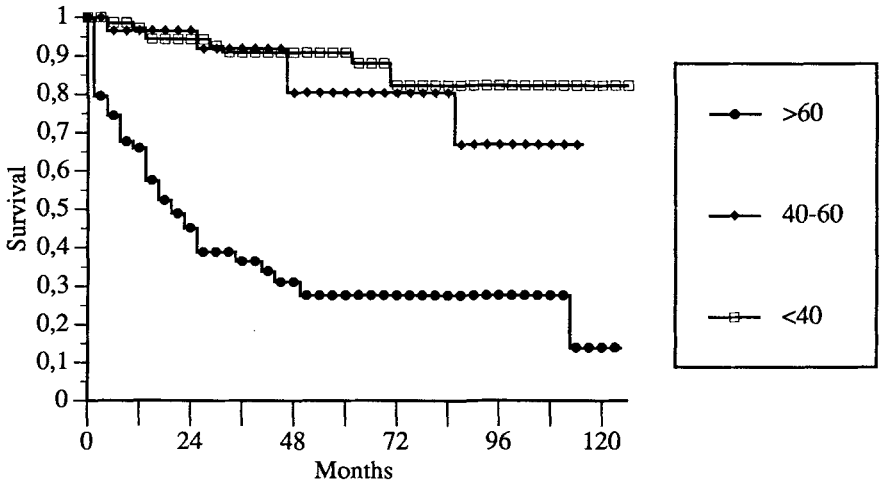


Figure 2. Cause-specific survival of all patients with HD below 40 years of age, 40-60 years and above 60 years.

The similarity in the HD-specific survival curves and the relative survival curves (figure 3) indicates that the poor survival is not attributable to an excess mortality due to high age per se.The elderly patients had MC and LD histology more often than the younger ($p<0.001$). The elderly patients were also less intensively staged, only 38% vs 69%, were considered adequately staged ($p<0.001$). The intensity of staging did not influence survival. In spite of the less intensive staging, the patients above the age of 60 were more often in stages IIB-IV, 66%, compared with 48% for the patients below that age ($p<0.02$). The survival of elderly patients in stages I-IIA was poorer than that of younger patients in stages IIB-IV ($p<0.02$).

Twelve patients above 60 years of age who were treated with radiotherapy with curative intent had the same estimated 5-year relative survival as the 41 young radiotherapy treated patients (84% vs 85%). These figures are in accordance with the results from Stanford (Austin-Seymour, 1984), where elderly patients in PS I-IIIA, adequately treated with radiotherapy, had an estimated 5-year survival of 86%.

In contrast, 37 patients above 60 treated with chemotherapy with curative intent had a 5-year relative survival of 33%, compared with 86% for the younger age group. The majority of the

elderly patients (54%) received less than 40% of the projected dose-intensity, compared with 5% in the young. The main reason for this were the adverse effects of therapy leading to dose reductions or premature termination of treatment in 22/37 (59%) of the patients (table 3) and also to eight treatment-related deaths. The elderly patients received less drugs and experienced considerably more toxicity; thus, the poor outcome in the elderly is not caused by the palliative treatment of this patients. It is instead, to a large extent, attributable to a very poor tolerability to treatment regimens generally used for the treatment of younger HD patients.

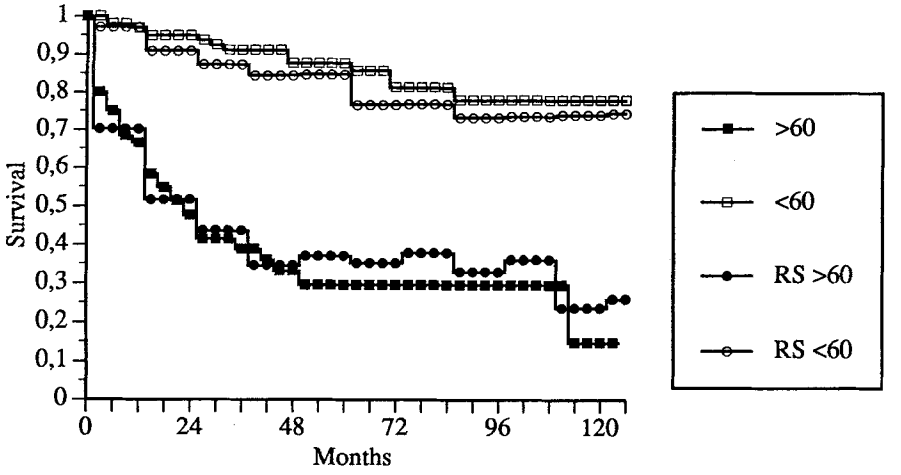


**Figure 3. Cause-specific and relative survival (RS) of all patients with HD above and below the age of 60.**

Table 3. Treatment and treatment results in relation to treatment dose and intensity for patients below and above the age of 60.

| | | <60 years | | | ≥60 years | | |
|---|---|---|---|---|---|---|---|
| | | No. | CR | CCR | No. | CR | CCR |
| Radiotherapy | A[1] | 41 | 41 | 27 | 1 | 1 | 1 |
| | B[1] | | | | 8 | 7 | 2 |
| | C[1] | | | | 3 | 1 | 0 |
| Chemotherapy | A[1] | 44 | 42 | 34 | 7 | 7 | 5 |
| | B[1] | 14[2] | 13 | 7 | 10 | 5 | 4 |
| | C[1] | 3[3] | 0 | | 20 | 7 | 2 |
| Palliative | | 0 | | | 3 | 1 | 0 |
| No treatment | | 0 | | | 6 | 0 | |
| Data missing | | 0 | | | 3 | | |
| 102 | | 61 | | | | | |

1) For definitions, see text. 2) Reasons for reduction: 13 patients bone marrow toxicity, 1 patient neuropathia. 3) Reasons for reduction: 2 patients progressive disease, 1 patient refusal.

A possible approach would be to start treatment at a lower dose intensity in elderly patients in order to be able to give a greater total amount of drug. This model has been tried by an Italian group (Botto, 1990) who reported treatment with ChlVP/CEB (table 4) in 19 patients above 65 years. In spite of the lower doses in the regimen, the relative dose intensity at 3 and 6 courses was significantly higher as that in a historical control. Eighty percent of the patients achieved a CR and there were 5% toxic deaths compared with 17% in the historical control material.

Table 4. The ChlVP/CEB and LVPP/OEPA regimens

| Regimen | Recommended dose mg/m$^2$ | Route | Cycle days |
|---|---|---|---|
| LVPP/OEPA (Cycle-length 8 weeks) | | | |
| Chlorambucil | 4-6 | oral | 1-14 |
| Vinblastine | 4-6 | intravenous (i.v.) | 1 |
| Procabazine | 50-75 | oral | 1-14 |
| Prednisone | 15-25 | oral | 1-14 |
| Vincristine | 1.0-1.4 | i.v. | 29 |
| Etoposide | 50-100 | i.v. | 29 |
| Etoposide | 100-200 | oral | 30 and 31 |
| Prednisone | 15-25 | oral | 29-43 |
| Doxorubicin | 15-25 | i.v. | 29 and 43 |
| ChlVP/CEB (Cycle-length 4 weeks) | | | |
| Chlorambucil | 6 | oral | 1-7 |
| Vinblastine | 6 | i.v. | 1 |
| Procarbazine | 100 | oral | 1-7 |
| Prednisone | 30 | oral | 1-7 |
| Cyclophosphamide | 500 | i.v. | 15 |
| Etoposide | 70 | i.v. | 15 |
| Bleomycin | 10 | i.v. | 15 |

A similar approach has been used within the National Health Care Program for HD since 1989. Briefly, the new staging and treatment recommendations for patients above 60 includes: Staging as intense as that used in yonger patients, except for staging laparotomy. Radiotherapy in CS IA, radiotherapy preceded by 2 courses of alternating chemotherapy in CS IB and IIA and 6-8 courses of alternating chemotherapy in stages IIB-IV. Since radiotherapy seems to be better tolerated, additional radiotherapy to bulky disease (defined as > 5cm) is recommended or if the patient responds poorly to chemotherapy or if chemotherapy cannot be given with reasonable intensity. A new, less intensive chemotherapy regimen, alternating LVPP/OEPA (table 4) has been developed. Preliminary results from 1989-1992 (unpublished data) show a higher complete remission rate, 82% as compared with 63% in patients treated 1985-1988, in patients treated with curative intent. A careful analysis of dose intensity and a longer follow-up is needed, since a lower dose intensity could increase the risk of relapses. A possible way of increasing dose intensity would be the routine use of hematopoethic growth factors in elderly HD patients.

