

Clinical experiences in Hodgkin lymphoma in particular with regard to pulmonary complications

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1. Introduction

Hodgkin lymphoma (HL) is the malignancy of the lymphatic system, it consists of 12-18% of all lymphomas. The most common symptoms are enlarged lymph nodes (lymphadenomegaly), liver- and spleen enlargement (hepato-splenomegaly). Lymph nodes are often involved in the cervical, supraclavicular and mediastinal region. Infradiaphragmatic, especially extranodal involvement is rare. General symptoms („B” symptoms) are weight loss exceeding 10%, night sweats, and undulant type – Pel-Ebstein-like – fever. Laboratory findings are increased erythrocyte sedimentation, eosinophilia, leukocytosis, lymphopenia, thrombocytosis, hypalbuminaemia, perhaps anemia.

Previously, the goal of treatment was to improve survival rate of patients. Nowadays, long-term side effects came to the front with improvement of survival; therefore avoiding over- or undertreatment is of interest. Our workgroup published data about cardiovascular, thyroid-, parathyroid, renal and ureter impairment, carotis stenosis, secondary malignancies, postirradiation cardiotoxicity, parodontological complications and quality of life of HL survivors. Our current work examined the pathobiology of HL, investigated clinical experiences gained in our Department with patients treated between 1983 and 2011 and long-term pulmonary side effects of HL treatment.

2. Literature background

2.1 Diagnosis

The diagnosis of HL is based on histology, which the biopsy of the involved lymph node is required for. Core biopsy could be considered, but fine needle aspiration is unacceptable due to the small number of the tumor cells compared to the tumor mass (1-5%) in contrast with solid tumors. The rest is of the mass in HL is inflammatory-fibrotic mass.

The 2016 World Health Organization (WHO) did not bring too much change in the histological classification of HL. It is still divided into nodular lymphocyte

predominant (NLPHL) and classical HL (cHL), which further subdivided into the known histological subtypes: lymphocyte rich (cLR), nodular sclerosis (cNS), mixed cellularity (cMC), lymphocyte depletion (cLD) and not determined (ND).

2.2 Pathogenesis

Although the B-cell origin of Hodgkin/ Reed-Sternberg (HRS) cells is now obvious, but the explicit reason of HL is still unknown. Genetic, immunologic and environmental factors can be blamed causing HL. HRS cells are monoclonal, germinal center originated pre-apoptotic B-cells, which carry rearranged, somatically hypermutated immunoglobulin heavy chains (IgVH), lost their B-cell phenotype, but express membrane markers characteristic for other lympho-hemopoetic cells and proteins regulating their abnormal transcription.

Several signaling pathways have been reported to prevent or modulate apoptosis, eventually causing progression of lymphoma. The most important is the constitutive upregulation of NF- κ B, JAK-STAT, which is an important mediator of cytokine signaling and active phosphatidil 3-kinase (PI3K)-Akt pathway, which inhibits apoptosis and activates cell cycle.

The pathogenic role of Epstein-Barr virus (EBV) is well known. Patients, who already underwent infectious mononucleosis develop EBV+ HL with 3-fold probability compared to the general population. EBV+ HRS cells express latent membrane protein -1 and -2 (LMP-1, LMP-2). LMP-1 imitated CD40 receptor, leading to NF- κ B activation and it can also stimulate infiltrating lymphocytes, while LMP-2 provides B-cell signaling by substituting B-cell receptor. FISH can evince clonal numeric aberrations in every cHL subtype. Recently, 9p24.1 chromosome amplification was reported to overexpress programmed cell death ligand 1 (PD-L1) in HRS cells – especially in cNS subtype. EBV infection further increases expression of PD-1L, therefore increased expression can be detected at the case of EBV+ HL patients. PD protein helps inhibiting T-cell mediated immunresponse, hence bonding PD-L1 and PD-L2 to PD-1 expressed by T-cells leads to apoptosis of cytotoxic T-cells, increasing

numbers of immunosuppressive Treg cells, thus leading to the inhibition of immuneresponse, which eventually results in immunescape and survival of the HRS cells. Microenvironmental cells help not only the proliferation of HRS cells but creating such environment in which effective immuneresponse against HRS cells cannot be carried out.

