Short thesis for the degree of Doctor of Philosophy (PhD)

Observations in Hodgkin lymphoma: the clinical role of late relapse and autoimmune cytopenias

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Debrecen, 2022

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The PhD defense will be held on 23 June 2022 at 10 a.m.

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1. Introduction

Hodgkin's lymphoma (HL) is a malignant lymphoproliferative disorder of monoclonal B-cell origin that accounts for 1% of all cancers worldwide.

HL can be divided into two distinct groups, both in clinical behavior and pathogenesis. Classical HL (cHL) comprises four histological subtypes with CD30 and/or CD15 antigen positivity. In contrast, the nodular lymphocyte-predominant form (NLPHL) carries surface markers (CD19, CD20) characteristic of B cells.

HL is characterized by a low number and proportion of tumor cells in the tumor tissue (about 1%). The niche microenvironment maintained by the high levels of cytokines and chemokines mediated signaling pathways produced by immune cells in tumor tissue contributes to HL's specific clinical features and the associated diverse immunological abnormalities such as autoimmune cytopenias (AICP).

With the modern risk- and response-adapted treatment methods, up to 80-90% of patients achieve a complete remission with the treatment modalities used. However, the management of primary refractory and relapsed (R/R HL) patients remains a significant challenge. In addition, a distinct group is those who experience late relapse (>5 years after diagnosis) (LR-HL).

Our study evaluated the experience gained during the management of HL patients in our department and the clinical characteristics of late relapses and HL-associated autoimmune cytopenias (HL-AICP) occurring in the past decades.

2. Literature review

2.1. Pathogenesis

The germinal B-cell origin of the distinctive tumor cells of HL is well established, but the etiology of the disease is not clearly understood. The pathogenesis of HL may result from genetic, immunological, and environmental factors operating individually or in combination. This includes defects in autocrine and paracrine signaling and inhibition of the anti-tumor immune response, a phenomenon known as immune escape.

The malignant cells of HL rarely have chromosome-level cytogenetic abnormalities. However, chromosome arm amplifications (2p, 9p, 16p, 17q) and deletions (13q, 6q, 11q) occur, and gain of function (REL, JAK2, STAT6) or loss of function (NFKB, TNFA, TP53)

gene mutations are common, which contribute to the atypical morphology, also altered growth, differentiation, and survival of tumor cells.

Chromosome 9p24.1 amplification is of particular importance in cHL, leading to overexpression of programmed cell death (PD-1) ligands on the surface of Hodgkin and Reed-Sternberg (HRS) cells. The PD-1-regulated signaling pathway is responsible for regulating the over-activation of T-cells during the normal immune response and for inhibiting autoimmune diseases, and preventing excessive allergic reactions. Of the two ligands of the PD-1 receptor (PD-L1 and PD-L2), PD-L1 is highly expressed on HRS cells. The binding of the ligand to the PD-1 receptor activates signaling pathways that inhibit T-cell activation and proliferation, causing a transient inhibition of the immune response. Overall, overexpression of PD-1 ligands on the surface of HRS cells promotes the formation of a microenvironment that leads to the depletion of cytotoxic T-cells, thereby contributing to HRS cell survival. Autocrine and paracrine cytokines and chemokines produced by tumor cells also contribute to the establishment of this environment. Interleukin (IL)- 13 and IL-4 also contribute to tumorigenesis by causing constitutive activation of the nuclear factor κB (NFκB) pathway, facilitating tumor cell proliferation, reducing apoptosis, and inducing the expression of cytokines that mediate immune cell migration into the microenvironment. Twin studies also demonstrate endogenous causes, in which the probability of HL in monozygotic twins was 100-fold higher than the risk in dizygotic twins.

The cellular immune deficiency and immune dysregulation observed in HL patients highlight the importance of immunological factors. According to the literature, HL is more common in various inherited (autoimmune lymphoproliferative syndrome) and acquired (AIDS, systemic autoimmune diseases) immunodeficiency conditions.

Epstein-Barr virus (EBV) is a notable environmental factor in the pathogenesis of HL. This ubiquitous pathogen infects nearly 90% of the population worldwide during their lifetime. Its role in the pathogenesis of HL is supported by the viral genome observed in HRS cells, and the presence of EBV-specific latent membrane proteins (LMP1 and LMP2) expressed by them. Following primary infection, proteins transcribed from viral genes can induce malignant transformation of resting B-cells by regulating the NFκB signaling pathway. Moreover, EBV infection enhances the expression of the aforementioned PD-L1 ligands on HRS cells, resulting in reduced T-cell activation and proliferation, contributing to the maintenance of the immune-escape phenomenon.

