THE IMMUNOHISTOCHEMICAL AND HIGH-PERFORMANCE IMAGING INVESTIGATIONS IN THE THERAPEUTIC DECISION LEADING TO THE CURE OF MALIGNANT HODGKIN LYMPHOMA

DOCTORAL THESIS SUMMARY

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General Considerations

Hodgkin Lymphoma (HL) is a relatively rare malignant lymphoproliferative disorder characterized by the presence of specific malignant cells derived from the germinal center of B lymphocytes: multinucleated Reed-Sternberg (RS) cells and mononucleated Hodgkin (H) cells – collectively known as Hodgkin Reed-Sternberg (HRS) cells. These cells are located within an inflammatory infiltrate composed of non-malignant cells that provide protection to the HRS cells against the immune system [1, 2, 3]. The malignant cells exhibit genetic transmission defects: they do not express certain antigen receptors specific to B lymphocytes (including impaired immunoglobulin expression) [1], and they demonstrate antigen expression without lineage specificity: CD 15 (Leu-M1) and CD 30 (Ki-1).

Immunohistochemistry aids in identifying the two main categories of Hodgkin lymphomas: classical Hodgkin lymphoma (cHL), which accounts for approximately 95% of cases, and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) – approximately 5% of cases. cHL can be further classified into four subtypes based on microscopic appearance, RS cell characteristics, their proportion, lymphocyte count, and degree of fibrosis [1]:

a) Nodular sclerosis – NS (70%)
b) Mixed cellularity – MC (20%)
c) Lymphocyte-rich – LR (5%)
d) Lymphocyte-depleted – LD (rare).

Hodgkin Lymphoma is a highly curable neoplasm in both adults and children through therapy tailored to risk factors, including both chemotherapy and radiotherapy [3, 4]. The use of cytotoxic chemotherapy and radiotherapy has short-term and long-term adverse effects that can impact patient survival: the development of secondary neoplasms, cardiac insufficiency, immunosuppression, pulmonary toxic effects, and various endocrine disorders. Effective and curable therapeutic regimens have an immense potential to improve long-term prognosis and quality of life.

From an imaging standpoint, positron emission tomography (PET) and PET/CT have improved both the quality and accuracy of staging in Hodgkin lymphoma, identifying 25% more disease determinations than conventional imaging methods, including extranodal determinations. PET scans are recommended for initial disease staging, restaging upon therapy completion, and assessing response at the end of the treatment protocol. Early intermediate PET scanning, after 2 cycles of ABVD-type polychemotherapy, has been shown to have a very high predictive value for the final outcome when using the combined therapeutic modality, though this appears to be more evident in advanced stages of the disease.

For the diagnosis of Hodgkin lymphoma, histopathological examination of biologic material extracted via biopsy from an affected lymph node is necessary. In a minority of cases,
extraganglionic biopsy is used to determine the disease [3, 4]. Occasionally, cells resembling RS cells can be present in other conditions such as infectious mononucleosis or other types of lymphomas. To diagnose Hodgkin lymphoma, HRS cells must be surrounded by non-malignant inflammatory cells. In questionable situations, immunohistochemical markers help establish a diagnosis.

A characteristic of Hodgkin Lymphoma is its contiguous spread, with the most frequently affected sites being the supradiaphragmatic lymph nodes (cervical, mediastinal, axillary, supraclavicular) [5, 6]. Less frequently, abdominal or inguinal lymph nodes, as well as non-lymphatic organs, can be involved [7]. Primary extraganglionic determinations are possible but rare, with commonly affected organs being the spleen, liver, pericardium, lungs, or bone marrow. Ten percent of patients present with splenomegaly at diagnosis, and splenic involvement in its absence has been detected using CT or PET scans in 20-30% of cases [8].