2.3 Treatment

HL represents one of the major success stories of hematology. The disease, which was fatal at the beginning, has nowadays 80% of survival rate nowadays. First line treatment is typically bleomycin-containing polychemotherapy. ABVD is used most likely in Hungary with cycle numbers depending stage and prognosis, which can be supplemented by involved field irradiation (IFRT). Patients with early favourable disease receive 2-4x ABVD+ 20-30 Gy IFRT. Early Unfavourable disease is usually treated 4-6x ABVD+ 30 Gy IFRT. Advanced stage disease is treated with 6-8x ABVD ± IFRT, where irradiation depends on the presence of initial bulky disease and when restaging ¹⁸FDG-PET/CT shows residual activity.

First line treatment gets 70% of patients into complete remission. About 10-15% of cases are refractory and 15-20% relapses, most likely in the first 2 years after completion of treatment. Half of the patients can be successfully salvaged by autologous stem cell transplantation (ASCT). However, treatment of those who relapse after ASCT is challenging and has a median survival of 2.5 years. For them anti-CD30 antibody conjugated with monomethyl auristatin E (MMAE) (brentuximab vedotin), PD-1 inhibitors and haploidentical allogeneic transplantation can be the chance to survive.

2.4 Brentuximab vedotin

Brentuximab vedotin (BV) is the first antibody drug conjugate for 30 years, which was approved by American Food and Drug Agency in 2011 (FDA) and European Medical Agency (EMA) in 2012 for the treatment of relapsed /refractory (R/R) HL patients: following ASCT failure, after two previous lines of therapy in the case of

patients unsuitable for ASCT. Recently, BV was approved for patients with high relapse risk after ASCT and maintenance therapy.

The conjugate itself is polymerase inhibitor; a chimera IgG1 type anti-CD30 antibody, which is covalently linked to 4 MMAE. The drug links to the surface of CD30 positive cells, then it's internalized and after proteolytic cleavage of MMAE it gets to lysosomes. Here, MMAE links to tubulin and causes the apoptosis of CD30 expressing cell by inhibiting cell cycle from getting G2 to M phase. Cleaved MMAE can get through cell membrane to the extracellular space effecting environmental CD30 negative cells.

Phase 1 trial revealed 36% overall response rate (ORR), when drug was administered on a 3 weekly basis. By choosing 1.8 mg/kg dose, phase 2 pivotal trial revealed 75% ORR when treating 102 heavily pretreated HL patients. Based on this result its use was approved. AETHERA was further important trial, when high-risk patients (primary refractory or remission duration less than 12 months or extranodal involvement at time of salvage treatment pre transplant) received maintenance treatment post ASCT on a 3 weekly basis 1.8 mg/kg BV or placebo. BV treated patients reached significantly superior PFS, than those receiving placebo (63% vs. 51%).

Unacceptable high pulmonary toxicity (44%) was revealed when BV was combined with first line ABVD treatment, which was not found, when combined with AVD. Failure free survival was found to be 96% through 3 years of follow up in examined advanced stage patients (II/A bulky or II/B-IV. stage). Based on these promising results, ECHELON-1 trial is currently investigating advanced stage patients worldwide comparing BV+AVD vs. standard of care ABVD. (ClinicalTrials.gov #NCT01712490)

The role of BV is currently investigated in several therapeutic settings and combinations, these results are currently evaluated: untreated early unfavourable patients, elderly patients, bendamustin, ICE, rituximab, gemcitabin, dacarbazin.

2.5 Pulmonary complications

Currently and recently used bleomycin containing polychemotherapy and IFRT involving the chest may cause potential pulmonary toxicity. Bleomycin is mainly excreted by the kidneys, but it can be also eliminated by its specific deactivating enzyme bleomycin hydrolase (BLMH). Against its chemotherapeutic effectiveness, its excessive use is limited by its potentially life-threatening pulmonary toxicity. It's toxic effect is due to its free radical producing capacity, while its pulmonary specificity is due to the fact, that it's absent in the lung.

