Diploma thesis

Distinct clinical, serological and histopathological parameters are associated with inferior survival of patients with primary central nervous system lymphoma

submitted by

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I. Statutory Declaration

I hereby formally declare that I have written the submitted thesis independently and without any outside support except for the quoted literature and other sources mentioned in the paper. I clearly marked and separately listed all literature and all the other sources which I employed when producing this academic work, either literally or in content.

Graz, 14.09.2023 Anna Mönch eh.

II. Acknowledgment

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III. Zusammenfassung

Hintergrund: Primäre Lymphome des zentralen Nervensystems (PZNSL) sind aggressiv wachsende und seltene, extranodal diffus großzellige B-Zell Non-Hodgkin-Lymphome. Sie machen ca. 4% aller neu diagnostizierten Hirntumore aus. Aufgrund des therapeutischen Fortschrittes müssen die meistgenutzten prognostischen Modelle, der IELSG- (International Extranodal Lymphoma Study Group) und MSKCC (Memorial Sloan-Kettering Cancer Center) -Score überarbeitet werden, um fortwährend eine gute Risikostratifizierung zu gewährleisten. Das primäre Ziel unserer Studie war es, den klinischen Nutzen bereits bestehender prognostischer Marker zu verifizieren, sowie neue potenzielle prognostische Faktoren für PZNSL Patient*innen zu identifizieren.

Material und Methoden: Wir inkludierten 74 neu diagnostizierte PZNSL Patient*innen in unsere Studie. Das mittlere Alter der Studiengruppe betrug 63 Jahre, davon waren 59.5% (n=44) männlich und 40.5% (n=30) weiblich. Sowohl das serologische als auch das histologische Material wurde vor Therapiebeginn entnommen. Wir führten eine retrospektive, univariate Überlebenszeitanalyse durch um klinische, serologische und histopathologische Parameter zu finden, die mit dem Gesamtüberleben assoziiert sind.

Ergebnisse: Auch in unserer Studienkohorte zeigte der MSKCC eine signifikante Assoziation mit dem Gesamtüberleben (p=0.01). Aufgrund fehlender Liquor Parameter konnten wir den IELSG weder anwenden noch analysieren. Die weiteren Untersuchungen zeigten, dass das Gehirn als primärer Entstehungsort des Tumors (Auge vs. Gehirn, p=0.013), ein hohes Alter (≤60 Jahre vs. >60 Jahre, p=0.009) und das frühe Fortschreiten der Krankheit (≤12 oder ≤24 Monate vs. >12 oder >24 Monate, p<0.0001) mit einem inferioren Überleben assoziiert sind. Darüber hinaus zeigten die folgenden serologischen Werte eine signifikante Assoziation mit unterlegenem Gesamtüberleben: Leukozytose (>11.3G/l, p=0.013), Neutrophilie (>7.7 G/l, p=0.0175), erhöhte GGT (>55U/l, p=0.037), Hypoalbuminämie (<3.5g/dl, 0.0048), Hypoproteinämie (<6.6g/dl, p<0.0001), erhöhter systemic immune inflammation index (p=0.0044), erhöhtes Neutrophilen-zu-Lymphozyten-Verhältnis (NLR, p=0.003), derived NLR (p=0.0009) und LDH-zu-Lymphozyten-Verhältnis (p=0.048). Zuletzt konnten wir den keimzentrumsartigen („germinal-center“) Subtyp (p=0.0463), einen hohen „double

expressor“ score (p=0.003), die fehlende Expression von CD30 (p=0.013), sowie die fehlende Expression von GCET-1 (p=0.0386) mit einem verkürzten Gesamtüberleben unserer Patienten in Verbindung setzen.

Diskussion: Unsere Daten indizieren, dass die hier genannten Parameter signifikant mit dem Gesamtüberleben der Patient*innen mit PZNSL assoziiert sind. Um die Ergebnisse zu verifizieren, planen wir multivariate Datenanalysen durchzuführen. Zusammenfassend, können die hier analysierten Faktoren für die Entwicklung zukünftiger prognostischer Modelle herangezogen werden.