2.2. Histological subtypes

The 2016 World Health Organization (WHO) lymphoma classification continues to separate HL into two subgroups, classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), which are different in both clinical behavior and pathogenesis. cHL can be further subdivided into four histological subgroups, mixed cell (cMC), nodular sclerosis (cNS), lymphocyte-poor (cLD), and lymphocyte-rich (cLR) histological variants.

The microscopic pattern of cHL is characterized by the presence of apoptosis-resistant, mononuclear HRS cells, which constitute only 1-2% of the infiltrated lymph node, and an abundant reactive cellular background of T- and B-lymphocytes, histiocytes, eosinophils, and plasma cells. HRS cells are derived from B-lymphocytes of germinal central origin, which undergo a transformation during maturation, loss of normal B-cell phenotype, and express the predominant immunoglobulins and transcription factors while displaying an abnormal set of cell surface markers and cytokines regulating their transcription. HRS cells do not correspond to any identified stage of normal B cell development. Only 20-40% of them show CD20 positivity, CD79a expression being even more scarce. In contrast, CD30 and/or CD15 antigen positivity is frequently observed, and their clonally rearranged immunoglobulin genes are inactive.

The tumor cells of the NLPHL subtype are known as "popcorn" or lymphocyte/histiocyte (L&H) cells. They express B-cell markers (CD19, CD20, CD79a); their immunoglobulin genes are intact and functional due to an ongoing somatic mutation; therefore, they are under continuous antigen selection activity.

2.3. Clinical features

The incidence of HL is 2-5/100 000 person/year, and the incidence in Hungary is average (2-3/100 000 person/year). The older population in developing countries is characterized by a unimodal age distribution with a peak in young adulthood (20-29 years) and a high incidence of the cMC subtype. On the other hand, developed industrialized countries are characterized by a bimodal distribution curve with peaks in young adulthood (20-29 years) and middle age (50-59 years) and a high predominance of the cNS subtype.

Most patients present to a physician with painless lymph node enlargement, nearly half of them being free of symptoms. Common accompanying symptoms include fever, night

sweats, and weight loss of more than 10% of body weight, collectively known as "B-symptoms". Laboratory abnormalities include increased erythrocyte sedimentation rate, leukocytosis, lymphopenia, anaemia, thrombocytopenia, hypalbuminaemia, and elevated LDH. The most common causes of the observed haematological abnormalities are insufficient haematopoiesis due to bone marrow infiltration of the underlying disease and AICPs as a complication of lymphoproliferation and immunological abnormalities.

The AICPs, namely autoimmune haemolytic anaemia (AIHA), autoimmune thrombocytopenia (AITP), and autoimmune neutropenia (AINP), often accompany the clinical course of lymphoproliferative disorders. According to previous data, the association of AICPs with HL is a rare complication of the disease, occurring in 0.5-4.2% of patients and is triggered either by paraneoplastic cytokine release or by an autoimmune response against the blood's cellular elements and consequent autoantibody production. These events are considered the pathological consequences of a persistent and prominent relationship between HL and immune cells.

2.4. Diagnosis

The diagnosis of HL is based on a comprehensive pathological evaluation of a sample from a lymph node or other affected tissue through morphological, immunohistochemical, and in some cases, molecular genetic analysis. Percutaneous core biopsy may be an alternative procedure if the sample contains a sufficiently large tissue sample. Histological diagnosis of HL is based on the detection of HRS or L&H cells and the presence of a microenvironment composed of representative microenvironmental cells. In immunohistochemical studies, CD15, CD20, CD30, and EBER are the preferred markers.

The diagnosis of HL should be followed by standard staging and full-body 18-fluorodeoxyglucose positron emission tomography (^{18}FDG-PET/CT). Staging also plays a crucial role in measuring the subsequent response to therapy by providing a basis for comparison with interim and restaging studies, thereby improving the accuracy of response-adapted treatment of HL.

For staging after histological diagnosis, we use the modified Ann Arbor, or Lugano, classification. In addition to lymph node involvement, the absence or presence of B-symptoms and a large ("bulky") tumor mass, defined as a lymph node conglomerate of more than one-third of the diameter of the thoracic diameter or 10 cm in diameter in the mediastinum, are further classification criteria.

Treatment strategy planning for HL patients is based on staging and the presence or absence of specific clinical prognostic factors, distinguishing between early favorable, early unfavorable, and advanced-stage HL. In advanced-stage HL, the International Prognostic Score (IPS), which identifies seven independent factors that additively predict failure-free survival (FFS), can be used to assess risk further. These markers indirectly predict the biological status of patients and their disease activity. These features are age over 45 years, male sex, stage IV disease, anaemia (haemoglobin <105 g/L), leucocytosis (white blood cell count $\geq 15x10^9$/L), lymphocytopenia (absolute lymphocyte count $<0.6x10^9$/L or relative lymphocyte count <8%), and hypalbuminaemia. The presence of each risk factor reduced the FFS rate by 7-8%, and IPS reliably identified a group of patients with a long-term FFS of less than 50%.