Due to the literature pulmonary toxicity occurs in about 20-46% of patients treated with bleomycin, while mortality rate is 1-3%. The radiological sign of interstitial fibrosis caused by bleomycin is typically bilateral reticular density and pure nodular infiltrates. During bleomycin induced pulmonary toxicity free radical formation, then DNA damage, pneumocyte and endothelial damage, abnormal cytokin cascade formation, inflammation, fibroblast activation, fibrous extraction, fibrin formation and finally interstitial pulmonary fibrosis can be observed. Factors predisposing for pulmonary toxicity are age, cumulative dose, smoking, abnormal kidney function, route of administration (intravenous or intramuscular). The role of granulocyte colony stimulating factor and chest irradiation are debated. In case of acute complication omission of bleomycin and parenteral steroid treatment is recommended. Favourable effect of imatinib was reported in one case.

Pulmonary toxicity of irradiation involving the chest is known, it used to be the most frequent cause of pulmonary impairment. Irradiation pneumonitis typically occurs 1-3 months after completion of irradiation. The probability of its development depends on size of the irradiated area, type of irradiation, daily and total dose of irradiation. Definitive fibrosis may occur after 6 months of irradiation. The probability of its development depends more on total dose and irradiated size, while daily dose is less important in this matter.

BLMH, the enzyme inactivating bleomycin may be a possible factor that can affect long-term pulmonary function of patients receiving bleomycin. SNP A1450G may be a potential candidate that can be responsible for enzymatic activity of BLMH,

which inactivates bleomycin, eventually pulmonary toxicity. It looks like SNP A1450G is responsible for exchanging isoleucine to valine at the amino acid residue 443. Although exact effect of SNP A1450G to BLMH is unknown, it looks like it could affect bleomycin induced DNA damage. The role of BLMH affecting pulmonary function of HL patients is unknown in the literature.

3. Aims

1. To investigate clinicopathological features of our currently followed HL patients (age, gender, histology, stage, general symptoms), to collect treatment modalities (chemotherapeutic protocols, type of irradiation and involved region) and to evaluate their pulmonary function by using questionnaire, chest X-ray, spirometry and lung scintigraphy
2. To investigate the role of irradiation involving the chest to develop pulmonary toxicity after the treatment of HL patients through retrospective evaluation.
3. To investigate the role of cumulative bleomycin dose to the pulmonary function HL patients after treatment through retrospective evaluation.
4. To investigate the factors reported in the literature that may potentially affect lung function of HL patients after treatment through retrospective evaluation.
5. To evaluate the role of the BLMH SNP A1450G polymorphism among previously bleomycin treated HL patients through retrospective pulmonary function evaluation.

4. Patients and methods

Evaluation of pulmonary complications of previously treated HL patients' was done at the Department of Hematology, University of Debrecen (UD), between November 2012 and October 2013. Patients were informed about the study and test methods and Local Research Ethics Committee of University of Debrecen gave approval of the study. The following data were collected: gender, age at time of diagnosis, clinical stage based on Cotswold modified Ann Arbor staging system, histological subtypes according to WHO 2008 classification, presence or absence of "B" symptoms, treatment modality (radiotherapy, chemotherapy, combined chemoradiotherapy), chemotherapy regimen (ABVD, COPP/ABV, CVPP, other) and radiotherapy (involved-field irradiation of the mediastinum or other region, mantle, inverted Y, etc.) and elapsed time from treatment. Pulmonary status was assessed by St. George Respiratory Questionnaire (SGRQ), spirometry, chest X-ray and dynamic inhalation lung scintigraphy.

SGRQ is a questionnaire validated for pulmonary diseases and it includes 3 parts: symptom, activity and impact scores, which make the total score, ranging between 0 and 100. The higher the value, the worse quality of life the patient reports. SGRQ was considered aberrant, when its value was above 10.