## IMMUNOHISTOCHEMICAL CARACTERISTICS OF THE HRS

The immunohistochemical characteristics of the HRS in 154 HD patients diagnosed in the Swedish Health Care Region of Uppsala/Örebro 1985-1988 were analyzed. The results of the staining are shown in table x. In total 105, cases (68%) were positive with either of the B-cell related antibodies and 57 (37%) with either of the T-cell related antibodies. Tumour specimens from patients above and below the age of 60 did not differ significantly in their antigen expression. There was also no difference between patients in advanced stage or patients with B-symptoms, when compared with patients with low stages and A-symptoms. Patients who expressed T-cell related antigens (UCHL 1 and CD 3) had a better DFS and survival but the differences were not statistically significant.

Table 5. Number of cases with positive staining of the Hodgkin and Reed-Sternberg cells in relation to histopathology. Percent in parantheses.

| No. | LN 1 | MB 2 | L26 | UCHL 1 | BerH2 | LeuM1 | CD3 |
|---|---|---|---|---|---|---|---|
| MC | 54 | 29 (54) | 10 (19) | 15 (28) | 13 (24) | 38 (70) | 22 (41) | 5 ( 9) |
| NS I | 54 | 39 (72) | 16 (30) | 13 (24) | 18 (33) | 46 (85) | 38 (70) | 9 (17) |
| NS II | 23 | 13 (57) | 6 (26) | 6 (26) | 6 (26) | 22 (96) | 17 (74) | 2 (9) |
| LP | 6 | 4 (67) | 3 (50) | 3 (50) | 4 (67) | 5 (83) | 2 (33) | 1 (17) |
| LPn | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| LD | 14 | 8 (57) | 5 (36) | 2 (14) | 4 (29) | 11 (79) | 7 (50) | 0 |
| uncl | 2 | 2 | 0 | 0 | 2 | 2 | 1 | 0 |
| Total | 154 | 96 (62) | 40 (26) | 40 (26) | 47 (31) | 124 (81) | 87 (57) | 17 (11) |

Abbreviations: MC = mixed cellularity, NS I = nodular sclerosis typ 1, NS II = nodular sclerosis type 2, LP = lymphocytic predominance, LPn= lymphocytic predominance, nodular subtupe, LD = lymphocytic depletion, uncl = unclassified.

No other study has correlated immunohistochemical characteristics of the HRS to age and clinical outcome and the clinical information in most papers is sparse or absent, rendering comparisons with other materials difficult. Antigen expression also differed significantly between different histopathological subgroups. Significantly fewer MC cases were positve as regards BerH2 (CD 30) and LeuM1 (CD 15) when compared with NS. This finding is also reported by certain authors (Werner, 1990, Hall, 1988, Kornstein) but not by others (Miettnen, 1992, Hall, 1987). It might indicate biological differences between MC and NS, possibly related to the histopathological picture. The difference in staining patterns between series might also be explained by slightly different interpretation of the criteria, especially the diagnosis of MC.

## EOSINOPHIL INFILTRATION IN TUMOUR TISSUE

When examining the immunohistochemical staining results, a great variability in the degree of eosinophilic infiltration in the tumour specimens was noted. The number of infiltrating eosinophils were therefore counted in all but autopsy cases and the patients were divided into three groups. Twenty-six patients (19%) showed intense, 51 (36%) moderate and 63 (45%) virtually no eosinophilic infiltration. There were significantly more NSI+II cases in groups 1 and 2 than expected (p=0.02). The proportion of cases in group 0 with stage 1A disease was higher (p= 0.02) than in the other stages. There were no differences in eosinophilic infiltration between patients with and without B-symptoms and between those above and below the age of 60.

Patients with intense infiltration of eosinophils (group 2) had a significantly poorer DFS (figure 4). This was true also in a multivariate analysis where eosinophil group 2 was the most important prognostic factor, also in patients below and above the age of 60, analyzed separately, although not statistically significant above 60 (table 6).

The mechanism behind the eosinophil infiltration in the lymph nodes of patients with HD is not known. The presence of mRNA for IL-5, the most potent stimulator of eosinophils, in the HRS in cases with tissue eosinophilia has been shown (Samoszuk,1990). IL-5 in HD tissue could also be produced by activated T-cells, which usually produce IL-5, but an inverse relation

between the degree of eosinophilia and the degree of T-cell activation has been described, thereby partly contradicting the theory (Ben-Ezra, 1989). Thus, it is reasonable to assume that the presence of eosinophils in tissues involved by HD is caused by factors produced by the tumour cells themselves and not as part of a general host response. This could explain the contrary results seen in cancers of the colon and rectum, where eosinophilic infiltration tends to predict a favourable outcome (Spry, 1988), possibly due to a more pronounced host response.

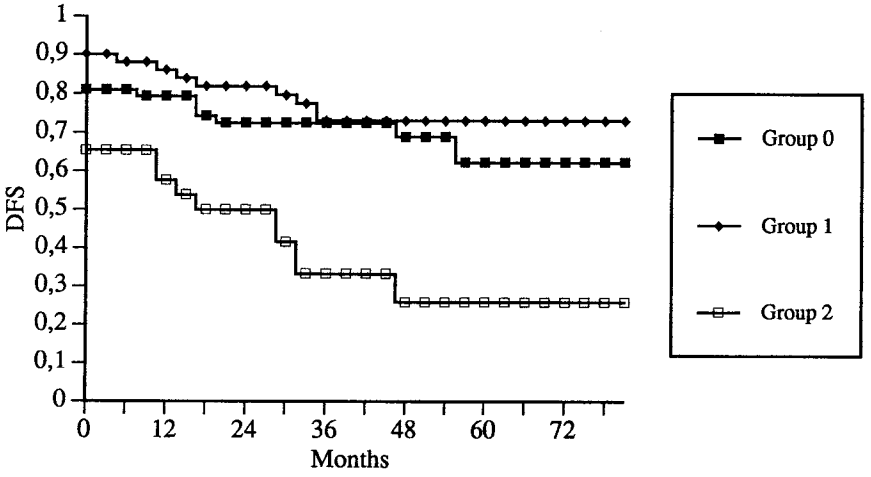


Figure 4. Disease-free survival of patients with different degree of eosinophilic infiltration in tumour specimens. Group 0=0-10/10 VF, group 1= 11-200 eosinophils/10 VF, group 2= >200 eosinophils/10 VF.

Table 6. Multivariate analysis of different prognostic factors in all cases with HD and in cases above and below 60 years, separately (t-value = beta/standard error of beta).

| Prognostic | All | | <60 years | | ≥ 60 years | |
|---|---|---|---|---|---|---|
| factor | t-value | p-value | t-value | p-value | t-value | p-value |
| EG-group 2 | 4.4 | <0.001 | 2.8 | <0.05 | 1.9 | n.s. |
| Age | 4.0 | <0.001 | 1.0 | n.s. | 1.7 | n.s. |
| Stage | 1.6 | n.s. | 2.1 | <0.05 | 1.6 | n.s. |
| Histology | 1.8 | n.s. | 1.7 | n.s. | | |
| B-symptoms | 0.5 | n.s. | 0.9 | n.s. | | |
| Bulky disease | 0.1 | n.s. | -0.8 | n.s. | | |

Abbreviations: n.s.= not statistically significant

An association between eosinophils and poor prognosis has previously been noted in one small study (Tóth, 1977), although the method applied for determining eosinophilic infiltration has not been accurately stated. dÀmore et al investigated the prognostic importance of several histopathological characterisitcs in 123 cases of NS in stages I-IIIA. Patients with a large number of eosinophils appeared to have a poorer survival but the difference was no statistically significant (d'Amore, 1992). The prognostic significance of intense eosinophilic infiltration seems to be at least partly unrelated to other prognostic factors, such as age, stage and B-symptoms. The reason for the association between eosinophils and prognosis is entirely

unknown. HD patients also exhibit blood and bone-marrow eosinophilia, and selective peripheral blood eosinophilia has been related to a more favourable prognosis (Vaughan Hudson, 1987) whereas bone-marrow eosinophilia was not related to clinical outcome (Macintyre, 1987). It is thus not likely that the presence of eosinophils in the tumour tissue is unfavourable per se but rather a phenomenon parallel to some unknown factor. Another possibility is an interplay between eosinophils and T-cells, since patients with hypereosinophilic syndrome have high serum levels of sIL-2R, a factor of great prognostic importance in HD (see below). The interaction with T-cells would, however, be equally important in HD patients with blood and bone-marrow eosinophilia.

The association between eosinophils and NS has also been described by Fuggle et al (Fuggle, 1984). The potential role of eosinophils in the formation of the sclerosis is substantiated by the observation that eosinophils stimulate fibroblasts (Hernäs, 1992) and by the detection of eosinophil peroxidase in the collagen bands of NS, but not in the tissue of reactive lymph nodes (Samoszuk, 1987). Furthermore, eosinophils are the major source of transforming growth factor beta (TGF-ß), a factor promoting the growth of fibroblasts and collagen synthesis in NS but not in MC using in situ hybridisation technique (Kadin, 1992).