The extent of disease has prognostic and therapeutic implications, and its determination currently uses the Ann Arbor staging system [9]. This system describes four stages of the disease:

1. Stage I - a single lymph node area involved
2. Stage II - at least two lymph node areas involved on the same side of the diaphragm
3. Stage III - at least two lymph node areas involved on different sides of the diaphragm
4. Stage IV - a non-lymphatic organ is diffusely affected

The therapeutic management of the disease is closely correlated with the stage of the disease and the presence of various prognostic factors. For an accurate diagnosis, a biopsy of the affected tissue is performed, followed by histopathological and immunohistochemical examinations. Additionally, imaging investigations, laboratory tests, anamnesis, and a complete physical examination are necessary. Imaging-wise, the preferred method is PET examination with integrated CT scanning, as the information provided is considered more accurate than that obtained through CT scanning alone [1, 3, 4]. If the PET-CT examination is negative, the myelogram and bone marrow biopsy can be omitted, as these have increased relevance in patients at advanced stages [10].

The therapeutic modalities of Hodgkin’s lymphoma have seen constant progress in recent decades due to advanced scientific research in the fields of radiotherapy and chemotherapy. Treatment is adaptable based on the stage of the disease and various prognostic factors with therapeutic impact. The use of positron emission tomography (PET) and positron emission tomography with CT sequencing (PET/CT) for staging, restaging, and treatment adaptation has led to more effective therapeutic management today [11, 12].

Thus, the cure rate for Hodgkin’s lymphoma has reached 70-80%. Radiotherapy and chemotherapy remain the foundation of the therapeutic approach, but, thanks to recent studies,
clinicians have also begun to use monoclonal antibodies, proteasome inhibitors, or histone deacetylase (HDAC) inhibitors [1, 2, 4, 13, 14, 15, 16].

**Purpose and Objectives of the Study**

This paper will pursue an innovative assessment of tumor microenvironment and malignant cell characteristics using advanced histopathology and immunohistochemistry (IHC) techniques. These methods are either already available or easily implemented in the laboratories of hematology clinics in the country. The goal is the detailed phenotypic characterization of tumor and non-tumor cells, highlighting including the association with the Epstein-Barr virus (EBV) and the study of specific gene expression. This approach promises to bring new fundamental and applied perspectives to oncology, marking a significant advance in the understanding and treatment of hematological neoplasias.

Our retrospective and prospective analytical observational study analyzes patients diagnosed with classical Hodgkin's lymphoma (LHC) between 2005 and 2018, under the supervision of the Hematology Clinic of the Colțea Clinical Hospital in Bucharest. Followed until December 2019 or until death, these patients were evaluated for clinical, biological and therapeutic response parameters with proven prognostic value. Through this research, we bring new insights into the evolution and treatment of LHC, contributing crucially to the advancement of knowledge in the field of hematology oncology.

The objectives of this study are:

This study follows the clinical course and response to treatment in Hodgkin's lymphomas, correlating these aspects with possible clinical and biological prognostic factors. Through a detailed analysis of these correlations, our research makes essential contributions to the understanding of prognostic mechanisms and to the optimization of therapeutic strategies in the treatment of Hodgkin lymphomas.

This research investigates how patients progress according to the histological subtype of Hodgkin's lymphomas, the stage of the disease and the treatment applied, while analyzing the prognostic factors initially identified.

This comparative research analyzes data from the literature to identify particularities related to incidence, course and response to treatment in various disease contexts.
A realistic assessment of a patient's prognosis at the time of diagnosis is the foundation of an optimal therapeutic approach, with the minimization of long-term side effects. We investigated certain clinical and paraclinical risk factors, evaluating the survival of patients with classical Hodgkin lymphoma according to these factors, the evolutionary stage of the disease and the applied treatments.

The diversity in the evolution of patients with Hodgkin lymphoma at the same clinical stage is influenced not only by the various treatments applied, but also by the unique characteristics of the host organism and the tumor proliferation process.

This research aims to create an innovative prognostic model based on the biological characteristics of classical Hodgkin's lymphoma, with the aim of predicting therapeutic response and allowing treatment to be personalized according to the degree of risk of each patient. The identification and evaluation of EBV expression in tumor cells brings new insights in determining the prognostic implications and developing a differentiated, advanced therapy in Hodgkin lymphomas. This complex approach integrates clinical, biological and therapeutic factors to optimize prognosis and provide each patient with the most effective and personalized therapy.

**Material and Method**

The study group consisted of 80 patients with classical Hodgkin's lymphoma, diagnosed over a period of 14 years (between 2005-2018) at the Hematology Clinic of the Colțea Clinical Hospital, followed until December 2019 or until death.