To investigate possible functional deviations, spirometry was done at Department of Pulmonology, UD, by EuTest Plus VT-17 spirometer. The main static parameters (FRC (functional residual capacity), RV (residual volume), TLC (total capacity), FVC (forced vital capacity) and dynamic parameters were investigated (FEV1 (forced expiratory volume in 1 second, Tiffenau (FEV1/FVC), and other parameters, like PEF (peak expiratory flow) and VC (vital capacity). Given financial and practical considerations spirometry was performed instead of gold standard DLCO. Spirometry results were calculated as percentage of age and gender adjusted healthy controls: value above 80% was considered as mild, 50-79% moderate, 30-49% severe and below 30% was considered as very severe alterations.

Chest X-rays were reviewed, looking for structural alterations, which may influence lung function. Chest X-rays with fibrotic shadows were considered as positive.

Dynamic inhalation lung scintigraphy was performed to collect data referencing membrane lesions at Department of Nuclear Medicine, UD with the use of Technetium-99m diethylene triamine penta-acetic acid (DTPA), results were compared to normal values of corresponding age and lung side. Results below normal values were considered as positive. The lower the result, the severe the membrane lesion was.

EDTA-anticoagulated peripheral blood samples were collected of previously treated HL patients, which were uniquely identified for encryption of patients' data. Genomic DNA was isolated using MagnaPure 96 System (Roche) according to manufacturer's protocol. Concentration of DNA samples was checked using Magna Pure 96 DNA and Viral NA small volume kit by Nanodrop UV photometer. SNP A1450G (rs1050565) of the BLMH gene was genotyped using TaqMan genotyping assays (Lifetechnologies). Measurements and genotype calling were performed on QuantStudio 12K flex instrument (Lifetechnologies) at the Biochemistry and Molecular Biology Institute, Genomic Medicine and Bioinformatics Service Provider Laboratory, UD. Experiments were done in duplicates.

Statistical analysis was performed using SPSS version 17 and 22 software. Analysis was done using the Fischer's exact test, Spearman rank correlation test, Mann-Whitney and Chi-square test, multivariate analysis as appropriate. Correlations were considered significant if $p < 0.05$.

5. Results

5.1 Patient characteristics

A total number of 137 patients were evaluated for pulmonary function over a one-year period. There were 75 male (54.74%) and 62 female (45.26%). Male to female ratio was 1.21. Median age was 29 years (range: 16-73 years) at time of diagnosis. 72 (52.56%) patients had early stage and 64 (47.72%) patients had advanced stage disease. 38 patients (27.74%) had a smoking history. 92 patients (67.15%) received combined modality treatment, while 42 patients (30.66%) received only chemotherapy and 3 patients (2.19%) irradiation alone. More than half of patients were treated with only first line ABVD regimen (56.2%), the remaining received COPP/ABV (13.87%), CVPP (5.84%) and other regimens (4.92%). Relapsing patients (18.98%) were treated according to institutional guidelines, 6 patients (4.37%) underwent ASCT. 65 patients received involved field irradiation, out of which 46 received to the mediastinal and 19 to the non-mediastinal region. Mantle field irradiation was used for 20 patients; other irradiation techniques included inverted Y and locoregional irradiation (10 patients). Median elapsed time since diagnosis was 11 years (2-30) at the time of our work.

5.2 Pulmonary function test results

Abnormal pulmonary function was found as the following, according to the different test methods: 49 patients (35.77%) had SGRQ score above 10 and 88 (64.23%) patients below 10 points. 75 patients (54.74%) had negative, 36 patients (26.28%) mild, 19 patients (13.87%) moderate, 3 patients (2.19%) severe and 4 patients (2.92%) had very severe spirometry alterations. 16 patients (11.68%) had restrictive and 61 patients (44.52%) had obstructive alterations. 14 patients (10.22%) had central and 47 patients (34.30%) had peripheral obstruction. Some patients had both restrictive and obstructive alterations. 108 patients (78.84%) had no alterations on chest X-ray, while 29 patients (21.16%) had fibrotic shadows. Lung scintigraphy revealed membrane lesions in 48 patients (35.04%), while 89 patients (64.96%) had no alterations.