## SERUM FACTORS

The serum levels of sIL-2R have recently been shown to be related to stage, B-symptoms and prognosis in young patients with HD. IL-2R is expressed on the HRS and the soluble form has retained its ability to bind circulating IL-2, a potent activator of T-cells in vivo. The levels of S-sIL-2R could therefore play a particular role by aggravating the immune-deficiency seen in HD. Since the immune-deficiency is most pronounced in elderly patients and the levels of S-sIL-2R in elderly patients have not been investigated, it was of interest to determine the levels in a material that also included elderly patients. Since the analyses required serum from the patients, the material could not be population-based. The results were correlated to stage, B-symptoms and routinely determined blood and serum factors (B-hemoglobin, leukocyte count, ESR, S-albumin, S-LDH, S-ALP, S-orosomucoid, S-haptoglobin) including also S-thymidine kinase (S-TK), an enzyme present in dividing cells, previously shown to be related to stage and prognosis in HD (Eriksson, 1985) and NHL (Hagberg, 1984, Martinsson,1988, Rehn).

S-sIL-2R levels were significantly higher in patients with advanced stage and B-symptoms but not in patients more than 60 years of age. Patients with high S-sIL-2R levels had a significantly poorer DFS and survival (figure 5).

In a multivariate analysis for DFS, S-sIL-2R and stage were the only prognostic factors in all patients. In patients below the age of 60, stage was the only prognostic factor and, in patients above the age of 60, S-sIL-2R was the only prognostic factor univariately. As regards HD-specific survival, the only significant factors for all patients were age, S-sIL-2R and S-orosomucoid and for patients above 60 years, S-sIL-2R was the only significant factor. No analysis was performed in patients below 60 years of age due to too few events (only four patients had died) (table 7+8). It was also possible to identify a group of patients below the age of 60 with very poor DFS, by a combination of S-sIL-2R and stage. Patients with stages IIB-IV were chosen. Those with S-sIL-2R > 8.0x $10^3$ units/ml had a significantly worse DFS (figure 6) and could possibly benefit from more intensive primary treatment, eg. ABMT.

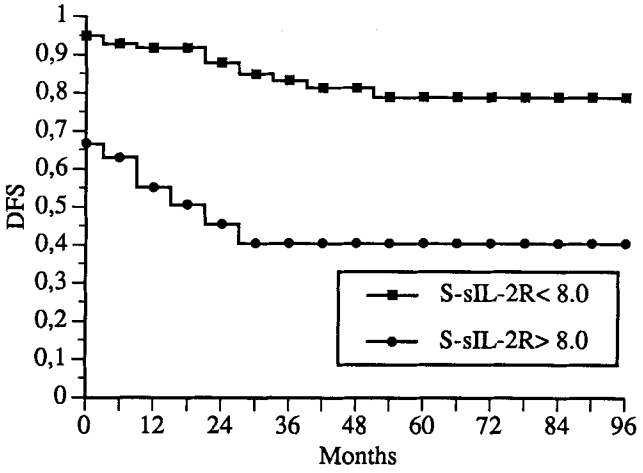


Figure 5. Disease-free survival for patients with S-sIL-2R levels above and below 8.0 x$10^3$ units/ml.

**Table 7.** Multivariate analysis with the Cox proportional hazard model of different prognostic factors, in relation to **disease free survival**, in all cases with HD and in cases below 60 years of age, separately.

| Prognostic factor | All t-value | All p-value | <60 years t-value | <60 years p-value |
|---|---|---|---|---|
| Age | 1.8 | n.s. | | |
| Stage | 2.7 | <0.01 | 2.5 | <0.05 |
| B-symptoms | 0.4 | n.s. | 1.0 | n.s. |
| S-sIL2-R | 2.7 | <0.01 | 1.8 | |
| S-TK | -0.4 | n.s. | -0.6 | n.s. |
| S-LDH | 0.5 | n.s. | -0.7 | n.s. |
| ESR | 1.0 | n.s. | | |
| B-hemoglobin | -1.4 | n.s. | 1.0 | n.s. |
| S-albumin | -0.4 | n.s. | -1.3 | n.s. |
| S-orosomucoid | 2.1 | <0.05 | 1.7 | |

Abbreviations: see table 3. No analysis was performed in patients ≥ 60 years since only S-sIL-2R was significant univariately.

**Table 8.** Multivariate analysis with the Cox proportional hazard model of different prognostic factors, in relation to **survival**, in all cases with HD and in cases above 60 years of age, separately.

| Prognostic factor | All t-value | All p-value | ≥60 years t-value | ≥60 years p-value |
|---|---|---|---|---|
| Age | 3.1 | <0.005 | | |
| Stage | 1.9 | n.s. | | |
| B-symptoms | 1.5 | n.s. | | |
| S-sIL2-R | 2.7 | <0.01 | 2.1 | <0.05. |
| S-LDH | 1.6 | n.s. | | |
| ESR | 2.0 | n.s. | 1.3 | n.s. |
| B-hemoglobin | 0.5 | n.s. | 0.5 | n.s. |
| S-albumin | 1.8 | n.s. | | |
| S-orosomucoid | 2.0 | <0.05 | | |

Abbreviations: n.s.= see table 3.

The strong prognostic significance of S-sIL-2R is interesting from a tumour-biological perspective. As mentioned above, high levels could aggravate the immune-deficiency seen in HD patients. Indeed, serum from HD patients can impair lymphocyte functions in the peripheral blood lymphocytes of healthy persons *in vitro* (Holm, 1979), and sIL-2R was the major cause of this inhibition in one study (Damle, 1992). If a relative deficiency of IL-2 is a contributing factor to the poor outcome in HD patients, it would be tempting to add IL-2 to conventional therapy. IL-2 treatment in HD has been tried in a few very advanced cases and remissions have been reported (West, 1987). IL-2 has not, however, been used in the adjuvant situation.

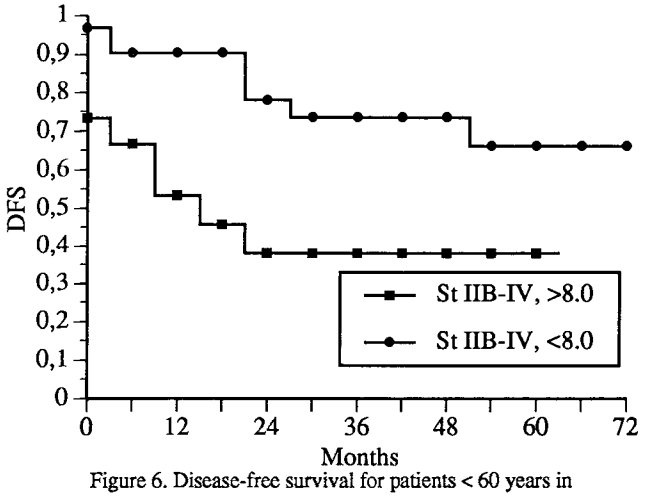


Figure 6. Disease-free survival for patients < 60 years in stages IIB-IV with S-sIL-2R levels below and above $8.0x10^3$ units/ml.

S-sIL-2R was the only factor of prognostic importance in elderly patients. The levels of S-sIL-2R were not significantly higher in the elderly patients, but this does not necessarily contradict the hypothesis that sIL-2R is an important prognostic factor in the elderly patients partly due to its interaction with the immune system. The amount of S-sIL-2R produced could be proportional to the tumour burden, but its effects in elderly patients could be more detrimental than in the young.

The prognostic importance of S-orosomucoid has also been noted in NHL and the finding is in accordance with other studies of inflammatory markers in HD (see above). The precise role of the inflammatory response in HD is not known and one might speculate that the inflammatory response is part of the general host response and that those factors are elevated by partly different mechanisms than those more closely correlated to tumour burden.

Serum levels of sIL-2R have also been reported to be elevated in patients with hypereosinophilic syndromes (Prin, 1991). Since S-sIL-2R was a very strong prognostic factor in this material and the infiltration of eosinophils was an equally strong factor in a partly different material, the number of eosinophils were counted in the tumour specimens form the patients in this material. In this material, patients with an intense infiltration had a significantly worse DFS ($p=0.01$). Patients with an intense infiltration of eosinophils did not have higher levels of S-sIL-2R. A multivariate analysis was also performed including age, stage and B-symptoms, traditionally the most important prognostic factors, and S-sIL-2R and eosinophil group 2. S-sIL-

2R and eosinophil group 2 turned out to be equally important and were the only significant factors. The correlation between sIL-2R and the eosinophil-group was rather weak ($r=0.2$, $p<0.05$) further supporting the independent value of those tests, possibly by identifying different groups with a poor prognosis.