N.B.: the patients included in the study were those mentioned above, but those who presented all the necessary data for the complete statistical analysis were those from the period 2007-2017.

In the research, we randomly included patients diagnosed with classical Hodgkin's lymphoma, covering all four histological subtypes (according to the WHO classification) previously, but also during the course of the study, based on the inclusion criteria defined before their selection:

**Inclusion Criteria:**

- Correct and complete diagnosis of the type of lymphoma through the HP and IHC examination of the biopsy piece
- Presence of all evaluated parameters in the patients’ observation records.
It is noteworthy that the study cohort did not include patients who were lost to follow-up during the study or those diagnosed with nodular lymphocyte-predominant Hodgkin lymphoma.

Data collection was performed from the patients’ observation records, which were subsequently recorded in a case research sheet. The study design was both retrospective and prospective, from the time of diagnosis to death or the end of the study. The unit of observation was the individual. Both quantitative (measurable) and qualitative (non-measurable), alternative and non-alternative characteristics were studied.

**Diagnostic Criteria**

The study began with detailed analysis of patient identification data, including name, age, sex, background, living and working conditions, and year of Hodgkin lymphoma diagnosis. After diagnosis, the clinical and paraclinical parameters of the patients were determined and evaluated. A complex analysis was performed to identify possible correlations between clinical and histological features of the disease, assessing their impact on prognosis and establishing the corresponding therapeutic implications.

In order to establish clinico-histological correlations in patients with cHL, the cohort was subdivided based on the treatment response at the end of the study into two subgroups:

- **Group 1**: Comprised of 53 patients with favorable disease progression, who achieved complete remission after the first line of treatment.
- **Group 2**: Comprised of 27 patients with unfavorable disease progression or refractory cases, including those in partial remission, with progressive disease, or with stable disease after the first line of treatment.

The study focused on analyzing patient-related factors (age, sex, clinical performance status, significant medical history), tumor mass evaluation parameters (biopsy location, presence of B symptoms, Ann Arbor stage, number of affected lymph node areas, presence of bulky tumor masses, type and number of extranodal involvements, bone marrow involvement, hepatomegaly/splenomegaly, serum LDH levels, serum beta-2 microglobulin levels), prognostic factors related to stage, histological subtype, biological parameters (blood count, serum iron, ESR, serum albumin levels, presence of Epstein-Barr virus), treatment-related prognostic factors (type of induction chemotherapy, tumor mass reduction of more than 50% after the first cycle, use of radiotherapy, monoclonal antibodies, autologous hematopoietic stem cell transplantation, need for interim PET-CT), and new prognostic factors assessing disease progression (BCL2 overexpression in malignant cells, PD1 presence in lymphocytes, CD68 antigen in macrophages within the tumor microenvironment, and presence of EGFR).
Diagnosis of Hodgkin Lymphoma

The diagnosis of Hodgkin lymphoma was established through several stages:

- Histopathological and Immunohistochemical Diagnosis
- Clinical Stage Evaluation

Histopathological diagnosis was based on the analysis of lymph node or other tissue samples collected via incisional or excisional biopsy. The initial stage in establishing the histopathological diagnosis involved the anatomical-pathological examination of representative tumor fragments, which aimed to exclude non-hematological proliferations and benign lymphoproliferations while attempting to define the histological type. Subsequently, the immunophenotypic profile of the lymphoproliferation was determined through immunohistochemical tests on paraffin-embedded tumor preparations. Gene expression profiling in malignant cells and cytogenetic studies were not conducted due to lack of access to these methods, although they would have significantly improved prognostic prediction accuracy and facilitated personalized therapeutic approaches based on tumor microenvironment characteristics.

Study Protocol

The patient's anamnesis included identification data (name, surname, age, sex, place of origin, year of diagnosis).