5.3 Evaluation the role of chemotherapeutic cycles and chest irradiation to pulmonary function

Patients were then subdivided into two groups. Group 1 included those receiving combined modality treatment and irradiation therapy alone (n=69 patients), when irradiation means exclusively chest irradiation (involved field irradiation to the mediastinum + mantle field). Group 2 included patients receiving combined modality treatment (with no chest irradiation) and chemotherapy alone (n=68 patients). Any kind of chemotherapeutic regimen was allowed in this comparison. No significant additive worsening effect of chest irradiation was confirmed with test methods. (SGRQ: p=0.73, spirometry: p=0.39, chest X-ray: p=0.69, scintigraphy: p=0.34)

When group 1 was narrowed to patients receiving only ABVD and chest irradiation (n=37 patients), a significant correlation could be detected between SGRQ total score and number of ABVD cycles and subsequent bleomycin dose (p=0.01). The more cycles the patient received, the higher score was reached at the SGRQ test.

When group 2 was narrowed to patients receiving only ABVD without chest irradiation (n=40 patients), a close correlation was found between scintigraphy results and number of ABVD cycles and subsequent cumulative bleomycin dose (left side: p=0.099, right side: p=0.051). The more cycles the patient received, the worse result was detected by scintigraphy.

Multivariate analysis was performed in patients receiving first line ABVD chemotherapy regarding potential risk factors of late lung impairment (n=77 patients). Smoking was a risk factor of SGRQ, spirometry and scintigraphy results. Age affected spirometry results. Bleomycin dose also significantly affected SGRQ and scintigraphy results. No correlation was found between chest irradiation and any of the test methods. All investigated patients had normal pretreatment kidney values; hence it didn't influence lung impairment in these cases.

5.4 Evaluating the role of bleomycin hydrolase gene polymorphism in Hodgkin lymphoma patients

To further complement our investigations a total number of 131 peripheral blood samples were collected from previously treated HL patients. Bleomycin was included in the treatment of 102 patients (ABVD, BEACOPP, COPP/ABV, relapsed/refractory patients). The treatment of the remaining 29 patients excluded bleomycin (irradiation only, COPP, CVPP), thus representing a control group. Out of the 102 bleomycin-treated patients 68 received ABVD chemotherapy alone. Median bleomycin dose and time elapsed from treatment completion differed significantly in the treatment groups. Other factors (smoking, age, bleomycin dose, chest irradiation, kidney function, use of colony stimulating factors) that could potentially affect lung function were equally represented in all investigated subgroups with no significant differences.

5.5 Distribution of bleomycin hydrolase gene polymorphism

BLMH A1450G genotype distribution was determined from blood samples collected from patients. Homozygous wild A/A genotype was found in 72 patients (55.0%), while 44 patients (33.6%) had heterozygous A/G and 15 patients (11.5%) had homozygous mutated G/G genotype, where “A” is the wild (allele frequency: 71.8%) and “G” is the mutated allele (allele frequency: 28.2%). Our results were comparable to the NCBI SNP database for BLMH SNP A1450G. Allele frequencies were in Hardy-Weinberg equilibrium. G/G genotype group alone would have been too small for relevant statistical analysis, therefore patients were then subdivided into subgroups: one containing the mutated allele: A/G+G/G (45.1%) and the other homogenous for the wild allele: A/A (55.0%), thus demonstrating the possible role of the wild “A” and mutated “G” allele.

Factors, which could potentially affect lung function, were equally represented between the subgroups containing the mutated allele (A/G+G/G) and those homogenous for the wild allele (A/A) even within the investigated treatment groups with no significant differences.

5.6 Investigation the role of bleomycin hydrolase gene polymorphism to pulmonary function

All bleomycin- treated patients (n=102) had more favorable lung function test results in the A/A genotype group, with every investigated test method with significant differences in the forced vital capacity results (FVC), $p=0.006$.

