Prognostic factors in Hodgkin’s lymphoma

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Risk-adapted treatment strategies have constituted a major issue since the beginning of clinical research into Hodgkin’s disease (HD). Various prognostic factors have been identified and several of those considered for staging procedures, resulting in strictly stage-dependent treatment recommendations for patients suffering from HD. These factors may be subdivided in host-related (e.g. age, sex) and tumour-related (e.g. number of tumour cells, growth characteristics, spread of tumour cells, resistance to apoptosis) factors. Owing to the striking improvement of the overall prognosis in HD patients it may be difficult to identify novel prognostic factors analysing the minority of patients with a fatal outcome. However, especially in advanced-stage disease, improved treatment results were achieved by the introduction of more aggressive treatment regimens, resulting in an increased toxicity rate. Thus, partially in contrast to earlier work in this field, future prognostic factors are needed for identification of those patients that have a good prognosis and might be susceptible to overtreatment. During the Fifth International Symposium on Hodgkin’s Lymphoma, promising results on several new prognostic markers were presented. Furthermore, a joint effort to design new studies on large, well characterised patient groups has been initiated.

Key words: Hodgkin’s lymphoma, prognostic factors, risk, treatment strategies

Introduction

Prognosis of patients with Hodgkin’s lymphoma (HL) has been impressively improved during recent decades. While until the 1960s only a minority of patients (i.e. patients with localised disease treated with radiotherapy) could be cured at all, the introduction of the mechlorethamine, vincristine, procarbazine, prednisone (MOPP) polychemotherapy regimen by de Vita and coworkers at the National Cancer Institute (NCI) and later the ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) regimen by Bonadonna and the Milan group resulted in a long-term survival of 66%, even including patients with stage IV disease [1, 2]. Simultaneously, improvement of radiotherapy techniques (i.e. megavoltage irradiation, linear accelerator) led to a consistent cure rate of 90% and more in patients with early and intermediate stage (i.e. early stage unfavourable) patients who received combined-modality treatment. Recently, the introduction of novel dose-escalated regimens like bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone (BEACOPP) resulted in improved survival of patients with advanced-stage disease indicated by 3-year survival rates of up to 92% [3].

Since the early days of HL treatment, therapeutic decisions have been made by subdividing this entity into different prognostic subgroups. Certainly, the Ann Arbor classification [4], based on the anatomical extent and the tumour activity (B-symptoms), had an enormous clinical impact, even though many further prognostic factors have been identified. Prognostic factors are defined as variables measured in patients that aid explanation of the heterogeneity of outcome and might predict, with more or less accuracy, the clinical outcome of a single patient. Furthermore, they may help the understanding of the biological features of the tumour. From a theoretical point of view the prognosis of a patient is influenced by the interaction between tumour (tumour-related factors) and ‘host’ (patient-related characteristics) [5]. Therefore, critical parameters are the amount of tumour cells as well as their specific characteristics (e.g. growth characteristics, chemoresistance, immune escape, resistance to apoptosis) and the ability of the ‘host’ to eliminate remaining tumour cells (e.g. immunocompetence) and tolerate therapeutic regimens (e.g. performance status, susceptibility to acute toxicity and late toxicity such as secondary malignancies). Prognostic factors reflect these interactions in a better or worse way.

In this review we want to summarise the data on prognostic factors in HL, emphasising their great impact on the improvement of prognosis but also showing their limits due to the outstanding overall survival (OS) rates of this entity today. In light of the price of high cure rates characterised by overtreatment of many patients, especially in advanced-stage HD, the importance of defining good prognosis in contrast to earlier approaches defining poor prognosis might gain more importance.
**Amount of tumour cells**