IV. Abstract

Background: Primary central nervous system lymphoma (PCNSL) is an aggressive and rare extranodal subtype of Diffuse Large B−Cell Lymphoma (DLBCL), representing approximately 4% of all newly diagnosed brain tumors. Because of the development of novel therapeutic protocols, the two most commonly used prognostic scoring systems, the International Extranodal Lymphoma Study Group (IELSG) score and Memorial Sloan-Kettering Cancer Center (MSKCC) score need to be updated to improve the risk stratification of these patients. Thus, we aimed on verifying already established prognostic markers and on identifying new prognostic markers in PCNSL.

Material and Methods: We included 74 newly diagnosed PCNSL patients in our study, wherefrom 69 received therapy. The median age of our study group was 63 years and the distribution of gender was as follows: 59.5% (n=44) male and 40.5% (n=30) female patients. Our primary endpoint was the overall survival (OS) and we used univariate analyses to determine clinical, serological and histopathological parameters associated with OS. The data we used were collected from patients prior to treatment.

Results: In our cohort we observed the clinical usefulness of the MSKCC score in association with inferior OS (p=0.01). However, we could not use the IELSG score because we largely lacked liquor samples. By further investigation, we detected that the brain as primary manifestation site (p=0.013), advanced age (>60years, p=0.009) and progression of disease within 12 or 24 months (p<0.0001) were correlated with reduced OS. Furthermore, the following serum factors: leukocytosis (>11.3G/l, p=0.013), neutrophilia (>7.7G/l, p=0.0175), high systemic immune inflammation index (p= 0.0044), high GGT (>55U/l, p=0.037), hypoalbuminemia (<3.5g/dl, 0.0048), hypoproteinemia (<6.6g/dl, <0.0001), high neutrophil-to-lymphocyte ratio (NLR, p=0.003), high derived NLR (p=0.0009) and high LDH-to-lymphocyte ratio (p=0.048) were associated with a poor clinical outcome. Finally, we found an association of the germinal center subtype (p=0.046), the double expressor lymphoma status (p=0.003), the lack of CD30 expression (p=0.013) as well as the lack of GCET-1 expression (p=0.0386) with inferior OS in our patients.

Discussion: Our data indicate that the above listed parameters are associated with OS in PCNSL patients. Thus, these factors represent promising tools to develop a more effective prognostic model for risk stratification and that is why we are planning to perform multivariate analysis to strengthen our results.

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VI. Abbreviations and Definitions

ABC Activated B-cells
AIDS *Aquired immunodeficiency syndrome*
ALC *Absolute lymphocyte count*
ASCT *Autologous stem cell transplantation*
BBB *Blood brain barrier*
BCL-6 *B-cell lymphoma 6*
BCR *B-cell receptor*
BMI *Body Mass Index*
BTK *Bruton's tyrosine kinase*
CAR *Chimeric antigen receptor*
CBC *Complete blood count*
CD10 Neprilysin
CHOP *Cyclophosphamide, doxorubicin, vincristine and prednisone*
CNS Central nervous system
CNSL Central Nervous System Lymphoma
COO *Cell-of-origin*
CR *Complete remission*
CRP *C-reactive protein*
CRu *Unconfirmed CR*
CSF *Cerebrospinal fluid*, Cerebrospinal fluid
CXCL12 *C-X-C ligand 12*
CXCR4 *C-X-C chemokine receptor 4*
Da *Dalton*
DEL Double expressor lymphoma
DEL1 *Double expressor lymphoma status 1*
DEL2 *Double expressor lymphoma status 2*
DHL *Double-hit lymphoma*
DLBCL Diffuse large B-cell lymphoma
DNA *Deoxyribonucleic acid*
dNLR *Derived NLR*
EBV *Epstein Barr Virus*
ECOG Eastern Cooperative Oncology Group
ESR *Erythrocyte sedimentation rate*
FISH Fluorescent in situ hybridization
GC Germinal center
GCB Germinal center B-cell-like
GCET-1 *germinal center B-cell-expressed transcript-1*
GEP Gene expression profiling
H *Heavy chain*
HD *High-dose*
HD-MTX *High dose methotrexate*
HIV *Human immunodeficiency virus*
I_E *Involvement of a single extralymphatic organ or site*
IELSG *International Extranodal Lymphoma Study Group experience*
Ig *Immunoglobulin*
Ig_H *Immunoglobulin heavy chain*
Ig_L *Immunoglobulin light chain*