Nowadays, the prognostic role of different biological markers (thymus and activation-regulated chemokine - TARC, circulating tumor DNA - ctDNA) is a major focus of attention. However, none of these markers has presented convincing evidence to be considered a routine tool in managing HL.

Today, early ^{18}FDG-PET/CT is considered the gold-standard method for estimating long-term response to treatment. The efficacy of therapy can be assessed by evaluating the scan after the second cycle of chemotherapy (interim) or after the completion of treatment (restaging). The Deauville score and the Cheson criteria can be used for this purpose. ^{18}FDG-PET/CT has an excellent negative predictive value (above 90%), so that in the case of a negative interim or restaging test, the treatment used is considered effective. However, the positive predictive value is lower (50-70%), in which case clinical evaluation and, if necessary, additional testing methods (e.g., repeated histological sampling) may be necessary.

2.5. Treatment

If the general condition of patients diagnosed with HL allows, the disease is always treated with curative intent. Surgical treatment is not an option for systemic disease, and polychemotherapy and radiotherapy are the cornerstones of HL care, complemented by immunotherapy. The modality chosen is directed by the histological subtype, the stage of the disease, and prognostic factors, which the age, general condition, comorbidities, and individual preference of the patients may modify.

The current practice guidelines in our country recommend 2-4 cycles of combined chemotherapy with ABVD (combination of doxorubicin, bleomycin, vinblastine, and dacarbazine) adjuvant irradiation (20-30 Gy total dose) in early, favorable-stage cHL. In the

early, unfavorable stage, 4-6 cycles of ABVD and, if necessary, irradiation of the affected field at a total dose of 30 Gy should be chosen.

In advanced cHL, six cycles of ABVD and radiotherapy to the residuals seen on restaging ^{18}FDG-PET/CT are required. In young patients with poor prognostic markers (IPS $\geq$ 4), the BEACOPP protocol (combination of bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) followed by irradiation can also be chosen. Although BEACOPP treatment may offer a better progression-free survival (PFS) than ABVD, it requires caution and longer follow-up due to its late toxicity, especially regarding the occurrence of secondary malignancies.

In early-stage NLPHL, radiotherapy alone is sufficient if the prognosis is favorable, but treatment similar to the approach used in cHL is needed in other cases.

Treatment of primary refractory and relapsed patients remains a significant challenge. Recurrence of HL is most common within one year of first-line treatment, with the vast majority of relapses occurring in the first three years. Intermediate-dose salvage chemotherapy is recommended for these patients, followed by autologous hematopoietic stem cell transplantation (AHSCT). In case of inadequate response to classical chemotherapeutic protocols, biological therapies, such as monoclonal antibody-drug conjugate against CD30, brentuximab vedotin (BV), or PD1 inhibitor treatment (nivolumab or pembrolizumab) are available. Both BV and pembrolizumab can be used in pretreated patients, for remission induction, before AHSCT, or in patients unsuitable for transplantation, also in post-transplant relapse. In the latter indication, nivolumab is also available.

Late relapse of HL, defined as relapse at least five years after initial diagnosis, is rare but clinically significant. Contrary to the many observations available on early relapse of HL, few cases of late relapse have been comprehensively documented and analyzed in the literature. The majority of the information available is in the form of individual case reports.

The incidence of LR-HL ranges from 3-8%, according to previous reports. Analyses by EORTC and GHSG concordantly state that HL survivors receiving curative therapy have an 84.5-150-fold increased risk of disease recurrence compared to age- and sex-matched healthy population. Clonality analysis and EBV genome persistence in tumor cells in LR-HL also suggest that late disease recurrence is a relapse of prior HL. Nevertheless, the possibility that a second HL, completely unrelated in clonality to the first one, may develop in the lifetime of an individual affected by predisposing genetic, lifestyle, and environmental risk factors.

The early identification of patients susceptible to late relapse is not yet resolved. There are no known clinical or laboratory parameters that can be considered as definite risk factors for the subsequent development of LR-HL, or that can be used to predict the possible outcome in cases of late relapse. Previous studies have suggested that male sex, age, B-symptoms, and mediastinal involvement at the initial diagnosis may increase the risk of developing LR-HL.

As patients with HL now represent a highly manageable population, research is focused on novel therapeutic options, improving quality of life, and avoiding overtreatment and late toxicities. A critical step in preventing the latter could be identifying patients who require more aggressive treatment at the time of diagnosis and selecting those who can be protected from late complications. The identification of new clinicopathological parameters with prognostic values can be of help in this area.