## TUMOUR BURDEN

There are several indications that the tumour cell burden is important for the clinical outcome in HD patients. Patients in advanced stages (Carbone, 1971, Kaplan, 1980) with bulky disease (Horwich,1986) and patients with many involved sites (Hoppe, 1980) have a poorer prognosis. Furthermore, histopathological subgroups which by definition contain more HRS, NS II and LD, are associated with a poorer outcome (Kaplan, 1980, MacLennan, 1989). Quantitative assessments of the tumour cell density and total tumour volume has recently been shown to be of prognostic importance (Specht,1990). The findings have, however, not been confirmed and tumour burden measurements in relation to tumour-specific serum markers have not been investigated. Furthermore, the tumour cell burden has not been studied in elderly patients, specifically.

In paper V, the tumour cell density and the total tumour burden (in stages I+II) was estimated and the findings related to each other as well as to other prognostic factors. In order to obtain an easy and reproducable measure of tumour burden, the serum levels of soluble CD 30 (S-sCD 30) was measured. It is likely that the HRS are the main source of S-sCD 30 in HD, since CD 30 is only detected on a small minority of reactive lymphocytes, and soluble CD 30 has not been detected in the serum in healthy controls (Gause, 1992). The results were related to tumour cell density and measurements of tumour volume. The results were also correlated to the serum levels of sIL-2R and TK; sIL-2R due to its strong prognositc importance and to its possible relation to the tumour burden and to the immunodeficiency present in many HD patients (paper IV), and TK due to its relationship with proliferation (Källander,1987) and the number of proliferating cells, i.e. also to some extent tumour burden.

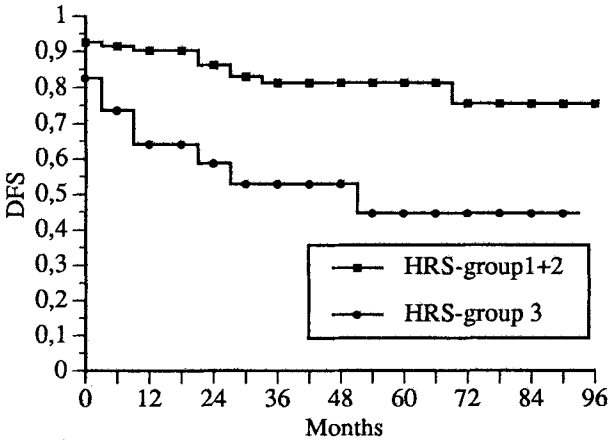


Figure 7. Disease-free survival for patients in HRS-group 3 vs groups 1+2. HRS-group 1=0-2 HRS / VF, HSR-group 2=2-8 HRS / VF, HRS-group 3= >8 HRS /VF.

Thirty-one (26%) patients with high tumour cell density had a sinificantly worse DFS ($p<0.01$) (figure 7). Patients in stages I+II with a high total tumour burden also had a significantly worse DFS ($p<0.05$), whereas patients with bulky disease did not. There was a statistically significant higher tumour cell density in patients with high total tumour burden ($p<0.001$) and stage IV $p<0.001$) (figure 8). The higher tumour cell concentration in advanced stage could imply biological differences between the HRS in early and advanced stages eg. differences in cytokine production, proliferation rates or in features facilitating escape from the immune defense.

The serum levels of sCD 30, sIL-2R and TK were significantly higher in patients with stages III-IV, B-symptoms, and bulky disease. They were also significantly higher in patients with high total tumour volume and combined tumour volume. S-sCD 30 and S-sIL-2R, were both significantly higher in patients with high tumour cell concentration. The levels did, however, not differ between patients below or above the age 60. The serum levels of not only sCD 30, but also sIL-2R and TK could thus all represent easy measurements of tumour burden.

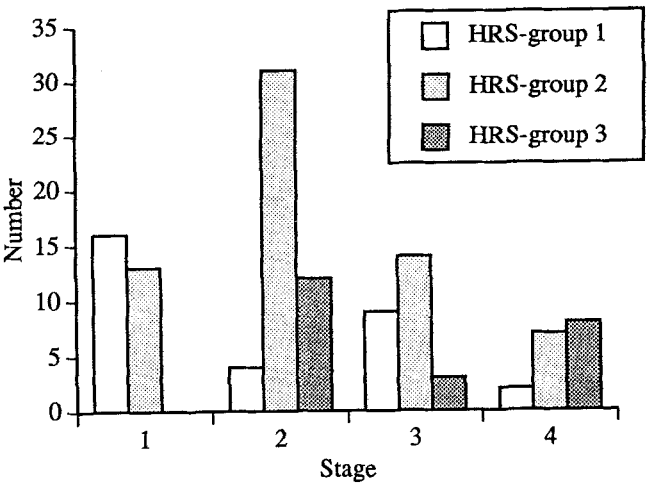


Figure 8. Relation between HRS-density and stage at diagnosis. HRS-group 1=0-2 HRS / VF, HSR-group 2=2-8 HRS / VF, HRS-group 3= >8 HRS /VF.

Patients with S-sCD 30 levels $>2.0x10^2$ U/mL had a significantly poorer DFS ($p<0.05$). The prognostic importance of S-sCD 30 was, however, weak in comparison with most other parameters investigated (table 9).

In a multivariate analysis, S-sIL-2R was the only significant factor for DFS and S-sIL-2R and age for HD specific survival. The prognosic impact of the tumour cell concentration and total tumour burden demonstrated in this study was similar to the results presented by Specht et al. The estimation of tumour cell concentration was, however, very laborious and counting of HRS can not be recommended in routine use as a single prognostic tool, since more accurate prognostic information most likely can be obtained by a combination of stage, possibly number of sites in certain stages, and a serum factor, preferably sIL-2R.

In the present study young and old patients were comparable in all measurements of tumour cell burden. The number of old patients is rather limited, but the findings do at least not indicate

that differences in tumour burden is responsible for the poor outcome in elderly patients. Tumour cell concentration was also estimated in the population-based material diagnosed 1985-1988 (papers II and III), including a higher proportion of elderly patients. Again, there was no difference in tumour-cell concentration between young and elderly patients. Patients with high HRS concentration had a significantly worse DFS (p=0.01) In a multivariate analysis HRS-concentration did not add any prognostic information.

Table 9. Univariate analyses with the Cox's proportional hazard model of different prognostic factors, in relation to **disease-free survival**, in all patients and in patients in stages I+II separately. t-value=beta/standard error beta.

| Prognostic factor | All | | Stages I+II | |
|---|---|---|---|---|
| | t-value | p-value | t-value | p-value |
| Age | 2.2 | <0.05 | 1.4 | n.s. |
| Stage | 3.7 | <0.001 | 1.0 | n.s. |
| B-symptoms | 3.5 | <0.001 | 3.0 | <0.01 |
| Bulky disease | 1.2 | n.s. | 0.1 | n.s. |
| S-sIL2-R | 4.5 | <0.001 | 3.6 | <0.001 |
| S-TK | 2.9 | <0.01 | 2.8 | <0.01 |
| HRS-group 3 | 3.0 | <0.01 | 2.9 | <0.01 |
| S-sCD-30 | 1.8 | n.s. | 0.9 | n.s. |
| Total t.b. 4 | | | 1.8 | n.s. |
| Comb t.b. | | | 2.4 | <0.05 |

Abbreviations: n.s.= not statistically significant ($p \geq 0.05$). S-sIL-2R= soluble interleukin-2 receptor, S-TK= thymidine kinase, S-LDH= lactate dehydrogenase, ESR =erythrocyte sedimentation rate, S-ALP= alkaline phosphatase.

# REFERENCES


Ambinder RF, Browning PJ, Lorenzana I, Leventhal BG, Cosenza H, Mann RB, MacMahon EME, Medina R, Cardona V, Grufferman S, Olshan A, Levin A, Petersen EA, Blattner W, Levine PH. Epstein-Barr virus and childhood Hodgkin´s disease in Honduras and the United States. Blood 1993;81:462-467.

d'Amore ESG, Lee CKK, Aeppli DM, Levitt SH, Frizzera G. Lack of prognostic value of histopathological parameters in Hodgkin´s disease, Nodular sclerosis type. Arch Pathol Lab Med 1992;116:856-861.

Anderson JE, Litzow MR, Appelbaum FR, Schoch G, Fisher LD, Buckner CD, Petersen FB, Crawford SW, Press OW, Sanders JE, Bensinger WI, Martin PJ, Storb R, Sullivan KM, Hansen JA, Thomas ED. Allogenic, syngenic, and autologous marrow transplantation for Hodgkin´s disease: The 21-year Seattle Experience. J Clin Oncol 1993;11:2342-2350.