Anamnesis assessed:

- Significant personal medical history.
- Living and working conditions.
- Clinical performance status.
- Onset mode with nodal or extranodal involvement.
- Presence of B symptoms (fever, profuse night sweats, weight loss of more than 10% of body weight over the past 6 months).
- Detection of complaints suggestive of extranodal involvement.
- Symptoms related to bone marrow involvement.
**Physical Examination** enabled:

- Examination of all lymph node areas (occipital, retro- and preauricular, submental, submandibular, laterocervical, supraclavicular, axillary, epitrochlear, inguinal, and popliteal) and description of adenopathy characteristics.
- Assessment of liver and spleen dimensions.
- Direct examination of oropharyngeal lymphoid tissue (Waldeyer’s ring) and/or indirect laryngoscopy.
- Identification of clinical signs suggesting extranodal involvement (salivary glands, skin, digestive system, orbit, thyroid, etc.).
- Detection of complications (infectious, autoimmune, metabolic, compressive, post-therapeutic).

**Clinical Performance Status Evaluation**

To perform a detailed evaluation of patients with Hodgkin's lymphoma, we highlighted clinical signs suggestive of extranodal determinations, such as involvement of salivary, skin, digestive, orbital, thyroid, and other glands. We also monitored and identified potential complications, including infectious, autoimmune, metabolic, compressive, and post-therapeutic.

Assessment of clinical performance status played a crucial role in establishing prognosis and choosing therapeutic strategy, illustrating the complex relationship between host and tumor. Performance status (SP) was rated numerically on a scale from 0 to 4, where a lower score indicates better health. According to Table no.10, ECOG groups 0-1 correspond to prognostic Karnofsky scores above 80, while ECOG groups 2-4 correspond to scores below 70 on the same Karnofsky scale.

**Staging of Lymphoma**

Staging of Hodgkin lymphoma, using the Ann Arbor classification (modified Cotswolds, 1989), was performed at the time of diagnosis to determine the extent of disease. This process is essential for establishing the prognosis and choosing the appropriate therapeutic protocol, distinguishing between the localized and the advanced form of the disease. This classification provides a standardized basis for assessing the extent of disease in different nodal and extranodal regions, thereby guiding clinical decisions for the optimal care of patients with Hodgkin lymphoma.

Thoraco-abdominal-pelvic CT examination and, less commonly, positron emission tomography (PET-CT), along with bone marrow aspiration-biopsy, were essential both for the initial staging of Hodgkin's lymphoma and for assessing response to treatment.
abdominal-pelvic CT allowed a detailed evaluation of the extent of the disease in various regions of the body, including the thorax, abdomen, and pelvis. Positron emission tomography (PET-CT) has provided additional information by metabolically detecting tumors, helping to identify affected areas that are not clearly visible on CT.

Bone marrow aspiration biopsy has been used to evaluate bone marrow involvement, being crucial for determining the stage of the disease and for monitoring the response to the administered treatment. These investigations have played a crucial role in the effective management of Hodgkin's lymphoma, ensuring that each patient receives the appropriate treatment based on their stage and response to therapy.

**Work Sheet**

The work sheet included the following information:

- General data (name, age, sex, environment of origin)
- Date of registration
- Personal pathological history
- Living and working conditions (occupational hazards/alcohol/tobacco)
- Reasons for hospitalization
- Disease history
- Clinical examination (ECOG performance status, presence of B symptoms, size and location of lymphadenopathy, degree of hepatomegaly, degree of splenomegaly, type and number of extranodal determinations)
- Routine biological tests: complete blood count with peripheral blood smear, ESR, fibrinogen, albumins, serum LDH, sideremia, alkaline phosphatase, liver tests (AST, ALT, GGT, bilirubins), kidney tests (serum uric acid, serum urea, serum creatinine)
- Beta-2 microglobulin
- Viral liver markers (HBsAg, anti-HCV antibodies), HIV
- Paraclinical explorations: Imaging explorations (mediastinum-heart-lung X-ray, abdominal ultrasound, PET-CT scan), lymph node biopsy and/or extranodal organ biopsy, histopathological and immunohistochemical examination, hematogenous bone marrow biopsy, marrow aspirate
- Biopsy site
- Clinical stage at diagnosis
- Diagnosis: (Histopathological, Immunohistochemical)
- Prognostic grouping (+/- calculation of IPI score for advanced stages)
- Applied treatment with doses, number of cycles, and possible side effects (polychemotherapy, radiotherapy, immunotherapy, autologous/allogeneic hematopoietic stem cell transplant)
• Treatment response (complete remission, partial remission, stable disease, progressive disease)
  • Duration of response to treatment
  • Relapse—site, time, applied treatment
  • Disease or post-therapeutic complications