3. Aims

1. To assess the clinicopathological characteristics (age, sex, histology, stage, general symptoms, comorbidities) of HL patients treated since 1981, to compare the therapeutic options (chemotherapy protocols, type, and modality of irradiation) used in their treatment, and to summarize the complications in their care through retrospective data collection.

2. To understand the clinical features of late relapses in patients with HL, investigate the risk factors of developing and treatment options for the management of LR-HL.

3. To investigate the prevalence of HL-associated AICPs and to compare the clinical characteristics of HL-AICP patients with the HL population, to gain a better understanding of the role of time of onset of cytopenias on the clinical course, and to assess the predisposing factors for the development of HL-AICPs in HL patients in a retrospective To understand

4. To understand the prognosis of late relapse of HL and autoimmune cytopenias associated with HL and to statistically analyze the expected relapse-free and overall survival.

4. Patients and methods

We retrospectively analyzed the demographic data and clinical characteristics of patients with HL, diagnosed and treated at the University of Debrecen, Department of Internal Medicine, between January 1, 1981, and December 31, 2017. The diagnosis of HL was based on histological sampling in all cases, and the stage and histological subtype of the disease were both according to the valid practice guidelines at the time of diagnosis. We assessed the age, sex, presence or absence of B-symptoms, treatment modality (radiotherapy, chemotherapy, combined radio-chemotherapy), and type of chemotherapy and radiotherapy used. The time from diagnosis to the end of treatment, the time of relapse, and the clinical parameters observed at the time of relapse were recorded. Demographic and clinical data were obtained from patient record databases. Our studies were approved by the Research Ethics Committee of the University of Debrecen (DE RKEB / 4881-2017).

The treatment modalities chosen were in accordance with the therapeutic guidelines in force at the time of diagnosis.

Late relapse was defined as relapse ≥5 years after primary diagnosis of HL. To better understand the prevalence of LR-HL, we examined all patients who received first-line treatment and remained in remission for 5 years after initial diagnosis. HL patients in sustained remission and relapsing within 5 years of the first diagnosis were used as a basis for comparison in terms of outcome.

AIHA was diagnosed on the basis of anaemia (haemoglobin levels below 130 g/l in men and below 120 g/l in women) and laboratory evidence of haemolysis (Coombs test positivity, elevated absolute reticulocyte count, elevated lactate dehydrogenase level, elevated indirect bilirubin level, and reduced serum haptoglobin level). The diagnosis of exclusion of AITP was made in patients with platelet counts below 100 G/L, in whom other causes of thrombocytopenia could be reliably excluded. The criteria for diagnosing AINP were an absolute neutrophil count below 1.5 G/L, exclusion of other clinical causes, and anti-neutrophil antibody testing if necessary. Other conditions associated with anaemia, thrombocytopenia, and neutropenia were excluded. All patients with HL had routine complete blood count and serum clinical chemistry analysis at the time of diagnosis, before treatment cycles, and during follow-up. Additional tests (Coombs test, serum haptoglobin level, absolute reticulocyte count, antibody levels, bone marrow biopsy) were performed in case of adequate clinical suspicion.

We determined the frequency, clinical significance, and response to treatment of AICPs associated with HL and compared the clinicopathological characteristics of affected patients with the vast majority of the HL patient population without a history of AICPs. We also compared patients with AICP at diagnosis of HL with those who developed AICP during treatment and follow-up. AICP was considered clinically significant if it led to a diagnosis of HL, indicated disease progression or relapse, or the development of a second malignancy.

Overall survival (OS) was defined as the time between the date of histological diagnosis of HL and the last presentation or the date of death. PFS was calculated based on the number of days between diagnosis and confirmation of progression or relapse.

Statistical analysis was performed using SPSS 22.0 and 25.0 computer software. Fisher's exact test and Chi-square test were performed to detect differences between discrete variables, and two-sample t-test and Mann-Whitney test were used to compare groups of continuous variables. Receiver Operating Characteristic (ROC) analysis determined the optimal cut-off values, and the relationships between variables were examined by linear and logistic regression. Survival times were compared using Kaplan-Meier analysis and log-rank test. Results were considered significant at $p<0.05$.

5. Results

5.1. Late relapse of Hodgkin lymphoma

To investigate late relapse of HL, data from patients diagnosed between 1981 and 2010 at the University of Debrecen, Department of Internal Medicine were processed. The majority of the 637 patients investigated were male (54%) and presented with cMC histological subtype (45%). Two hundred and fifty patients (39%) had early-stage disease, and 361 patients (57%) had the advanced-stage disease (staging was not available for 26 patients). Five hundred and eighty-four (91%) patients were in complete remission after first-line treatment. During a median follow-up of 9.08 years, 176 (28%) patients relapsed, of which 26 (4%) relapsed ≥5 years after primary diagnosis. The cumulative incidence of LR-HL at 10 and 15 years after diagnosis was 4.9% and 5.3%, respectively.