Different measurable parameters describe the dissemination and number of tumour cells. Each therapy intended to kill tumour cells reduces these cells in a given proportion [6]. Therefore, the total number of tumour cells is one parameter to predict the effect of an anti-neoplastic therapy. Furthermore, the growing fraction, which is vulnerable to chemo- or radiotherapy, is smaller in large tumours [7]. Bulky mediastinal disease describes the number of tumour cells in the mediastinum. Consensus exists on its prognostic value in early stage HD as demonstrated for disease-free survival (DFS) in different studies for patients treated with radiotherapy alone [8–12]. By comparison, in advanced-stage HD treated with more aggressive therapies there are less consistent data on the prognostic significance of mediastinal bulk [13–17]. Splenic involvement was shown to be of prognostic significance in a univariate analysis of patients treated with radiotherapy alone. But the statistical significance disappeared when performing multivariate analysis and after the introduction of multimodal therapy [18–21]. In non-Hodgkin’s lymphoma (NHL), lactate dehydrogenase (LDH) is a surrogate parameter of tumour mass and is an accepted prognostic factor [22]. An elevated serum LDH, which might reflect the total amount of tumour cells, has also been shown to be of prognostic value in HD [23].

To define the number of tumour cells more precisely, different methods were developed for measuring the size of macroscopically involved areas. In several studies analysing patients with different disease stages and treated with different therapies, the overwhelming prognostic relevance of the tumour burden was confirmed, as pointed out by Specht during the Fifth International Symposium on Hodgkin’s Lymphoma [24–33]. The combination of macroscopic measurement of the tumour with a microscopic estimation of the percentage of tumour cells might even increase the predictive value of tumour burden [34].

In the last decade, new parameters have been investigated to predict prognosis. CD30, a member of the tumour necrosis factor (TNF) family, although not specific for HD, is consistently expressed on Hodgkin’s and Reed–Sternberg (HRS) cells and the CD30/CD30 ligand interaction is believed to be crucial for the growth of HRS cells. In different studies, soluble CD30 had independent prognostic significance [35–37]. Data on 307 ABVD treated patients presented at the Fifth International Symposium on Hodgkin’s Lymphoma further underlined the independent prognostic value of this marker [38]. As soluble CD30 correlates with stage and tumour burden and is elevated in lymphocyte depleted HD [37, 39] it might correlate with the number of HRS cells and therefore predict clinical outcome.

**Spread of tumour cells**

In nearly all carcinomas spread of tumour cells equal to metastatic disease is the worst prognostic factor predicting the incurability of the patient. The biological mechanisms underlying metastasis are the independency of tumour cells from growth promoting stimuli in the microenvironment, the ability to circulate through the blood and lymph system and the invasive potential of these tumour cells [40]. Although different biological mechanisms might underlie the dissemination of carcinoma and malignant lymphoma cells, disseminated, i.e. stage III/IV, disease is also a negative prognostic factor in malignant lymphomas [41].

HRS cells seem to be strongly dependent on their microenvironment, since they are extremely difficult to grow in vitro. The majority of HD-derived cell lines were established from cells in peripheral blood, pleural effusion, pericardial effusion or bone marrow [42, 43] suggesting an in vivo adaptation to liquid phase cultivation conditions in the course of tumour progression. The Ann Arbor classification [4] describing the anatomic distribution and thus the dissemination of tumour cells has been demonstrated to be of prognostic relevance for DFS as well as for OS in several studies testing different therapeutic regimens [23, 41, 44–49]. Consequently the (modified) Ann Arbor classification is used in most therapeutic studies in HL to subdivide patients into different prognostic groups.