Anagnostopoulos I, Herbst H, Niedobitek G, Stein H. Demonstration of monoclonal EBV genomes in Hodgkin´s disease and Ki-1-positive anaplastic large cell lymphoma by combinedSouthern blot and in situ hybridisation. Blood 1989;74:810-816.

Ambrosetti A, Nadali G, Vinante F, Carlini S, Veneri D, Todeschini G, Morasoto L, deSabata D, Chilosi M, Maggi E. Serum levels of interleukin-2 receptor in Hodgkin´s disease.Relationship with clinical stage, tumor burden, and treatment outcome. Cancer 1993;72(1):201-206.
Audouin J, Diebold J, Pallesen G. Frequent expression of Epstein-Barr virus latent membrane protein-1 in tumour cells of Hodgkin´s disease in HIV-positive patients. J Pathol 1992;167:381-383.

Austin-Seymor M M, Hoppe R T, Cox R S, Rosenberg S A, Kaplan H S. Hodgkin´s disease in patients over sixty years old. Ann Int Med 1984;100:13-18.

Ben-Ezra J, Sheibani K, Swartz W, Stroup R, Traweek ST, Kezirian J, Rappaport H. Relationship between eosinophil density and T-cell activation markers in lymph nodes of patients with Hodgkin´s disease. Hum Pathol 1989;20(12):1181-5.

Bennett MH, MacLennan KA, Easterling MJ, Vaughan Hudson B, Jelliffe AM, Vaughan Hudson G. The prognostic significance of cellular subtypes in Nodular Sclerosing Hodgkin´sdisease: An analysis of 271 non-laparotomised cases (BNLI report No. 22) Clin Rad 1983,34:497-501.

Bergmann L, Mitrou PS, Demmer-Dieckmann M, Ruhmann FT, Weidmann E. Impaired T-and B-cell functions in patients with Hodgkin´s disease. Cancer Immunol Immunother 1987;25:59-64.

Bernhards J, Fischer R, Hübner K, Schwarze E-W, Georgii A. Histopathological classification of Hodgkin´s disease. Ann Oncol 1992;3(suppl 4):31-33.

Björkholm M, Wedelin C, Holm G, Essy-Ehsing B. Familial longevity and prognosis in Hodgkin´s disease. Cancer 1984;54:1088-1092.

Björkholm M, Holm G, Mellstedt H. Immunocompetence in patients with Hodgkin´s disease. In Lacher MJ and Redman JR (eds). Hodgkins Disease. The consequences of survival. Lea & Febiger, Philadelphia, USA, pp 112-150, 1990.
Blum R H, Carter S K, Agre K. A clinical review of bleomycin- a new antineoplastic agent. Cancer 1973;31:903-913.

Bonadonna G, Zucali R, Monfardini S, DeLena M, Uslenghi C. Combination chemotherapy of Hodgkin´s disease with adriamycin, bleomycin, vinblastine and imidazole carboxamide versus MOPP. Cancer 1975;36:252-259.

Botto B, Levis A, Depaoli L, Bertini M, Mairone L, Orsucci L, Vitilo U, Gavarotti P, Salomone A, Buchi G, Resegotti L. Preliminary results of an alternating chemotherapy regimen

(ChlVP/CEB) for elderly patients affected with Hodgkin´s disease. Meeting Abstract, Fourth International Coference on malignant lymphoma, June 1990, Lugano; Switzerland.

Boyle P, Soukop M, Scully C, Robertson AG, Burns HJG, Gillis CR, Kaye SB. Improving prognosis of Hodgkin´s disease in Scotland. Eur J Cancer Clin Oncol 1988;24:229-234.

Brinker M, Poppema S, Buys C et al. Clonal immunoglobulin gene rearrangements in tissues involved by Hodgkin´s disease. Blood 1987;70:186-191.

Bristow M R, Mason J W, Billingham M E et al. Doxorubicin cardiomyopathy: evaluation by phonocardiography, endomyocardial biopsy and cardiac catheterisation. Ann Int Med 1978;88:168-175.

Butler JJ. The histologic diagnosis of Hodgkin´s disease. Semin Diag pathol 1992;9:252-256.

Cabanillas F. A review and interpretation of cytogenetic abnormalities identified in Hodgkin´s disease. Hematol Oncol 1988;6:271-274.

Cannellos GP, AndersonJR, Propert K et al: Chemotherapy of advanced Hodgkin´s disease with MOPP, ABVD or MOPP alternating with ABVD. N Engl J Med 1992;19:1478-1484.

Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report on the committee on Hodgkin´s disease staging. Cancer Res 1971;31:1960-61.

Chang KL, Albújar PF, Chen Y-Y, Johnson RM, Weiss LM. High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of Hodgkin´s disease occurring in Peru. Blood 1993;81:496-501.

Cimino G, Lo Coco F, Cartoni C, Gallerano T, Luciani M, De Rossi L, De Rossi G. Immune-deficiency in Hodgkin´s disease: a study of patients anf healthy relatives in families with multiple cases. Eur j Cancer Clin Oncol 1988;24:1595-1601.

Connors JM, Klimo P, Adams G et al. MOPP/ABV hybrid versus alternating MOPP/ABVD in advanced Hodgkin´s disease. Proc Am Soc Clin Oncol 1992;11:317-

Connors JM. Is cyclical chemotherapy better than standard four-drug chemotherapy for Hodgkin´s disease? Yes. Principles and Practice of Oncology updates 1993;7(2):1-6.

Cordell JL, Falini B, Erber WN et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984;32:219-229.

Correa P, O´Conor GT. Epidemiologic patterns of Hodgkin´s disease. Int J Cancer 1979;8:192-201.

Damle RN, Advani SH, Gangal SG. Analysis of T-cell responses by soluble inhibitory factors from patients with Hodgkin´s disease. Int J Cancer 1992;50:192-196.

Dawson PJ. The original illustrations of Hodgkin´s disease. Arch Int Med 1968;121:288-90.

DeVita VT, Serpick A. Combination chemotherapy in the treatment of advanced Hodgkin´s disease. Proc Am Assoc Cancer Res 1967;8:13. Abstract.

DeVita VT, Simon RM, Hubbard SM, Young RC, Berard CW, Moxley JH, Frei E, Carbone PP, Canellos GP. Curability of advanced Hodgkin´s disease with chemotherapy. Long-term follow-up of MOPP-treated patients at the National Cancer Institute. Ann Int Med 1980;92:587-595.

DeVita VT, Hellman S, Rosenberg SA. Principles and Practice of Oncology. Third edition. JB Lippincott Company, Philadelphia, USA, 1989.

DeVita VT, Hubbard SM. Hodgkin´s disease. Drug therapy. N Engl J Med 1993;328:560-565.

DeVita VT. Is alternating cyclic chemotherapy better than standard four-drug chemotherapy for Hodgkin´s disease? No. Principles and Practice of Oncology updates 1993;7(1):1-11.

Diehl LF, Hopper KD, Giguere J, Granger E, Lesar M. The pattern of intrathoracic Hodgkin´s disease assessed by computed tomography. J Clin Oncol 1991;9:438-443.

Drexler HG, Amlot PL, Minowada J. Hodgkin´s disease-derived cell lines- conflicting clues for the origin of Hodgkin´s disease. Leukemia 1987;1:629-637.

Eghbali H, Hoerni- Simon G, deMascarel I, Durand M, Chauvergne J, Hoerni B. Hodgkin´s disease in the elderly. Cancer 1984;53:2191-2193.

Erdkamp FL, Breed WP, Bosch LJ, Winjen JT, Blijham GB. Hodgkin´s disease in the elderly. Cancer 1992;70:830-834.

Eriksson B, Hagberg H, Glimelius B, C Sundström, S Gronowitz and C Källander. Serum thymidine kinase as a prognostic marker in Hodgkin´s disease. Acta Radiol Oncol 1985; 2:167-71.

Ferrini P R, Bosi A, Casini C, Messori A, Bellesi G. Hodgkin´s disease in the Elderly: a retrospective clinicopathologic study of 61 patients aged over 60 years. Acta haemat 1986;78;suppl 1:163-170.

Forni M, Hofman FM, Parker JW, Lukes RJ, Taylor CR. B- and T-lymphocytes in Hodgkin's disease. Cancer 1985;55:728-737.

Fox H. Remarks on microscopical preparations made from some of the original tissue described by Thomas Hodgkin, 1832. Ann Med Hist 1926;8:370-374.

Franssila KO, Heiskala MK, Heiskala HJ. Epidemiology and histopathology of Hodgkin´s disease in Finland. Cancer 1977;39:1280-1288.