Treatment Response

Treatment response was defined as complete remission (CR), partial remission (PR), progressive disease (PD), and stable disease (SD). Complete remission (CR) was defined as the disappearance of clinical signs of disease, normalization of paraclinical and imaging tests considered abnormal before treatment initiation. Patients whose tumor mass decreased by more than 75% and all other parameters normalized were also considered in CR. Partial remission (PR) was defined as a reduction of the nodal tumor mass by 50-75% of the initial volume, decreased spleen and liver size, with bone marrow examination positive or irrelevant. The term “stable disease” (SD) means that the disease has not worsened but has not improved after treatment either. Progressive disease (PD) means that the disease has worsened, the tumor load has increased, or lymphoma has spread during therapy or observation.

Study Evaluation

The response to treatment in the patients included in the study was evaluated after the first chemotherapy cycle and at the end of induction chemotherapy through clinical examination, repetition of biological tests, and imaging explorations considered abnormal at onset. The duration of response to treatment was measured in months and defined as the time from obtaining remission (CR or PR) to relapse, end of follow-up period, loss to follow-up, or death. Relapse was defined as the reappearance of the disease in patients who initially responded to induction therapy.

Statistical Data Processing

The statistical processing and interpretation of the obtained data were carried out using Microsoft Excel 2021 Professional (for some of the descriptive statistics charts), SPSS Statistics 15.0.0 (SPSS Inc – 2006) – for ANOVA analysis, Crosstabulation - Chi-square tests and some charts, survival time (Kaplan-Meyer), Odd Ratio (OR), and MedCalc Version 14.2.1 (1993-2014) for ROC and Cronbach’s Alpha analysis. A p-value < 0.05 was considered statistically significant.
Results

80 patients with a confirmed diagnosis of classical Hodgkin's lymphoma were selected from the database of the Hematology Clinic of the Colțea hospital, according to the following criteria:

- international WHO criteria
- analysis of a series of clinical and paraclinical parameters with a proven prognostic role
- potential prognostic factors noted in the dynamics of the disease evolution.

Response to therapy was tracked at various times during treatment between 2005 and 2018.

The entire cohort was divided into two subgroups:

- Group 1 = Patients with favorable outcomes (53 cases; 66.25%)
- Group 2 = Patients with refractory cases (27 cases; 33.75%)

This categorization was maintained throughout the entire analysis. It should be noted from the outset that all statistical processing was performed on the cohort of 80 patients. If the number of patients were larger, some conclusions might differ. It should also be noted that statistical significance is labeled as Sig in SPSS and p in MedCalc, the two statistical processing programs used in this analysis.

Almost all analyses were conducted by comparing the two groups: patients with favorable outcomes (Group 1) and patients with refractory outcomes (Group 2). All data existing in the database were analyzed using various methods, from age to survival/death.

We will now make brief comments only on those data for which the analysis was statistically significant.

- **Toxic Environment** - Influenced the distribution in the two groups with a statistical significance of $p = 0.008$. Of the 21 patients who lived/worked in a toxic environment, 12 (57%) were in Group 2 and only 9 (43%) in Group 1. This is an important risk factor with an OR = 3.911.

- **Disease stage** - The distribution of the four stages (I, II, III, IV) between the two groups shows a prevalence of the first three stages in Lot 1 and a prevalence of stage IV in Lot 2, with statistical significance $p = 0.003$. 


• **General signs** - p = 0.006. Half of the patients did not present general signs (47.5%), while the other half did (52.5%). The SG- / SG+ ratio is approximately 0.8 in Lot 1 and approximately 3 in Lot 2. Therefore, SG is also a risk factor with OR = 4.026.

• **Form** - We have 55 patients with an extensive form and 25 with a limited form. 60% of patients in Lot 1 have an extensive form, whereas in Lot 2, 80% of patients have an extensive form. p = 0.024 and OR = 3.773, thus Form is identified as an important risk factor.

• **Serum iron** - p = 0.033, OR = 2.870. The presence of serum iron constitutes a risk factor for the distribution of patients between the two groups.