The median age of LR-HL patients at the time of primary diagnosis was 23 (11-58) years. In their case, the median time to late relapse was 9 (5-22) years. At the time of primary diagnosis, the majority of patients had advanced-stage disease (54%), histological subtype of

cMC (42%), with few (21%) presenting with B-symptoms. Nine (34.5%) patients received only radiotherapy as first-line treatment, with 2 cases of total-nodal field, six extended-field, and one case of affected-field irradiation.

Eleven (43%) LR-HL patients changed from the baseline histological subtype at the time of relapse. From 15 (57%) patients with identical histological subtypes at the time of LR-HL, the relapse location also coincided in 8 (31%) patients.

A strong correlation was found between the age of the patients at diagnosis and their susceptibility to late relapse. Using ROC analysis, a cut-off point at the age of 24 was defined, dividing HL patients into two groups regarding the time of relapse. The 143 (23%) patients who were adolescents or young adults at diagnosis had a higher incidence of LR-HL than those diagnosed with HL after the age of 24 years (493 patients - 77%) ($p<0.001$). The frequency of relapses did not differ between the two age groups ($p=0.102$), but the time to relapse was significantly prolonged in the under-24 age group ($p=0.002$).

The era in which the diagnosis of HL was made influenced the risk of developing LR-HL. Among HL patients diagnosed and treated between 1981-1990 and 1990-2000, the odds of late relapse were 6.3-fold and 4.7-fold higher, respectively, compared to patients diagnosed and treated after 2000 ($p=0.004$ and $p=0.019$, respectively).

A higher proportion of LR-HL patients received radiotherapy alone during first-line treatment than those who relapsed early ($p=0.018$) or remained in complete remission ($p=0.001$).

Patients who relapsed early were more likely to have advanced-stage disease at diagnosis than those who went into sustained remission ($p<0.001$) or relapsed late ($p<0.026$). HL patients diagnosed with advanced-stage disease had a one-third risk of relapsing late, compared to experiencing early relapse ($p=0.017$). Similarly, HL patients with B-symptoms at the time of the first diagnosis had a higher rate of early relapse than late relapse ($p=0.015$). Neither advanced stage disease nor a diagnosis of HL with B-symptoms was associated with an increased risk of late relapse compared to achieving durable remission.

Multivariate analysis confirmed the age less than 24 years at first diagnosis ($p<0.001$), first detection between 1981-1990 and 1991-2000 ($p=0.025$ and $p=0.023$, respectively), and first-line treatment with radiotherapy alone ($p=0.034$) as independent risk factors for developing LR-HL.

In subgroup analysis, we investigated how the presence of factors potentially predisposing to LR-HL at the time of primary diagnosis influences the outcome of late relapse. Age of fewer than 24 years ($p=0.027$) and absence of B-symptoms ($p=0.004$) positively affected overall survival.

Overall, the 5-year overall survival of early relapsed HL patients was significantly lower compared to patients with late relapse and persistent remission ($p<0.001$). Conversely, the 5-year overall survival of late-relapsing HL patients was not significantly different from the long-term disease-free population.

5.2. Association of Hodgkin lymphoma and autoimmune cytopenias

Based on the quality and detail of the available data, patients diagnosed with HL between January 1, 1990, and December 31, 2017, at the University of Debrecen, Department of Internal Medicine were included in the study of HL associated autoimmune cytopenias. During the period under review, a total of 563 patients with HL started first-line treatment, among whom a total of 8 AIHA and 8 AITP were confirmed, and no AINP occurred. AIHA and AITP developed simultaneously in one patient, i.e., a diagnosis of Evans syndrome was established. In total, 15 autoimmune cytopenic events were recorded in 14 patients with HL.

In two (13%) cases, the onset of AICP preceded the diagnosis of HL by 3 and 19 months, respectively. In both cases, the evaluation of AICP led to the diagnosis of HL. In two (13%) additional cases, the presence of AICP was confirmed at the same time as the diagnosis of HL. In the remaining 559 patients, 11 additional AICPs developed during more than 5000 person-years of follow-up.

The incidence of AICPs associated with HL was 2.8%, with 0.71% of patients experiencing an AICP at diagnosis and 1.96% subsequently developing an AICP during follow-up. The cumulative incidence of HL-AICPs increased in a linear fashion at 5, 10, and 15 years after diagnosis. However, the incidence of clinically significant HL-AICPs reached a plateau 10 years after primary diagnosis.