IHC Immunohistochemistry
IL-1 *Interleukin-1*
IL-10 *Interleukin-10*
IL-6 *Interleukin-6*
IPI *International Prognostic score*
IRF4 *Interferon regulatory factor 4*
KPS *Karnofsky performance status*
L *Light chain*
LDH *Lactate dehydrogenase*
LMR *Lymphocyte to monocyte ratio*
MDSCs *Myeloid-derived suppressor cells*
MMSE *Mini Mental State Examination*
MRI *Magnetic resonance imaging*
MSKCC *Memorial Sloan-Kettering Cancer Center*
MUM1 *Multiple Myeloma oncogene 1*
NCCN *National Comprehensive Cancer Network*
NCCN-IPI *National Cancer Network IPI*
NCCS-NNI *National Cancer Center Singapore-National Neuroscience Institute*
NHL *Non-Hodgkin-Lymphoma*
NK *Natural Killer*
NLR *Neutrophil-to-lymphocyte ratio*
NMR *Neutrophil-to-monocyte ratio*
NR *Not reached*
OS *Overall survival*
PCNSL Primary central nervous system lymphoma
PD *Programmed cell death protein*
PD-1 *Programmed death 1*
PD-2 *Programmed death 2*
PD-L1 *Programmed cell death ligand 1*
PD-L2 *Programmed cell death ligand 2*
PFS *Progression free survial*
PIOL *Primary intraocular lymphoma*
PLR *Platelet-to-lymphocyte ratio*
PNS *Peripheral nervous system*
PR *Partial remission*
PS *Performance status*
rdWBRT reduced-dose Whole-Brain Radiotherapy
R-IPI *Revised IPI*
R-MVP *Rituximab, methotrexate, vincristine, procarbazine*
RPVI *Reactive perivascular T-cell infiltration*
SII *Systemic inflammation index*
SIRI *Systemic inflammation response index*
SLOs *Secondary lymphoid organs*, *Secondary lymphoid organs*
SPSS *Statistical Package for Social Sciences*
THL Triple-hit lymphoma
TMZ *Temozolomide*, Temozolomide

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1 Introduction

Lymphoma is a neoplasm that arises from a clonal proliferation of a lymphocyte within the body's immune system.(1) Lymphomas are broadly divided into two different groups, the Hodgkin`s-lymphoma and the Non-Hodgkin lymphoma.(2,3)

Only about 10% of all lymphomas are Hodgkin`s lymphomas.(3,4) They are usually B-cell lymphomas, characterized by the presence of Reed-Sternberg-cells. There is a bimodal age distribution with a predominance seen in younger patients aged 20-30 years.(5–7) Due to the development of a stage-adapted treatment, more than 80% of these patients can be cured from this type of cancer. (8)
The Non-Hodgkin-Lymphoma (NHL) on the other side represents the most frequent hematologic malignancy and constitutes a heterogeneous group of more than 80 different subtypes. Non-Hodgkin lymphomas derive from either B- or T-cells and are hence divided into two major groups. The B-cell lymphomas which account for about 85% of all NHLs and the natural killer (NK)/ T-cell lymphomas.(9) Due to the great variety of NHLs, there is a broad spectrum of treatment strategies and the cure rates differ widely. Unfortunately, some NHLs remain incurable up until now. Therefore, clinical trials are constantly submitted, aiming on identifying more promising therapeutic approaches for this disease. (10)

1.1 B-cell function and development

Non-Hodgkin B-cell lymphomas use the biologic features of normal B-cells. Therefore, the function of the lymphoma highly depends on the differentiation state of the B (*b*one marrow derived)- lymphocyte (B-cell) it originated from.(11)

B-cells are cells with diverse cell surface immunoglobulin (Ig) receptors that recognize specific antigenic epitopes. Their main function is the secretion of antibodies, targeting invasive pathogens. Furthermore, they activate T-cells through antigen presentation and cytokine production, influencing both the humoral and cellular immune response.(12,13)

The B-cell evolves from a hematopoietic stem cell, which originates from the bone marrow and runs through sequential maturation steps. The B-cell develops within the bone marrow and traverses through the following stages: pro-B-cell to pre-B-cell to an immature B-cell, which enters the circulation.(14,15)