Different primary localisations of HD were shown to be of prognostic significance. Bone marrow involvement, for example, had adverse prognostic value in patients with advanced-stage HD treated with combination chemotherapy [50–52]. Other studies could not confirm these findings in previously untreated patients [47, 53]. The rare pleural involvement has also been shown to be of prognostic relevance in a mixed population of patients with HD (primary and relapsed HD) [1, 54]. Presentation of HD in the bone marrow as well as pleural involvement may reflect the independency of HRS cells from their physiological lymphoid microenvironment. Further localisations of HD were only analysed in a few studies and thus their prognostic value is questionable. Splenic involvement and extranodal disease is taken into account by the Ann Arbor classification, but overall impact on prognosis seems to be small [1, 47–49, 55, 56]. In summary, whereas the data on the prognostic value of the localisation of HD (bone marrow, inguinal involvement, pleural involvement) are controversially discussed and seem to have only little impact on prognosis, there is consensus about the pronounced prognostic impact of dissemination of HD according to the Ann Arbor classification.

**Growth characteristics**

The growth fraction of tumour cells can be assessed by staining the Ki-67 antigen. In several neoplasias Ki-67 has been shown to have a negative prognostic value [57, 58]. In addition, in HD the growth fraction of the malignant cells is of prognostic relevance [59–61]. Similar to Ki-67, proliferating cell nuclear antigen (PCNA) is a protein present only in prolif-
berating cells, which has been shown to be of prognostic value in some studies [62]. The retinoblastoma tumour suppressor gene is a protein interacting with the cell cycle influencing the prognosis of several neoplasias [63, 64]. Only a few studies have analysed retinoblastoma gene expression in HD [59, 65, 66]. In the study by Morente and colleagues a prognostic impact of Rb expression is pointed out [59]. P53 is a further protein that interacts with the cell cycle and often is mutated in malignant cells. Data on the prognostic role of p53 expression are controversial. A high index of p53 expressing cells has been reported to have negative impact on the outcome of patients suffering from HD [62]. Differing results were obtained by Xerri et al. [67], as summarised by Nieder and colleagues [68]. Analysis of soluble p53 did not show prognostic significance in HD [69] and mutations within the p53 gene are rare events, which do not play a major pathogenetic role and are not associated with EBV [70].

Resistance to apoptosis is a frequent characteristic of malignant cells. One of the proteins preventing cells from undergoing apoptosis is Bcl-2. Bcl-2 is expressed in a proportion of HRS cells. An elevated index of Bcl-2-positive cells was shown to have a negative prognostic impact [62, 71–73], which was confirmed on a large data set by Sarris and coworkers [38]. Van Spronsen further specified, that high amounts of Bcl-2-expressing HRS cells surrounded by a low percentage of Bcl-2-expressing T cells is associated with clinical prognosis [74]. Smolewski and colleagues further measured apoptotic cells by TUNEL staining, revealing a positive prognostic value of a high frequency of apoptotic HRS cells [71]. The proapoptotic Bax protein is expressed in HRS cells [75], but no consistent data about its prognostic relevance exist [38]. Caspase-3 is a downstream element of the apoptotic cascade. As Dukers and coworkers showed at the Fifth International Symposium on Hodgkin’s Lymphoma, a high amount of caspase-3-positive HRS cells predict a favourable clinical outcome of these patients [76]. About 50% of HD cases are Epstein–Barr virus (EBV) positive. LMP-1, an EBV-encoded protein, is another protein that interacts in the apoptotic cascade. In several studies LMP-1 expression was shown to influence the prognosis of HD [59, 61, 77–79], but differing results were obtained in other study groups [80].

Summarising these data one may conclude that differences between the growth characteristics of HRS cells exist. A high proportion of proliferating HRS cells and a small number of apoptotic HRS cells or HRS cells susceptible to apoptosis seem to have a negative prognostic impact. However, data were collected in relatively small retrospective analyses and the comparisons between different studies are difficult due to differences in the populations analysed, the technical procedures and the clinical data presented.