Frederick P, Lokich J, Costanza M, Moloney W C, Hellman S. Hodgkin´s disease in the elderly. Lancet 1973:774.

Fuggle WJ, Crocker J, Smith PJ. A quantitative study of eosinophil polymorphs in Hodgkin´s disease. J Clin Pathol 1984;37:267-71.

Gause A, Roschansky V, Tschiersch A, Smith K, Hasenclever D, Schmits R, Diehl V, Pfreundschuh M. Low serum interleukin-2 receptor levels correlate with a good prognosis in patients with Hodgkin´s lymphoma. Ann Oncol 1991;2(suppl 2):43-47.

Gause A, Jung W, Schmits R, Tschiersch A, Scholz R, Pohl C, Hasenclever D, Diehl V and Pfreundschuh M. Soluble CD 8, CD 25 and CD 30 antigens as prognostic markers in patients with untreated Hodgkin´s lymphoma. Ann Oncol 1992;3(suppl 4):49-52.

Glaser S L. Recent incidence and secular trends in Hodgkin´s disease and its histologic subtypes. J Chron Dis 1986; 39:789-798.

Glaser SL. Regional variation in Hodgkin´s disease incidence by histologic subtype in the US Cancer 1987;60:2841-2847.

Glatstein E, Trueblood HW, Enright LP, Rosenberg SA, Kaplan HS. Surgical staging of abdominal involvement in unselected patients with Hodgkin´s disease. Radiology;1970:425-432.

Glimelius B, Enblad G. Hodgkin´s disease. In Macieira-Coelho A, Nordenskjöld B (eds) Cancer and Aging. CRC Press, pp227-237, 1990.

Glimelius B, Kälkner M, Enblad G, Gustavsson A, Jacobsson M, Branehög I, Lenner P. Treatment of early and intermediate stage supradiaphragmal Hodgkin´s disease: The Swedish National Care Program experience. Submitted to annals of Oncology.

Greenfield WS. Specimens illustrative of the pathology of lymphadenoma and leucocythaemia. Transactions of the Pathological Society of London 1878;29:272-304.

Greisser H, Mak T. Immunogenotyping in Hodgkin´s disease. Hematol Oncol 1988;6:239-245.

Grimfors G, Holm G, Mellstedt H, Schnell P-O, Tullgren O, Björkholm M. Increased blood clearance rate of Indium-111 oxine-labeled autologous CD4+ blood cells in untreated patients with Hodgkin´s disease. Blood, 1990;76:583-589.

Grimfors G. Lymphocyte kinetics and function in Hodgkin´s disease. Thesis, Stockholm, 1990.

Gruss H-J, Brach MA, Drexler H-G, Bross KJ, Herrmann F. Interleukin 9 is expressed by primary and cultured Hodgkin and Reed-Sternberg cells. Cacer Res 1992;52:1026-1031.

Guthenson N M. Social class and age at the diagnosis of Hodgkin´s disease: New epidemiologic evidence for the "two-disease-hypothesis". Cancer Treat Rep1982;66:689-695.

Hagberg H, Glimelius B, Gronowitz S, Killander A, Källander C. Biochemical markers in non-Hodgkin´s lymphoma stages III and IV - a multivariate analysis. Scand J Haematol 1984;33:59-67.

Hagemeister FB, Tannir N, McLaughlin P, Salvador P, Riggs S, Velasquez WS, Cabanillas F. MIME chemotherapy (methyl-gag, ifosfamide, methotrexate, etoposide as treatment for recurrent Hodgkin´s disease. J Clin Oncol 1987;5:556-561.

Hakulinen T, Abeywickrama KH. A computer program package for relative survival analysis. Comput Progr Biomed 1985;19:197-207.

Hall PA, D´Ardenne AJ. Value of CD 15 immunostaining in diagnosing Hodgkin´s diseae: a review of published literature. J Clin pathol 1987;40:1298-1304.

Hall PA, D´Ardenne AJ, Stansfeld AG. Paraffin section immuno-histochemistry. II. Hodgkin´s disease and large cell anaplastic (Ki1) lymphoma. Histopathology 1988;13:161-169.

Hansmann M-L, Stein H, Fellbaum C, Huo PK, Parwaresch MR, Lennert K. Nodular paragranuloma can transform into high-grade malignant lymphoma of B type. Hum Pathol 1989;20:1169-1175.

Henry-Amar M, Friedman S, Hayat M, Somers R, Meerwaldt JH, Carde P, Burgers JMV, Thomas J, Monconduit M, Noordijk EM, Bron D, Regnier R, de Pauw E, Tanguy A, Cosset J-M, Dupouy N, Tubiana M. Erythrocyte sedimentation rate predicts early relapse and survival in early-stage Hodgkin´s disease. Ann Int Med 1991,114:361-365.

Herbst H, Pallesen G, Weiss LM, Delsol G, Jarret RF, Steinbrecher E, Stein H, Hamilton-Dutoit S, Brousset P. Hodgkin´s disease and Epstein-Barr virus. Ann Oncol 1992;3(suppl 4):27-30.

Hernnäs J, Särnstrand B, Lindroth et al. In: Eosinophil cationic protein alters proteoglycan metabolism in human lung fibroblast cultures. Metabolism of proteoglycans in fibrosis, studies on the regulation in vivo and in vitro. Thesis, Lund 1992.

Hodgkin T. On some morbid appearances of the absorbent glands and spleen. Med Chir Trans 1832;17:68-114.

Holm, Angelin B, Björkholm M, Ericsson P, Mellstedt H, Pettersson D. Immunosuppressive serum factors and lymphocyte deficiency in Hodgkin´s disease. J Clin Lab Immunol 1979;1:269-275.

Hoppe RT, Rosenberg SA, Kaplan HS. Prognostic factors in pathological stage IIIA Hodgkin´s disease. Cancer 1980;46:1240-1246.

Horwich A, Easton D, Nogueira-Costa R, Liew K H, Colman M Peckham M J. An analysis of

prognostic factors in early stage Hodgkin´s disease. Radiotherapy and oncology 1986;7:95-106.

Hryniuk W. The impact of dose intensity on the design of clinical trials. Semin Oncol 1987;14:65-74.

Jaffe ES, Zarate-Osorno A, Kingma DW, Raffeld M, Medeiros LJ. The interrelationship between Hodgkin´s disease and non-Hodgkin´s lymphomas. Ann Oncol 1994;5(Suppl 1):7-11.

Jarret RF, Gallagher A, Jones DB, Alexander FE, Krajewski AS, Kelsey A, Adams J, Angus B, Gledhill S, Wright DH, Cartwright RA, Onions DE. Detection of Epstein-Barr-virus genomes in Hodgkin´s disease: relation to age. J Clin Pathol 1991,44:844-848.

Jackson H, Parker F. Hodgkin´s disease and allied disorders. new York, Oxford University Press 1947.

Johansson B, Klein G, Henle W, Henle G. Epstein-Barr virus (EBV-) associated antibody patterns in malignant lymphoma and leukemia. Hodgkin´s disease. int j Cancer 1970;6:450-462.

Jücker M, Abts H, Li W. Expression of interleukin-6 and interleukin-6 receptor in Hodgkin´s disease. Blood 1991;77:2413-2418.

Kadin M, Butmarc J, Elovic A, Wong D. Eosinophils are a major source of transforming growth factor-beta (TGF-ß) in nodular sclerosing Hodgkin´s disease. Meeting Abstract 5th meeting of the European Association for Haematopathology, 21-25 September 1992, Bologna, Italy.

Kaldor JM. Day WE, Clarke EA et al. Leukemia following Hodgkin´s disease. N Eng J Med 1990;311:7-13.

Kaplan HS. Evidence for a tumoricidal dose level in the radiotherapy of Hodgkin´s disease. Cancer res 1966;26:1221-1224.

Kaplan HS. Hodgkin´s disease. Second edition. Harvard University Press. Cambridge, Massachusetts, USA, 1980.

Kennedy B J. Aging and cancer. J Clin Oncol 1988;6:1903-1911.

Klimo P, Connors JM. MOPP/ABV Hybrid program: Combination chemotherapy based on early introduction of seven effective drugs for advanced Hodgkin´s disease. J Clin Oncol 1985;3:1174-

Knowles DM. Neoplastic Hematopathology. Williams & Wilkins, Baltimore, Maryland, USA, 1992.

Kornstein MJ, Bonner H, Bethann Gee HT, Cohen R, Brooks JJ. Leu M1 and S 100 in Hodgkin´s disease and non-Hodgkin´s lymphomas. Am J Pathol 1986;85:433-437.

Källander CFR, Simonsson B, Gronowitz JS, Nilsson K. Serum deoxythymidine kinase correlates with peripheral lymphocyte thymidine uptake in chronic lymphocytic leukemia. Eur J Haematol 1987;38:331-337.