Simultaneously, a rearrangement of hundreds of genes, the V(D)J genes takes place during this early B-cell development. This rearrangement is crucial for the immune system to generate the vast repertoire of antibodies to fight the biologic diversity of invasive pathogens. The genes, namely variable (V), diversity (D) and joining (J), get cut, rearranged and ligated in an ordered fashion. The exons within these gene segments encode for the antigen binding domains of the immunoglobulins and are responsible for the antigen receptor diversity. Structurally, immunoglobulins are composed of two light chains (IG_L) and two heavy chains (IG_H).Therefore, within this recombination process a rearrangement of both immunoglobulin heavy (Ig_H) and light (Ig_L) chain gene segments occur.(11,13–21) In order to mediate this recombination process the V(D)J recombinase, a collection of diverse enzymes chooses a pair of segments, causes double-stranded breaks to each segment, deletes the intervening DNA and ligates them together. This rearrangement is tightly regulated and starts at the stem cell state as depicted in Figure 1. D_H joins J_H before V_H joins the rearranged D_HJ_H segment to form a functional heavy chain protein (Ig) in a late pro-B-cell. Hereafter, the light chain recombination follows, as D_L joins J_L. These light chains are than associated with the recombined heavy chains to form the pre-B-cell receptor, which can be found on the surface of a large pre-B-cell. Consequently, V_LJ_L rearrangements allow for the production of a complete functional B-cell receptor (BCR). This BCR is expressed as an IgM on the surface of an immature B-cell and yields a unique specificity. Whenever the B-cell has reached the stage of an immature B-cell, it is released into the circulation and migrates towards the secondary lymphoid orangs (SLOs), such as the spleen and lymph nodes. (11,13–21)

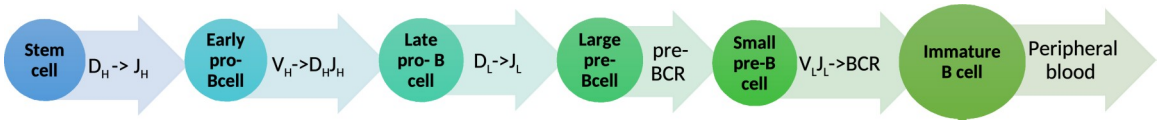


Figure 1 Simplified presentation of the B-cell development within the bone marrow(19)

Meanwhile, the B-cell develops through transitional stages (T1 and T2) before it becomes a mature B-cell.(14) The activation of a B-cell occurs within the SLOs as an antigen binds

to the BCR of the B-cell. Hereafter the B-cell has two main developmental possibilities as graphically demonstrated in Figure 2. One is the plasmocytic differentiation, forming an IgM secreting plasma cell that is short-lived but enables a rapid response to the antigen. The other possibility entails the formation of a germinal center (GC) within the lymphoid tissue. The GC is organized into two functionally and physically distinct zones, namely the dark and light zone. The reaction which takes place within the GC is the basis of T-dependent humoral immunity.

The primary function of the GC is the production of high-affinity antibodies through plasma cells and memory B-cells. Within the GC, B-cells undergo rapid proliferation as so-called centroblasts in the dark zone of the germinal center.

Throughout the maturation process within the dark zone, somatic hypermutations cause the Ig-variable region to change its affinity towards the antigen.

Afterwards, the centroblasts repeatedly enter the light zone, transforming into non-dividing centrocytes. The centrocyte processes the antigen in order to present it to T-cells. It undergoes selection and may then convert back into a proliferating centroblast or turns into a memory B-cell or plasma cell, based on the BCR affinity and access to T-helper-cells. At the same time, class switch recombination induces changes of the heavy chain, converting IgM to IgA, IgE or IgG.(14,15,23–26)