Seven (46%) cases of AICPs (4 AIHA, 2 AITP, and 1 Evans syndrome) were clinically significant events. Five (33%) cytopenias led to a diagnosis of HL or indicated relapse of the underlying disease. Two (13%) additional events were associated with the development of a second malignancy during the follow-up of HL.

The median age of patients with HL-AICP at the time of HL diagnosis was significantly higher than the rest of the HL population (37.5 years and 33 years, respectively). In addition, compared to HL patients without AICP, both HL-AIHA patients and HL-AICP patients presented more frequently with advanced-stage disease ($p=0.010$ and $p<0.004$, respectively).

Four (26%) autoimmune events (2 AIHA, 1 AITP, and Evans syndrome) were recorded at HL diagnosis. Additional 5 AIHAs and 6 AITPs developed in 549 patients with HL during a median follow-up of 141 months. HL patients who developed AICP during follow-up had a higher proportion of advanced-stage disease at the time of primary diagnosis than patients in the HL population without AICP ($p=0.004$).

Ten (71%) patients with HL-AICP went into complete remission after first-line treatment, two (14%) of them relapsed later. Two patients underwent AHSCT for primary refractory HL, and one patient underwent AHSCT in the second complete remission after relapse. Ten (71%) patients were still in remission at the end of the analysis, after a median follow-up of 171.5 (91-252) months. Three (21%) patients with HL-AICPs were lost due to progression or complication of the underlying disease, and one (7%) was lost due to unrelated causes of HL.

The 5-year OS and PFS did not differ significantly between HL-AICPs patients and the HL population without AICPs, but early (1-year) mortality was higher among HL-AICPs patients ($p<0.022$). The development of AIHA and AITP alone was not associated with a survival disadvantage compared to the overall HL population.

The 5-year expected overall survival of patients presenting with AICP at the time of primary diagnosis was inferior to that of HL patients with AICP during follow-up and without AICP ($p=0.005$ and $p<0.001$, respectively).

6. Discussion

The management of patients with primary refractory and relapsed HL remains a significant challenge. After diagnosis or early response assessment, identifying those who require more aggressive treatment can be crucial in improving their long-term survival. In our studies, we looked for clinical parameters that, if present or absent, may predict a more severe course of HL, increased susceptibility to early or late relapse, or a more complicated course of the disease. Our investigations focused on late relapse of HL and AICPs associated with HL.

Although HL can only be declared cured after 10 years of remission, most HL patients experience relapse within 3 years after first-line treatment. Therefore, in our study, we considered relapses occurring at least 5 years after the diagnosis of HL as LR-HL.

We identified a younger patient population diagnosed with HL in adolescence or young adulthood (<24 years), who had an increased risk of developing LR-HL after first-line treatment compared to older patients. There was no difference in the relapse rate between the two age groups identified, but younger HL patients (<24 years) were more likely to develop LR-HL. In addition, they had higher estimated survival rates after relapse compared to older patients. The shift towards late relapse in younger patients is consistent with our knowledge on age-related changes in immune competence, which implies that the distribution and function of immune cells are deteriorating with age.

The confirmation of the diagnosis and first-line treatment of HL between 1981-1990 and 1991-2000 as an independent prognostic marker for LR-HL reflects the continuous evolution of diagnostic methods and treatment modalities in HL. With the development of imaging studies and the advent of ^{18}FDG-PET/CT, the lower limit of residual disease detection has allowed the isolation of an increasingly smaller tumor mass, and newer therapeutic protocols offer the prospect of deeper and longer-lasting remission. Therefore, it is not surprising that the significant decrease in the incidence of LR-HL coincides with the introduction of ABVD, which is now the standard treatment protocol.

We have found that patients with cHL initially treated with radiotherapy alone have an increased risk of developing LR-HL. According to today's clinical practice guidelines, first-line treatment consisting of radiotherapy alone for curative purposes is now considered an inadequate therapy. However, representatives of this treatment era can be found in the HL patient population under care. Patients receiving irradiation therapy alone had a better OS than patients receiving first-line chemotherapy or combination therapy during late relapse. This observation can, of course, be explained by the fact that tumor cells are considered naive to any systemic treatment, the selection pressure of these agents could not yet be exerted on persisting HRS cells.

We observed a significantly better OS in LR-HL patients compared to the early relapsing population, while survival in late relapsing patients was not inferior to patients in persistent remission. This trend was explained by the more aggressive nature of the primary refractory or

early relapsing disease and, in our opinion, reflects the need for a more conscious choice of the modality and duration of first-line therapy in this vulnerable patient population.