Lavey RS, Eby NL, Prosnitz LR. Impact on second malignancy risk of the combined use of radiation and chemotherapy for lymphomas. Cancer 1990;66:80-88.

Liberati AM, Ballatori E, Fizzotti M, Schippa M, Proietti MG, Di Marzio R, Pecci A, Biscetti L, Sbarretti R, Cini L, Grinani F. Immunologic profile in patients with Hodgkin´s disease in complete remission. Cancer 1987;59:1906-1913.

Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC, Rosenberg SA, Coltman CA, Tubiana M. Report of the committee convened to discuss the evaluation and staging of patients with Hodgkin´s disease: Cotswolds Meeting. J Clin Oncol 1989;7:1630-1636.

Lokich J J, Pinkus G S, Moloney W C. Hodgkin´s disease in the elderly. Oncology

1974;29:484.500.

Lukes RJ, Butler JJ. The pathology and nomenclature of Hodgkin´s disease. Cancer Res 1966;26:1063-1081.

Lukes RJ, Craver LF, Hall TC, Rappaport H, Ruben P. Report of the Nomenclature Committee. Cancer Res 1966; 26;1311.

Löffler M, Mauch P, MacLennan K, Specht L, Henry-Amar M. Review on prognostic factors. Ann Oncol 1992;3(suppl 4):63-66.

MacIntyre EA, Vaughan Hudson B, Vaughan Hudson G, Jelliffe AM, Linch DC. Incidence and clinical importance of bone marrow eosinophilia in Hodgkin´s disease (BNLI Report No 29). j Clin Pathol 1987;40:245-246.

MacMahon B. Epidemiology of Hodgkin´s disease. Cancer Res 1966;26:1189-1200.

Mantovani G, Coiana A, Massida A, Locci F, Loy M, Piludu G, Piras MC, Macciò A, Del Giacco GS. Peripheral blood lymphocyte response to recombinant interleukin 2 in previously treated patients with Hodgkin´s disease, long-time off-therapy. Eur J Haematol 1987;38:179-186.

Martinsson U, Glimelius B, Hagberg H, Sundström C. Prognostic relevance of serum-markers in relation to histopathology, stage and initial symptoms in advanced low-grade non-Hodgkin lymphomas. Eur J Haematol 1988;40:289-298.

Mauch P, Somers R. Controversies in the use of diagnostic staging laparotomy and splenectomy in the management of Hodgkin´s disease. Ann Oncol 1992;3(suppl 4):41-43.

Mauch PM, Kalish LA, Kadin M, Coleman CN, Osteen R, Hellman S. Patterns of presentation of Hodgkin´s disease. Cancer 1993;71:2062-2071.

Merk K, Björkholm M, Tullgren O, Mellstedt H, Holm G. Immune deficiency in family members of patients with Hodgkin´s disease. Cancer 1990;66:1938-1943.

Merk K, Björkholm M, Rengifo E, Gavilondo J, Holm G, Rivas H. Epidemiological study of Hodgkin´s disease in Cuba and Sweden. Oncology 1990;47:246-250.

Miettinen M. CD 30 Distribution. Immunohistochemical study on formaldehyde-fixed, paraffin-embedded Hodgkin´s and non-Hodgkin´s lymphomas Arch Pathol Lab Med 1992;116:1197-1201.

Mueller N. Epidemiological studies assessing the role of Epstein- Barr virus in Hodgkin´s disease. Yale J Biol Med 1987;60:321-327.

Mueller N, Evans A, Harris NL, Costock GW, Jellum E, Magnus K,Orentreich N, Polk BF, Vogelman J. Hodgkin´s disease and Epstein-Barr virus. Altered antibody pattern before diagnosis. N Engl J Med 1989;320:689-695.

Mueller N. An epidemiologist´s view of the new molecular findings in Hodgkin´s disease. Ann Oncol 1991;2(suppl 2):23-28.

National Health Care Program for Hodgkin´s disease (Vårdprogram för Hodgkin´s sjukdom ). Regional Oncological Centre in the Uppsala-Örebro Health Care Region. 1984.

Norberg B, Dige U, Johansson H, Roos G, Johansson H, Lenner P. Hodgkin´s disease in Northern Sweden 1971-1981. Acta Oncol 1991;30:697-701.

Nordentoft A M, Pedersen-Bjergaard J, Brincker H, Andersen E, Pedersen M, Boye Nielse J, Björn Jensen K, Nissen N I, Skov Jensen T, Vidbaek Aa, Krogh jensen M, Walbom-Jörgensen S. Hodgkin's disease in Denmark. A national clinical study by the Danish Hodgkin study group, LYGRA. Scand J Haematol 1980;24:321-334.

Paietta E. Cell adhesion molecules in Hodgkin´s disease. Ann Oncol 1992;3(suppl 4):17-19.

Pedersen-Bjergard J, Specht L, Larsen SO et al. Risk of therapy-related leukemia and preleukemia after Hodgkin´s disease. Lancet 1987;7:83-88.

Peters MV, Middlemiss KHC. A study of Hodgkin´s disease treated by irradiation. Am J Roentenol 1958;79:114-121.

Peterson B A, Pajak T F, Cooper M R, Nissen N I, Glidewell O J, Holland J F, Bloomfield C D, Gottlieb A J. Effect of age on therapeutic response and survival in advanced Hodgkin´s disease. Cancer treat rep 1982;66:889-898.

Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer1977;35:1-39.

Pinkus GS, Said JW. Hodgkin´s disease, lymphocytic predominance type, nodular- A distinct entity. Am J pathol 1985;118:1-6.

Pizzolo G, Chilosi M, Vinante F, Dazzi F, Lestani M, Perona G, Benedetti F, Todeschini G, Vincenzi C, Trentin L and Semenzato G. Soluble interleukin-2 receptors in the serum of patients with Hodgkin´s disease. Br J Cancer 1987;55:427-428.

Pizzolo G, Vinante F, Chilosi M, Dallenbach F, Josimovic-Alasevic O, Diamantenstein T, H Stein. Serum levels of soluble CD 30 molecule (Ki1-antigen) in Hodgkin´s disease: Relationship with disease activity and clinical stage. Br J Haematol 1990;75:282-4.

Pizzolo G, Vinante F, Nadali G, Ricetti MM, Morosato L, Marrocchella C, Vincenzi C. ICAM-1 tissue overexpression associated with increased serum levels of its soluble form in Hodgkin´s disease. Br J Haematol 1993;84:161-162.

Poppema S. Lymphocytic predominance Hodgkin´s disease. Sem Diag Pathol 1992;4:257-264.

Poppema S, Kaleta j, Hepperle B, Visser L. Biology of Hodgkin´s disease. Ann Oncol 1992;3(suppl 4):5-8.

Poppema S, Kaleta j, Hepperle B. Chromosomal abnormalities in patients with Hodgkin´s disease: Evidence for frequent involvement of the 14Q chromososmal region but infrequent BCL-2 rearrangement in Reed-Sternberg cells. JNCI 1993;84:1789-1793.

Prin L, Plumas J, Gruart V, Loiseau S, Aldebert D, Ameisen JC, VermerschA, Fenaux P, Bletry O, Capron M. Elevated levels of soluble interleukin-2 receptor: a marker of disease activity in the hypereosinophilic syndrome. Blood 1991;78(10):2626-2632.

Proctor SJ, Taylor P, Mackie MJ, Donnan P, Boys R, Lennard A and prescott RJ. A numerical prognostic index for clinical use in identification of poor-risk patients with Hodgkin´s disease at diagnosis. Leukemia and Lymphoma 1992;7(suppl):17-20.

Pui CH, Hudson M, Luo X, Wiliams J, Evans W, Crist WM. Serum interleukin-2 receptor levels in Hodgkin´s disease and other solid tumors of childhood. Leukemia 1993;7(8):1242-1244.

Rapoport AP, Rowe JM, Kouides PA, Duerst RA, Abboud CN, Liesveld JL, Packman CH, Eberly S, Sherman M, Tanner MA, Constine LS, DiPersio JF. One hundred autotransplants for relapsed or refractory Hodgkin´s disease and lymphoma: Value of pretransplant disease status for predicting outcome. J Clin Oncol 1993;11:2351-2361.

Reed DM. On the patholgic changes in Hodgkin´s disease, with special reference to its relation to tuberculosis. John Hopkin´s Hosp. Rep. 1902;10:133-196.

Rehn S, Glimelius B, Sundström C. A comparative study of proliferation-associated parameters in B-cell non-Hodgkin lymphomas. Hematol Oncol 1991;9:287-298.