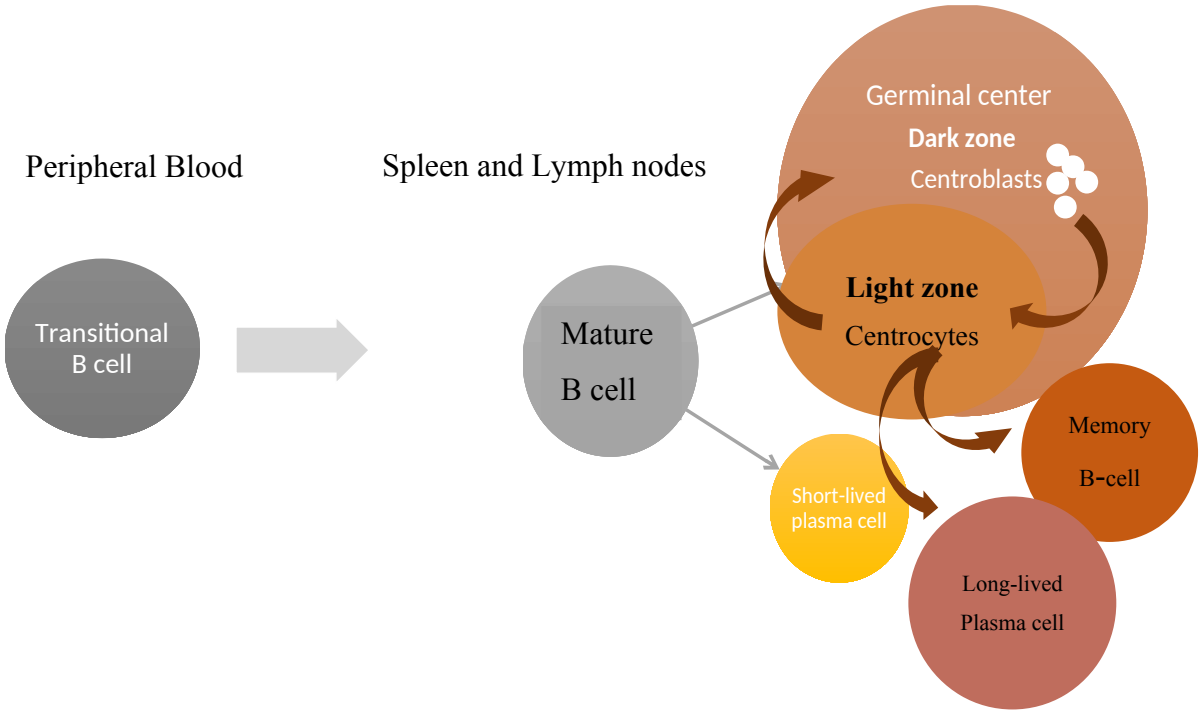


Figure 2 Graphic representation of B-cell development(22)

To establish and maintain the function of a germinal-center, several transcription factors such as the B-cell lymphoma 6 (BCL-6) and interferon regulatory factor 4 (IRF4) are needed. BCL 6 is a repressor of transcription and has many genetic targets. For instance, it mediates the suppression of sensing DNA breaks associated with somatic hypermutations. Consequently, BCL 6 translocations or mutations that occur during the GC-reaction or alterations of genes that regulate the expression of BCL-6 lead to a persistent activity of BCL 6, promoting the development of GC-derived lymphomas. On the other hand, IRF4 also known as Multiple Myeloma oncogene 1 (MUM1) is needed and upregulated as germinal center B-cells differentiate into plasma cells and regulates the immunoglobulin class switch recombination. IRF4 rearrangement can result in a strong IRF4 expression which has been described to be found in numerous B-cell lymphomas.(13,14,25,27,28)
Moreover, the necessity of repetitive DNA breaks during the V(D)J recombination process in the early B-cell development may lead to errors, causing subsequent alterations of survival and proliferation of these cells. V(D)J-driven oncogenic events have been discovered in lymphoid neoplasms and contributes to B-cell lymphomagenesis as well. (21)

Aberrant events during the B-cell development can give rise to B-cell derived lymphomas such as follicular lymphoma, Burkitt's lymphoma and Diffuse Large B-Cell Lymphoma. All of them carry the differentiation program of the original B-cell they derived from. Chromosomal mutations cause them to display antiapoptotic properties and the activation of oncogenes allows the cell metabolism and growth to subvert the differentiation program of the normal B-cell.(11,21)

1.2 Diffuse Large B-Cell Lymphoma

The Diffuse Large B-Cell Lymphoma (DLBCL) is an aggressive B-cell lymphoma. It accounts for 30% of all adult non-Hodgkin-lymphomas and is the most common lymphoid malignancy worldwide. This lymphoproliferative disease may appear in patients of all age groups but most commonly presents at a median age of 60 years with a slight male predominance.(29) The group of DLBCLs is very heterogeneous, showing differences in clinical behavior, morphology, immunophenotype and molecular mechanisms. This wide

variety makes it crucial to identify and distinguish the different groups, aiming on specifying and improving prognostic factors and treatment strategies.(30,31)