Our results suggest that LR-HL's clinical behavior is more similar to newly confirmed HL than to its early relapse. HL's late relapses should be managed in a risk- and response-adapted manner, tailored to the previously administered therapy, with particular attention to cumulative anthracycline dose and previously irradiated areas. Retreatment with first-line chemotherapy protocols is therefore often limited by increasing toxicity. Biotherapies and novel agents such as BV and checkpoint inhibitors may be preferred.

Two of our patients died in disease progression, and two other patients died due to causes unrelated to HL.

A total of 5 patients underwent AHSCT after LR-HL. In this subgroup, the time to late relapse was shorter (5.9 years) than in late relapsed patients who did not require transplantation (9 years). No LR-HL was detected after AHSCT. Keller et al. investigated the incidence of late relapse, defined as relapse at least 3 years after transplantation, in HL patients refractory to ABVD treatment or subsequently relapsing and undergoing AHSCT. They found that the time to relapse after transplantation did not affect the outcome, in contrast to the trend seen in non-transplanted patients. Similarly to an Italian research team, we found no difference in the estimated overall survival of late relapsing patients who underwent AHSCT for LR-HL.

Anaemia, thrombocytopenia, and neutropenia often complicate the course of HL. Various mechanisms can contribute to the cytopenias, including the destruction of blood cell components by autoantibodies, impaired haemopoiesis, side effects of radiotherapy or chemotherapy, and bone marrow infiltration of the underlying disease.

Previous reports suggest that the prevalence of HL-AICPs is between 0.5-4.2% and that in these cases, cMC histological subtype and advanced disease (stage III/IV, B-symptoms, bone marrow involvement) are common. Comparing the studies of Lechner & Chen and Dimou et al. is difficult because while Lechner and Chen compare cases based on the type of cell line involved, Dimou et al. try to draw conclusions from the temporal pattern of AICPs. Therefore, our study aimed to address the issues raised above by considering both perspectives.

Although HL is rarely associated with AICPs, we recorded an incidence of 2.8%, representing a moderate rate, and the 14 patients thus identified represent the largest cohort of

patients presented by a single center. We report a remarkable predominance of advanced-stage disease among patients with HL-AICP. Stage III/IV disease was also frequent in the HL-AIHA and HL-AICP populations and cases with AICP during follow-up, demonstrating the complexity of advanced-stage HL and suggesting an increased potential for autoimmune processes to develop as the disease progresses. The female predominance observed among patients with HL-AICPs was attributed to the well-documented differences in immune response between the two sexes, with women presenting with a higher incidence and prevalence of autoimmune disease than men.

Immunologic abnormalities and prolonged T-cell dysfunction also explain the increased incidence of cMC in HL-AICP patients and the higher rate of EBV-associated histological subtypes overall. HRS cells often carry part of the EBV monoclonal viral genome, especially in cMC and cLD histological subtypes. As EBV can rescue pre-apoptotic B cells from apoptosis, it is suggested to play an initiating role in both the pathogenesis of HL and the induction of HL-associated autoantibody production. Although our study did not confirm a significant difference in the increase of the proportion of EBV-associated histological subtypes, a clear trend towards cMC and cLD subtypes was observed.

In our study, with a median follow-up time of more than 11 years, the incidence of HL-AICPs increased linearly, but the number of clinically relevant events reached a plateau at 10 years. This time interval is precisely the same as the time after which Hodgkin lymphoma in persistent remission is considered cured.

Most patients with HL-AICP responded well to immunosuppressive treatment, but in the case of cytopenias with metabolically active lymphoma, a durable therapeutic response was not achieved with methylprednisolone as first-line treatment. Because of the anti-lymphoma effect of steroids, the methylprednisolone used presumably exerts a transient control, not only on the autoimmune response but also on the lymphoma provoking it. Thus, although its early effect is beneficial, overall, it may even delay a definitive diagnosis. In the presence of HL and AICP, anti-lymphoma treatment has provided a definitive solution for both the underlying disease and AICP without exception. In patients with steroid-refractory HL-AICP in whom HL activity was excluded, azathioprine, rituximab and romiplostim were effective.

The development of autoimmune cytopenic events associated with HL had an impact on survival. Short-term (1-year) overall survival of the HL-AICP population was inferior to HL patients without cytopenias. Similarly, the prognosis of patients already presenting with AICP

at the time of HL diagnosis was significantly worse than those who did develop an autoimmune event during follow-up or did not develop AICPs at all. Both factors, i.e., the development of AICP associated with HL itself and the presence of AICP at the time of HL diagnosis, were confirmed as independent risk factors for lower OS compared to the general population with HL. Overall, we explain the survival disadvantage associated with HL-AICPs with the increased activity and complication of the underlying disease, with the detailed cytopenias as severe manifestations of a complex cytokine storm induced by lymphoproliferation. This is well illustrated by one of the subjects of our study, who was lost to fulminant AIHA during the completion of the diagnostic workup for HL.