Romagnini S, Ferrini PLR, Ricci M. The immune derangement in Hodgkin´s disease. Semin Hematol 1985;22:41-55.

Rosenberg SA, Kapölan HS. Evidence for an orderly progression in the spread of Hodgkin´s disease. Cancer Res 1966;26:1225-1231.

Rosenberg SA. The continuing challenge of Hodgkin´s disease. Ann Oncol 1991;2(Suppl 2):29-31.

Rosenthal SA. Significance of tissue lymphocytes in the prognosis of lymphogranulomatosis. Arch Pathol lab med 1936;21:628-646.

Samoszouk M and Nansen L. Detection of Interleukin-5 messenger RNA in Reed-Sternberg cells of Hodgkin´s disease with eosinophilia. Blood 1990;75:13-16.

Santoro A, Viviani S, Valagussa P, Bonfante V, Bonadonna G. CCNU, etoposide and prednimustine (CEP) in refractory Hodgkin´s disease. Semin Oncol 1986;13(suppl 1):23-26.

Serraino D, Franceschi S, Talami R, Bara S, Negri E, Carbone A, La Vecchia C. Socio-economic indicators, infectious diseases and Hodgkin´s disease. Int J Cancer 1991;47:352-357.

Silverman D T, Correra P, O´Connor G, Myers M H, Axtell L M, Bragg K U. A comparison of Hodgkin´s disease in Alameda county, California, and Connecticut. Cancer 1977;39:1758-1763.

Smetana HF, Cohen BM. Mortality in relation to histologic type in Hodgkin´s disease. Blood 1956;11:211-224.

Smithers DW. Spread of Hodgkin´s disease. Lancet 1970;1:1262-1267.

Specht L, Nordentoft AM, Cold S, Töffner Clausen NA, Nissen NI. Tumor burden as the most important prognostic factor in early stage Hodgkin´s disease. Cancer 1988;61:1719-1727.

Specht L, Nissen NI. Prognostic factors in Hodgkin´s disease stage III with special reference to tumour burden. Eur J Haematol 1988;41:80-87.

Specht L, Nissen NI. Hodgkin´s disease and age. Eur J Haematol 1989;43:127-135.

Specht L, Falensteen Lauritzen A, Nordentoft AM, Kragh Andersen P, Egelund Christensen B, Hippe E, Hou-Jensen K and Nissen NI. Tumor cell concentration and tumor burden in relation to histopathologic subtype and other prognostic factors in early stage Hodgkin´s disease. Cancer 1990;65:2594-2601.

Spry CJF: Eosinophils: A Comprehensive review and guide to the scientific and medical literature. Oxford, UK, Oxford University Press, 1988.

Sternberg C. Über eine eigenartige unter dem Bilde der Pseudoleukämie verlaufende Tuberculose des lymphatischen Apparates. Z Heilkunde 1898;19:21-90.

Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG. The unlabeled atibody enzyme method of immunohistochemistry. J Histochem Cytochem 1970;18:315-333.

Sweetenham JW, Williams CJ. Malignant lymphoma in the elderly. Ballière´s Clinical Haematology 1987;1:493-511.

Swerdlow AJ, Douglas AJ, Vaughan Hudson G, Vaughan Hudson B, MacLennan KA. Risk of secondary primary cancer after Hodgkin´s disease in patients in the British National Lymphoma Investigation: relationships to host factors, histology and stage of Hodgkin´s disease, and splenectomy. Br J Cancer 1993;68:1006-1011.

Symmers W S. Survey of the eventual diagnosis in 600 cases referred for a second histological opinion after an initial biopsy diagnosis of Hodgkin´s disease. J Clin Pathol 1968;21:650-653.

Tesch H, Feller AC, Jücker M, Klein S, Merz H, Diehl V. Activation of cytokines in Hodgkin´s disease. Ann Oncol 1992,3(suppl 4):13-16.

Tóth J, Dwórak O, Súgar J. Eosinophil predominance in Hodgkin´s disease. Z Krebsforsch 1977,89:107-111.

Tubiana M, Henry-Amar M, van der Werf-Messing B, Henry J, Abbatucci J, Burgers M, Hayat M, Somers R, Laugier A, Carde P. A multivariate analysis of prognostic factors in early stage Hodgkin´s disease. Int J Radiation Oncology Biol Phys 1985;11:23-30.

Tucker MA, Coleman CN, Cox RS et al. Risk of second cancers after treatment for Hodgkin´s disease. N Eng J Med 1990;311:7-13.

Tullgren O, Grimfors G, Holm G, Johansson B, Svedmyr E, Wedelin C, Mellstedt H, Merk K, Björkholm M. Lymphocyte abnormalities predicting a poor prognosis in Hodgkin´s disease. Cancer 1991;68(4):768-775.

Urba WJ, Longo DL. Hodgkin´s disease. Review article. N Eng J Med 1992;326:678-687.

Walker A, Schoenfeld ER, Lowman JT, Metlin CJ, MacMillan J, Grufferman S. Survival of the older patient compared with the younger patient with Hodgkin´s disease. Cancer 1990;65:1635-1640.

Van Rijswijk REN, de Meijer AJ, Sybesma B, Kater L. Five-year survival in Hodgkin´s disease. The prospective value of immune status at diagnosis. Cancer 1986;57:1489-1496.

Vaughan Hudson B, Mac Lennan KA, Easterling MJ, Jelliffe AM, Haybittle JL, Vaughan Hudson G. The prognostic significance of age in Hodgkin´s disease. Examination of 1500 patients (BNLI Report No. 23). Clin Rad 1983;34:503-506.

Vaughan Hudson B, Mac Lennan KA, Bennet MH, Easterling MJ, Vaughan Hudson G, Jelliffe A M. Systemic disturbance in Hodgkin´s disease and its relation to histopathology and prognosis (BNLI report No. 30). Clin Radiol 1987;38:257-261.
Vaughan Hudson B, Linch DC, MacIntyre EA, Bennet MH, MacLennan KA, Vaughan Hudson G, Jelliffe AM. Selective peripheral blood eosinophilia in Hodgkin´s disease (BNLI Report No 31). J Clin Pathol 1987;40:247-250.

Wedelin C, Björkholm M, Biberfeld P, Holm G, Johansson B, Mellstedt H. Prognostic factors in Hodgkin´s disease with special reference to age. Cancer 1984;53:1202-1208.

Weiss LM, Strickler JG, Warnke RA, Purtilo T, Sklar J. Epstein-Barr viral DNA in tissues of Hodgkin´s disease. Am J Pathol 1987:129:86-91.

Werner M, Georgii A, Bernhards J, Hübner K, Schwarze EW, Fischer R. Characterization of giant cells in Hodgkin´s lymphomas by immunohistochemistry applied to randomly collected diagnostic biopsies from the German Hodgkin trial. Hematol Oncol 1990;8:241-250.

West WH, Tauer KW, Yannelli JR, Marshall GD, Orr DW, Thurman GB, Oldham RK. Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Eng J Med 1987;316:898-905.

Vestlev PM, Pallesen G, Sandvej K, Hamilton-Dutoit SJ, Bendtzen SM. Prognosis od Hodgkin´s disease is not influenced by Epstein-Barrvirus latent membrane protein. Int J Cancer 1992;51:1-3.

Vianna NJ, Polan AK. Epidemiologic evidence for transmission of Hodgkin´s disease. N Eng J Med 1973;10:499-502.

Vijayakumar S, Myrianthopoulos LC. An updated dose-response analysis in Hodgkin´s disease. Radiother Oncol 1992;24:1-13.

Wijlhuizen TJ, Vrints LW, Jairam R, Breed WPM, Wijnen TM, Bosch LJ, Crommelin MA, van Dam FE, de Konig J, Verhagen-Teulings M. Grades of nodular sclerosis (NSI-NSII) in Hodgkin´s disease. Cancer 1989;63:1150-1153.

Wilks Sir S. Cases of enlargement of the lymphatic glands and spleen, (or, Hodgkin´s disease), with remarks. Guy's Hosp Rep 1865;11-56-57.

Viviani S, Santoro A, Ragni G, Bunfante V, Bestetti O, Bonadonna G. Gonadal toxicity after combination chemotherapy for Hodgkin´s disease. Comparative results of MOPP vs ABVD. Eur J Cancer Clin Oncol 1985;21:601-605.

Wolf J, Diehl V. is Hodgkin´s disease an infectious disease? Ann Oncol 1994;5 (suppl 1):105-111.

Zhou X-G, Hamilton-Dutoit S, Yan Q-H, Pallesen G. The association between Epstein-Barr virus and Chinese Hodgkin´s disease. Int J Cancer 1993;55:359-363.

Correspondense to:

Gunilla Enblad
Department of Oncology
University Hospital
S-751 85 Uppsala
Sweden