Interaction of tumour and host
Interaction between host and tumour cells is reflected by the unique pathomorphological appearance of HD. The HRS cells are surrounded by a large number of non-malignant cells. Since the early days of HD research, pathologists have searched for morphological characteristics to describe HD and to categorise patients into prognostic groups. The classification established in the 1960s by Lukes and Butler [81] was confirmed in many studies [16, 21, 46, 82–84]. In a large data set of the international database on HD, Gobbi and colleagues demonstrated the prognostic value of histology stressing the unfavourable prognosis of patients with lymphocyte depleted HD [85]. Similarly a negative prognostic value was demonstrated for the lymphocyte depleted subtype in different studies [40, 86], but the number of patients suffering from this subtype of HD is very small and even decreasing in recent years. When comparing the nodular sclerosis (NS) and the mixed cellularity (MC) subtype, respectively, with lymphocyte predominant HD (LPHD), histiotype added significant prognostic value to tumour burden, the main prognostic factor in the cited study [51]. The different biological behaviour of LPHD has been considered in the new Revised European–American Lymphoma (REAL) Classification opposing LPHD to classical HD (CHD) [87]. As up to 70% of the study populations suffer from NS subtype, different efforts were made to subdivide this subgroup. The British National Lymphoma Investigation (BNLI) group categorised the NS type in two subgroups, of which the NSII subtype with areas depleted from lymphocytes and a large number of pleomorphic HRS cells was associated with a decreased survival independently of stage [88, 89]. However, other groups could not confirm this prognostic difference [90]. At the Fifth International Symposium on Hodgkin’s Lymphoma a further subclassification of NS histology was suggested using CD15, tissue eosinophilia, lymphocytic depletion, extended necrosis and atypical morphology of HRS cells as parameters [91].

Some groups focused on the non-malignant cells in HD for the establishment of prognostic factors. The HRS cells are surrounded by a high number of CD4+ T cells. Oudejans and coworkers analysed CD8 granzyme B-positive T cells, demonstrating the negative prognostic impact of these cells [92]. Tissue eosinophilia was also shown to influence prognosis of HL in a stage stratified model [93]. During the Fifth International Symposium on Hodgkin’s Lymphoma, Molin and coworkers presented data on the prognostic value of mast cells present in the affected lymph nodes [94]. Despite these retrospective analyses the predictive value of the histopathological subtype is not pronounced enough to include it into therapeutic decisions. Thus, in the current clinical trials of nearly all major study groups, stage-dependent therapeutic strategies are being clinically tested regardless of the histological subtype. A pathological grading with major prognostic impact like in solid tumours has not been established.

B-symptoms have been known to be of clinical importance for many years, and thus have been included in the Ann Arbor classification and were confirmed in several studies [1, 8, 52, 56, 84, 85, 95–99]. B-symptoms correlate with other parameters used to predict prognosis, e.g. stage, erythrocyte
sedimentation rate (ESR) [27, 41, 56, 99], serum albumin [38, 41, 85] and haemoglobin [41]. All these parameters may have their biological correlate in the spectrum and amount of cytokines secreted by the tumour cells and the surrounding lymphoid infiltrate. Specht pointed out that there is an inverse relationship between accuracy of tumour mass assessment and prognostic value of B-symptoms [44]. This observation stresses once more the great impact of the amount of tumours cells compared with a rather weak impact of the characteristics of the tumour cells in this special neoplasia. Several further attempts were made to assess the interaction between tumour cells and the host, for example, which is thought to be the result of a complex cytokine profile expressed by the host and the tumour cells, was shown to have independent prognostic significance for DFS and OS for different stages and several therapeutic regimens [83, 100]. Albumin [41, 101, 102], haemoglobin [23, 41, 103, 104], leukocytosis [41] and lymphocytopenia [26, 86, 103] are further parameters describing the effect of cytokines secreted by malignant and non-malignant reactive cells. Many further markers were correlated with prognosis. Factors like ferritin [105–107], coeruleoplasmin, serum copper [107, 108], β2-microglobulin [32, 109–111] and thymidin kinase [112] may also reflect the inflammatory re-action of the host.