A limiting factor of our studies is the retrospective nature of data collection, which may imply incomplete knowledge of the medical history of some patients. Another limitation of our study is the relatively low number of patients in the respective subgroups (LR-HL and HL-AICP), which is explained by the patient numbers in our center and the rarity of the observed pathologies. In our single-center studies, incidence data for both conditions were in line with the international literature. In addition, we believe that the patient selection without regard to age group, histological subtype, and comorbidities reflects the real-life experience of routine patient care and complements the conclusions drawn from the filtered patient population of clinical studies, thus being the main strength of the analysis.

In conclusion, our analyses extend the previously available data on both late relapses of Hodgkin lymphoma and autoimmune cytopenias associated with Hodgkin lymphoma, thus providing a more complete picture of the rare but clinically important manifestations of HL.

Late relapse of HL is comparable to de novo disease in clinical course and response to treatment. Diagnosis under the age of 24 years, diagnosis between 1981 and 2000, and first-line treatment with radiotherapy alone increase the risk of developing LR-HL. This delineated patient group may benefit from conventional-dose chemotherapy tailored to previous treatment and regular follow-up beyond 10 years. With the use of modern therapeutic options, the prognosis of LR-HL is more favorable than in early relapse of HL.

Patients presenting with AICP associated with HL also have specific, well-defined clinical features. The association is more likely to occur in advanced-stage HL and EBV-associated histological subtypes. The development of HL-AICP is of prognostic significance, increasing mortality. The presence of autoimmune cytopenias at the time of HL diagnosis is also an independent risk factor for overall HL survival. Among HL patients in remission, the

possibility of relapse of the underlying disease and the presence of a second malignancy should be raised in the clinician when AICP develops.

7. New findings

1. Advanced stage Hodgkin's lymphoma, or Hodgkin's lymphoma with B symptoms, increases the risk of early relapse of the underlying disease compared to achieving sustained remission or late relapse.
2. Hodgkin's lymphoma confirmed in adolescence and young adulthood (<24 years of age), primary diagnosis between 1981 and 2000, or baseline disease treated with first-line radiotherapy alone are independent risk factors for late relapse of Hodgkin's lymphoma, i.e. relapse at least 5 years after diagnosis.
3. In the ABVD era, early relapsed Hodgkin lymphoma patients have an inferior expected 5-year overall survival compared to patients in remission and late relapsed patients.
4. In the case of late relapse, a better prognosis is indicated if Hodgkin's lymphoma was present in a patient under 24 years of age or in the absence of B symptoms at first diagnosis.
5. Nearly half of the autoimmune cytopenias in the course of Hodgkin lymphoma are clinically significant, indicating relapse or the development of a second malignancy. Such an event was encountered only within 10 years after diagnosis of Hodgkin lymphoma.
6. Without exception, anti-lymphoma treatment is the definitive solution for autoimmune cytopenias that develop in the presence of active underlying disease.
7. The occurrence of autoimmune cytopenia associated with Hodgkin's lymphoma during the course of the disease or the presence of concurrent autoimmune cytopenia at diagnosis is an independent risk factor for shorter overall survival.

8. Acknowledgements

I would like to thank my thesis supervisor, Professor Árpád Illés, who from my first day of work has been determined but always consistent in his efforts to guide me to become better not only in everyday medical tasks, but also in the important things of academic work and life.

I would like to thank Zsófia Miltényi for being a mentor and a friend I can always rely on, and I can safely listen to her advice and guidance, which is not only guiding me on the bumpy road of my profession, but also of life.

My grateful thanks go to Ferenc Magyari and Ádám Jóna, senior lecturers, who have shared their experience with me with unending patience and enthusiasm and selflessly helped me in my daily life.

I thank Professor László Rejtő and Dr. Anna Selmeczi for the opportunity to learn about haematology through their eyes as a student, which led to a lifelong commitment to the field.

I would like to thank Katalin Hodosi for her help with statistical analysis and for her refined critical sense, which is a constant source of scientific rigor.

I thank the co-authors of my communications, my colleagues and all the staff of the Department of Hematology for their help in my work so far.

Thank you to my Parents, Partner, Family and Friends for supporting me with patience and understanding throughout my work.

9. References

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Registry number: DEENK/440/2021.PL
Subject: PhD Publication List

Candidate: László Imre Pinczés
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

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Total IF of journals (all publications): 22,945
Total IF of journals (publications related to the dissertation): 5,822

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

17 September, 2021

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