When focusing on patients who received ABVD regimen (n=68) alone, significantly more favorable results were seen in the A/A genotype group, with every investigated test methods. The SGRQ score was significantly more favorable in the A/A genotype group (11.90 pts. vs. 4.20 pts., $p=0.035$). Right-sided lung scintigraphy results were also significantly more favorable in the A/A genotype group (74.81 vs. 57.56, $p=0.045$). Among spirometry results FVC ($p=0.020$) and forced expiratory volume in 1 s (FEV1) results were significantly more favorable ($p=0.028$) in the A/A genotype group. A linear regression analysis also confirmed these results.

As a control group (n=29), patients treated with agents excluding bleomycin (irradiation or chemotherapeutic regimens not containing bleomycin) were tested with no significant differences between A/G+G/G and A/A genotype groups.

6. Discussion

Currently used first-line treatment of HL, bleomycin containing ABVD and/or radiotherapy involving the chest is reported to produce pulmonary side effects such as bleomycin-induced lung injury (BILI) and radiation-induced pneumonitis.

In our study, abnormal pulmonary function was detected in between 21-45% of patients and found to be correlated with smoking, age and cumulative bleomycin dose.

Bleomycin is usually administered 10 mg/m² intravenously or intramuscularly. While the half-life of bleomycin is comparable with iv. and im. administration, peak plasma level of im. administration is only one tenth of iv. administration. In the current study bleomycin was administered exclusively intramuscularly and dose was maximized in 15 mg, hence providing basis, that only one acute pneumonitis occurred, which was considered bleomycin toxicity. Our workgroup also published our patient's survival data, which is comparable to international survival rates. The reason may be, that more clear associations can be found in patients receiving ABVD, that they were treated later (1999-present), hence there are less confounding factors that could contribute to pulmonary functions.

Combination of brentuximab vedotin with first-line ABVD revealed an additive lung damage effects of bleomycin, hence administering brentuximab vedotin is recommended only with AVD (adriamycin, vincristine, dacarbazine) chemotherapy. The German Hodgkin Study Group is currently investigating the role of brentuximab vedotin by developing BEACOPP^{escalated} (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) to two experimental variants BrECAPP (brentuximab vedotin, etoposide, cyclophosphamide, doxorubicin, procarbazine, prednisone) and BrECADD (brentuximab vedotin, etoposide, cyclophosphamide, doxorubicin, dacarbazine, dexamethasone). The product description of brentuximab vedotin also reports pulmonary toxicity as single agent, like pneumonitis, interstitial lung disease, acute respiration distress syndrome (ARDS).

Nevertheless, in these cases an obvious relationship was not established, but the risk of pulmonary toxicity could not have been excluded.

Nowadays, due to the combined modality treatments, only stage I/A nodular lymphocyte predominant HL patients are treated with radiotherapy alone, furthermore irradiated area is also notably smaller (involved-field or involved-node irradiation). According to the literature, chest irradiation does not significantly contribute to the pulmonary damage of bleomycin, however it prolongs the duration of reversible damage. Nevertheless, the additive negative effect of thoracic irradiation is debated. Our results confirm this statement; currently used chest irradiation did not significantly worsen pulmonary function.

A biomarker would be mandatory, which would be of help even in combination with other pulmonary risk factors. In the presence of such biomarker patients could be chosen to eliminate bleomycin from their treatment and could be replaced with targeted therapeutic agents.

BLMH gene SNP A1450G has been investigated previously in testicular germ-cell cancer (TC) patients in correlation with survival data. Mutant variant (G/G) genotype was associated with decreased survival compared to the heterozygous (A/G) and the wild type variants (A/A). SNP A1450G was also investigated in correlation with pulmonary toxicity of TC patients in a subsequent study. Interestingly, no association was found of gene polymorphism either with acute bleomycin-induced pneumonitis or with pulmonary function tests performed during treatment.

In the current study BLMH gene SNP A1450G polymorphism led to significant differences in the follow-up pulmonary test results of ABVD-treated HL patients. The authors believe, that the observed difference in the ABVD only group is due to the genotype differences, and not due to other factors, that could possibly affect pulmonary function, since these factors were equally represented between the treatment groups and also in the control group.