VCAM-1 and ICAM-1 are adhesion molecules from the immunoglobulin gene superfamily. Soluble forms of these factors were analysed in HL by Christiansen and colleagues and prognostic significance was shown [36, 113]. Soluble VCAM-1 and ICAM-1 may help HRS cells to escape the immune system by competitively inhibiting the function of ligand receptors [114, 115]. HRS cells are surrounded by multiple lymphoid cells. An intensive interaction between these predominantly T-helper (Th) 2 cells and HRS cells is reflected by multiple cytokines and chemokines secreted by both cell types. Several studies were performed measuring these cytokines [interleukin (IL)-2r, TNFr, TNFα, IL2Rα, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13] and correlating them with clinical outcome of patients [69, 116–121]. IL-10 is one of these cytokines produced by HRS cells and able to suppress a Th1 proliferation, which turned out to be of prognostic significance in advanced-stage HD [35, 120, 122, 123], which was confirmed by data on 307 ABVD-treated patients presented by Sarris and coworkers at the Fifth International Symposium on Hodgkin’s Lymphoma [38]. IL-10 was further shown to be much more prominent in the EBV-associated cases [124]. A further marker, which was analysed in several studies, is sCD25, the soluble IL-2 receptor and its correlation to clinical stage and prognosis was shown [36, 125–129]. Inconsistent data exist about further cytokines and their prognostic value. Primary results on cytokines in HD are promising but further studies need to be conducted in a randomised, multicentre setting with more patients to prove the additive prognostic value of these parameters.

**Patient characteristics**

Age is not only a risk factor in HD. Several aspects have to be taken into account when analysing age as a biological risk factor. First, increased morbidity and mortality in older patients due to other causes has to be considered and results have to be matched to the general population. Secondly, increased co-morbidity results in augmented therapy-related morbidity (acute toxicity as well as late adverse effects as cardiac disease and secondary neoplasia) [130, 131]. Therefore, taking co-morbidity into account when analysing age as a prognostic factor is of major interest [132]. Nevertheless, in studies matching patients to the general population, considering additional prognostic factors and performing multivariate analysis age has been shown to have adverse prognostic impact for different stages and therapies [46, 84, 88, 133–136]. Reduced capacity of the immune system in older age has been hypothesised to play a role for the worse outcome of these patients.

Sex is a further well known but pathophysiologically not well understood prognostic factor [10, 27, 41, 46, 83, 88]. Difference in HD between males and females is not only a reflection of the predominance of NS histology, which may explain part of the better prognosis in female patients, as convincingly demonstrated by Specht [44].

**Perspectives**

Several factors have been identified as prognostic factors in HL. However, in view of the excellent prognosis of the majority of HD patients, prognosis for patients with stage adapted modern therapy is nearly equal for each clinical stage as demonstrated by Hasenclever [137]. This observation retrospectively underlines the great importance of the ‘classical’ prognostic factors that have already been included into staging procedures. Concerning future attempts to identify new prognostic factors some difficulties may arise. As measurable events such as treatment failure and death of HD become rare, an enormous number of patients has to be analysed in prospective studies for the establishment of new prognostic factors. To circumvent this problem, retrospective material from patients treated with less aggressive therapies might be examined. New information, however, will only be obtained if the markers studied will stratify patients better than the existing prognostic scores. Similar conclusions were drawn by Fannitto, Henry-Amar and colleagues after a comparison of different prognostic scores [138, 139]. They confirmed the need for the improvement of existing prognostic scores while assuming that single new prognostic factors will have little clinical impact.

To overcome the problem of the low numbers of patients in prognostic factor studies the combined effort of all interested researchers is crucial. Therefore, at the Fifth International Symposium on Hodgkin’s Lymphoma great interest was expressed in planning joint studies to create large data sets that would allow further refinement of existing prognostic factors.
The importance of prospectively collecting biological material in ongoing and planned clinical studies was underlined. Furthermore, a large consensus existed about the importance of defining risk factors for late therapy-related adverse events, especially therapy-induced leukemia.

References


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