• **ESR** - 74% of the 80 patients had elevated ESR. Among the 53 patients in Lot 1, 66% had elevated ESR, while in Lot 2, 89% of the 27 patients had elevated ESR. The differences are statistically significant with p = 0.028. Elevated ESR is a risk factor between the two groups, with OR = 4.114.

• **Lymphnode involvement** - Comparison of the number of cases is statistically significant with p = 0.001 and is an important risk factor with OR = 5.113.

• **Infections** - The statistical significance of the chi-square test is p = 0.027, indicating that infections influence membership in one of the two sub-lots. The estimated OR is 3.055 with p = 0.030, highlighting infections as a risk factor. The proportion of infections is higher compared to Lot 1 (44.44% versus 20.75%).

• **Neurological effects** - The proportion of neurological effects is higher compared to Lot 1 (18.52% versus 1.89%). The statistical significance of the chi-square test is p = 0.008, showing that neurological effects influence membership in one of the two sub-lots. The estimated OR is 11.818 with p = 0.028, emphasizing neurological effects as a significantly high-risk factor.

Regarding treatments, we have the following variables with statistical significance in the comparison between groups:

**First-line treatment** - ABVD was the predominant regimen (71.25%), with BEACOPP accounting for 28.75%. In Cohort 1, ABVD was administered in 64.15% of cases, while BEACOPP was used in 35.85%. In Cohort 2, ABVD constituted 85.19%, and BEACOPP
14.81%. The statistical significance was $p = 0.049$, indicating a correlation between treatment type and the two sub-cohorts.

**Number of treatment lines** - Patients receiving 1-2 lines of treatment were predominant in Cohort 1 (77.35%), whereas those with 3-5 lines were prioritized in Cohort 2 (55.56%). The number of treatment lines influenced cohort assignment significantly ($p < 0.001$ by Chi-square test).

**Brentuximab vedotin** - The use of Brentuximabvedotin was 48.15% in Cohort 2 compared to 7.55% in Cohort 1. There is a significant association between Brentuximabvedotin usage and cohort assignment ($p < 0.001$). OR = 11.375 indicates a high-risk factor.

**Final treatment response** - Across the entire cohort, over half of the patients (66.25%) achieved complete remission at the end of the study (after at least one line of treatment), while 1.25% achieved partial remission. Disease progression despite treatment occurred in 25.00% of cases, with 7.50% remaining stable. In Cohort 2 (unfavorable disease), 74.07% showed progressive disease, 22.22% stable disease, and only 3.70% partial remission. Significant statistical differences were found via Chi-square test ($p < 0.001$).

**Survival** - The survival rate was 82.50%, with 17.50% of patients deceased. Cohort 1 had 1 deceased patient, while Cohort 2 had 13. The majority of deaths occurred in Cohort 2. Chi-square test yielded $p < 0.001$ and OR = 48.286, indicating a very high-risk factor.

For estimating survival time and comparing between cohorts and sexes, Kaplan-Meier analysis was used.

Overall, the estimated mean survival time was 62.562 months, with a median of 59 months. In Cohort 1, the estimated mean survival was 63.750 months, median 62 months, and in Cohort 2, it was 58.90 months mean, median 49 months. There were no statistically significant differences between the two cohorts (all comparison tests $p > 0.05$).

For other variables such as sex, environment, smoking status, ECOG stage, age, histological subtypes, bulky tumor mass, Hb, Le, Ly, LDH, albumin, bone marrow involvement, comorbidities, EBV, and PET-CT, there were no statistically significant comparisons.

**Personal contribution**

I have devised an algorithm to calculate a Patient Evaluation Indicator (IEP) that is straightforward and aids in the initial allocation of patients into groups. The purpose of this algorithm is to mitigate initial subjectivity in patient allocation.

I analyzed the 22 variables coded as 0-1 in the database and added a 23rd variable encoding Stage as follows: 0 - stages I-II and 1 - stages III-IV. These 23 variables include Stage,
Bulky Tumor, Comorbidities, Gender, Living Environment, Lymph Node Involvement, Smoking, Toxic Environment, Morphology, General Signs, Hepatosplenomegaly, Hemoglobin, Leukocytes, Lymphocytes, ESR, LDH, Albumin, Beta 2mg, Infections, Neurological Effects, Serum Iron, Marrow Det., and Node Areas.