Dutch authors explained the lack of correlation with barely injured kidney function, when the effect of altered BLMH activity is negated by the rapid elimination

of bleomycin. Because the serum half-life of bleomycin is relatively short, the effect of altered BLMH activity could have been negated by the rapid elimination of bleomycin. The fact, that homozygous variant (G/G) TC patients in the Dutch-study were associated with decreased survival could be explained by the fact that in this genotype group, there were more patients with refractory disease, than in other groups. This suggests, that it may not have been an independent parameter in terms of survival. Authors describe, that G/G genotype variant could have been associated with other factors that causes tumor resistance. The fact that they didn't find association of SNP A1450G and acute pulmonary toxicity can be explained also, that they investigated the occurrence of acute pulmonary toxicity, whereas, our study investigated quantitative features of long-term pulmonary toxicity. Compared to the TC patients of the Dutch study, our patients were treated with lower cumulative bleomycin doses administered im., and still had normal kidney function results. All of these factors represent major differences. Previously, the difference between im. and iv. administration was described and the maximized 15 mg dose of bleomycin. Given a median 120 mg/m² bleomycin dose, a maximum of 180 mg bleomycin could have been administered, which is far less, than what the TC patients received (3-400 mg). Overall, BLMH gene SNP A1450G polymorphism seems to have significance on the long-term. Obviously, the different nature of the diseases may also have contributed to these conflicting results.

Limitation of the control group is, that control patients cannot be matched in elapsed time since treatment with the investigated (ABVD) patient population. We were looking for a patient group, whose treatment did not included bleomycin to show, that BLMH had no role to pulmonary function in these patients. However such treatment modalities existed only in the reported 15-44 years of follow up period. We did not find any other historical, patient group with hematological disease, who could have been matched in either age or general condition to our investigated HL patient population. We are planning to compare the BLMH polymorphism data of brentuximab vedotin-AVD and standard of care ABVD arms of currently running clinical trials. Evaluation of these results is currently in progress.

As a summary of our work, it can be concluded, that chest irradiation – as part of current treatment of HL patients – doesn't significantly worsen pulmonary side effects. In terms of pulmonary side effects avoiding chest irradiation is not recommended – if other pulmonary risk factors are not present. However, increasing cumulative bleomycin dose may cause further side effects. Our results further confirm current endeavor, which try to reduce chemotherapeutic cycles, while maintaining favourable survival rates. Because the investigated BLMH polymorphism led to significant differences in long-term pulmonary side effects of patients, further study is planned to confirm our results.

7. Novel statements

1. Currently used involved field irradiation for the treatment of HL patients doesn't contribute to the pulmonary toxicity in case of irradiation involving the chest after treating HL patients, through retrospective analysis. The decrease or abandonment of irradiation is irrelevant – based on these results.
2. Increasing chemotherapeutic cycles and hence cumulative bleomycin dose pulmonary function worsened after treating HL patients, through retrospective analysis. Bases on these results, reducing bleomycin-containing chemotherapy is recommended, while maintaining curative efficacy.
3. Multivariate analysis confirmed that smoking and age are potentially pulmonary toxic factors after treating HL patients, through retrospective analysis.
4. BLMH gene SNP A1450G polymorphism led to significant differences in the long-term follow-up pulmonary test results of ABVD-treated HL patients. Based on our results, investigating the further role of BLMH gene SNP A1450G polymorphism is recommended.
5. Clinical significance of our work would be, that based on BLMH gene SNP A1450G polymorphism exact cases could be chosen even with multiple pulmonary risk factors, whose treatment did not include bleomycin but would be replaced by targeted therapeutic options.

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List of publications related to the dissertation

1. **Jóna, Á.**, Miltényi, Z., Póliska, S., Bálint, B. L., Illés, Á.: Effect of bleomycin hydrolase gene polymorphism on late pulmonary complications of treatment for Hodgkin lymphoma. *PLoS One*. 11 (6), e0157651, 2016.
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Total IF of journals (all publications): 24,285

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