These 23 variables can be likened to a questionnaire where responses are either Yes or No. To assess the consistency of this set of variables, Cronbach’s alpha analysis was employed. It calculates an Alpha indicator for the entire set, sequentially removing each variable and recalculating Alpha. If the resulting Alpha is higher than that of the entire set, the variable may be considered for removal. A smaller difference suggests the variable is important and should remain.

Applying the Cronbach’s test resulted in the removal of the following variables: Bulky Tumor, Comorbidities, Gender, Living Environment, and LDH, as they were deemed non-significant for the overall consistency. This left us with 18 variables.

The IEP indicator is calculated as the sum of values assigned to these 18 variables. Theoretically, it ranges between 0 and 18.

The following table shows the final situation of the Cronbach’s Alpha analysis

<table>
<thead>
<tr>
<th>Variable dropped</th>
<th>Alpha</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extranodal determination</td>
<td>0.7944</td>
<td>-0.02298</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.8098</td>
<td>-0.007542</td>
</tr>
<tr>
<td>Lymphnodes</td>
<td>0.8074</td>
<td>-0.009932</td>
</tr>
<tr>
<td>Beta 2mg</td>
<td>0.8096</td>
<td>-0.007754</td>
</tr>
<tr>
<td>Bone marrow determination</td>
<td>0.8121</td>
<td>-0.005282</td>
</tr>
<tr>
<td>Neurological effects</td>
<td>0.8108</td>
<td>-0.006531</td>
</tr>
<tr>
<td>Form</td>
<td>0.8017</td>
<td>-0.01568</td>
</tr>
<tr>
<td>Smoker status</td>
<td>0.8169</td>
<td>-0.0004956</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.8153</td>
<td>-0.002072</td>
</tr>
<tr>
<td>Hepato/Splenomegaly</td>
<td>0.8122</td>
<td>-0.005172</td>
</tr>
<tr>
<td>Infections</td>
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<td>Leukocytes</td>
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<td>Lymphocytes</td>
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<td>Toxic environment</td>
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<td>General signs</td>
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<tr>
<td>Sideremy</td>
<td>0.7988</td>
<td>-0.01857</td>
</tr>
<tr>
<td>Stage</td>
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<td>-0.01568</td>
</tr>
<tr>
<td>ESR</td>
<td>0.8010</td>
<td>-0.01640</td>
</tr>
</tbody>
</table>
The calculated value $\text{Alpha}=0.8174$ is a very good value.

In the following table we have displayed these 18 variables ordered by the Alpha value calculated when they are omitted from the set, this being their importance in this set. For each of them, we calculated the tiebreaker criteria and its statistical significance through ROC analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cronbach’s alpha</th>
<th>ROC Analyze</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alpha</td>
<td>Change</td>
</tr>
<tr>
<td><strong>Extranodal determination</strong></td>
<td>0.794</td>
<td>-0.02298</td>
</tr>
<tr>
<td><strong>Sideremy</strong></td>
<td>0.799</td>
<td>-0.01857</td>
</tr>
<tr>
<td><strong>ESR</strong></td>
<td>0.801</td>
<td>-0.0164</td>
</tr>
<tr>
<td><strong>Form</strong></td>
<td>0.802</td>
<td>-0.01568</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td>0.802</td>
<td>-0.01568</td>
</tr>
<tr>
<td><strong>General signs</strong></td>
<td>0.806</td>
<td>-0.01134</td>
</tr>
<tr>
<td><strong>Lymphnodes</strong></td>
<td>0.807</td>
<td>-0.009932</td>
</tr>
<tr>
<td><strong>Beta 2mg</strong></td>
<td>0.81</td>
<td>-0.007754</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>0.81</td>
<td>-0.007542</td>
</tr>
<tr>
<td><strong>Neurological effects</strong></td>
<td>0.811</td>
<td>-0.006531</td>
</tr>
<tr>
<td><strong>Bone marrow determination</strong></td>
<td>0.812</td>
<td>-0.005282</td>
</tr>
<tr>
<td><strong>Hepato/Splenomegaly</strong></td>
<td>0.812</td>
<td>-0.005172</td>
</tr>
<tr>
<td><strong>Infections</strong></td>
<td>0.813</td>
<td>-0.004376</td>
</tr>
<tr>
<td><strong>Toxic environment</strong></td>
<td>0.813</td>
<td>-0.003956</td>
</tr>
<tr>
<td><strong>Leukocytes</strong></td>
<td>0.814</td>
<td>-0.003109</td>
</tr>
<tr>
<td><strong>Lymphocytes</strong></td>
<td>0.815</td>
<td>-0.002309</td>
</tr>
<tr>
<td><strong>Hemoglobin</strong></td>
<td>0.815</td>
<td>-0.002072</td>
</tr>
<tr>
<td><strong>Smoker status</strong></td>
<td>0.817</td>
<td>-0.000496</td>
</tr>
</tbody>
</table>

ROC Analyze in tabel Cronbach’ Alpha

The average value of the criteria is 7.66, i.e. 8, and we will use this value to separate the patients into the 2 groups. Therefore, patients with $\text{IEP} \leq 8$ will be in Lot 1 and those with $\text{IEP} > 8$ will be in Lot 2.

Comparing by the Chi square test the 2 batches Calculated batch*Initial batch, there is a statistically significant connection between them $p=0.008$.

We will present below a comparative synthesis of the statistical significances resulting from the chi-square analysis for the variables in the two batches: The initial batch and the calculated batch (using $\text{IEP}$)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Initial Lot (p)</th>
<th>IEP Lot (p)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.522</td>
<td>0.146</td>
<td>No significant statistical significance in both groups. An increase in age is observed in batch 2 in the calculated batch</td>
</tr>
<tr>
<td>Stage</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Extranodal det.</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Lymphnodes</td>
<td>0.024</td>
<td>&lt;0.001</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Smoker status</td>
<td>0.058</td>
<td>0.004</td>
<td>Statistically significant at IEP batch. Changes to the number of cases</td>
</tr>
<tr>
<td>Toxic environment</td>
<td>0.008</td>
<td>0.002</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Form</td>
<td>0.024</td>
<td>&lt;0.001</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>General signs</td>
<td>0.006</td>
<td>&lt;0.001</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Hepato/splenomegaly</td>
<td>0.048</td>
<td>0.008</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.934</td>
<td>&lt;0.001</td>
<td>Statistically significant at IEP batch. Changes to the number of cases</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>0.122</td>
<td>0.003</td>
<td>Statistically significant at IEP batch. Changes to the number of cases</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.306</td>
<td>0.012</td>
<td>Statistically significant at IEP batch. Changes to the number of cases</td>
</tr>
<tr>
<td>ESR</td>
<td>0.028</td>
<td>&lt;0.001</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.723</td>
<td>&lt;0.001</td>
<td>Statistically significant at IEP batch. Changes to the number of cases</td>
</tr>
<tr>
<td>Beta 2mg</td>
<td>0.086</td>
<td>0.007</td>
<td>Statistically significant at IEP batch. Changes to the number of cases</td>
</tr>
<tr>
<td>Infections</td>
<td>0.027</td>
<td>0.002</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Neurological effects</td>
<td>0.008</td>
<td>0.003</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Sideremy</td>
<td>0.033</td>
<td>&lt;0.001</td>
<td>Statistically significant in both types of lots. Changes to the number of cases</td>
</tr>
<tr>
<td>Bone marrow det.</td>
<td>0.241</td>
<td>0.001</td>
<td>Statistically significant at IEP batch. Changes to the number of cases</td>
</tr>
</tbody>
</table>
Comparisons in the calculation of Survival in the Kaplan-Meyer analysis between the two types of batches:
In the Initial Lot for Favorable Evolution- Average survival is $63.750 \approx 64$ months, and for Unfavorable Evolution the average is $58.900 \approx 59$ months. The comparison of the two is not statistically significant.
For the Lot calculated for Favorable Evolution we have the Average survival of $66.857 \approx 67$ months, and for Unfavorable Evolution the average is $55.979 \approx 56$ months. The comparison of the two is not statistically significant.

The final conclusion is that using the IEP for the distribution of patients in the two subgroups, the results of the statistical analysis are better with 2-3 exceptions. The method can be further refined, for example using modified algorithms from EQ3D or EQ5D.

**Bibliography**