One approach to differentiate the diverse group of DLBCLs is based on gene expression profiling (GEP), which divides DLBCLs into two groups according to their cell-of-origin (COO). These subgroups are morphologically overlapping but show distinct gene expression patterns, indicating them to originate from different B-cell differentiation stages. The Germinal Center B-cell-like (GCB) DLBCL exhibits genes that are similar to those observed in a normal germinal center B-cell, showing a high expression of BCL-6 and hypermutated immunoglobulin genes. The second group on the other hand, resembles an activated B-cell and is therefore classified as activated B-cell (ABC) DLBCL. Similar to activated B-cells that are in the transition of being differentiated into plasma cells, they show an activation of NF-κB as well as BCR signaling pathways and upregulated genes that are needed for plasmocytic differentiation.(30–33) However, about 15% of the DLBCLs remain unclassifiable according to this COO classification.(1)

New immunohistochemical (IHC) algorithms have been developed trying to surrogate GEP. One of them, the Hans algorithm used antibodies targeting neprilysin (CD10), MUM-1/IRF4 and BCL-6 and identified two groups: the GCB and non-GCB group. The markers of the GCB group are BCL-6 and CD10. Thus, cells with positivity for either BCL-6 or CD10 or both, were assigned to the GCB group. On the contrary, if both markers were negative, the case was assigned to the non-GCB group. Plasma cells and late stages of the B-cell development display MUM-1, which is associated with the ABC group in GEP studies. Thus, if lymphomas stain positive for BCL-6 but negative for CD10, MUM-1 determines the group.(34) However, the Hans algorithm shows reproducibility issues. Thus, its utility is still a matter of debate.(34,35) Therefore, new antibodies have been developed, expanding the IHC panel and establishing new algorithms such as “Choi”, “Visco Young” and “Tally” to better discriminate between non-GCB and GCB DLBCL and therewith improving the replicability.(36–38)

Moreover, a digital gene expression test named Lymph2Cx was established, using formalin-fixed paraffin-embedded tissue, assigning 20 genes to either the GCB or ABC subtype. This test shows to have a high concordance and consistency with GEP results and may in future facilitate the integration of GEP into daily practice.(35,39)

Beside these COO studies, gene rearrangements affecting oncogenes and tumor suppressor genes are investigated using fluorescent in situ hybridization (FISH). In about 10% of

newly diagnosed DLBCL, a MYC translocation can be observed. MYC is a proto-oncogene and regulates the expression of genes that are involved in the cell cycle, metabolism, protein synthesis and the repair of DNA damage. Hence, its rearrangement leads to a dysregulated cell survival and proliferation.(40,41) In 50% of these cases an additional rearrangement of the antiapoptotic proto-oncogene BCL-2 and/or its transcription repressor BCL-6 could be detected. If a lymphoma presents both MYC and BCL-2 and/or BCL-6 translocations, it is assigned to be a double-hit or triple-hit lymphoma (DHL/THL), accordingly. These DHLs and THLs are classified as high-grade B-cell lymphomas.

Moreover, immunohistochemical studies detected a concurrent overexpression of BCL-2 and MYC proteins in one third of all diagnosed DLBCLs. This subtype of DLBCLs, without the above-mentioned rearrangements that define a DHL are characterized as Double Expressor Lymphomas (DEL).(41–43)

1.3 Extranodal Diffuse Large B- Cell Lymphoma

The majority of DLBCLs originate in lymph nodes. If these show extranodal involvement they are classified as secondary extranodal DLBCLs. In contrast, only one third of all primary NHLs arise from an extranodal origin. These primary extranodal DLBCLs represent a distinct entity due to a different molecular pathogenesis and clinical presentation.(44) The most commonly involved extranodal site is the gastrointestinal tract but lymphomas may also evolve from the skin, soft tissues, bone, central nervous system (CNS) and testis.(45,46)

1.4 Primary CNS lymphoma

1.4.1 Definition

The primary central nervous system lymphoma (PCNSL) is a highly aggressive and rare subtype of an extranodal non-Hodgkin lymphoma. It is strictly confined to the brain, leptomeninges, cerebrospinal fluid (CSF) and eyes, without the involvement of extracranial structures.(47) The primary intraocular lymphoma (PIOL) is a subtype of PCNSL and initially develops within the retina, optic nerve or vitreous chamber with or without CNS

involvement.(48) Histologically, about 90 to 95% of all PCNSLs are identified as DLBCLs, the rest being Burkitt, low-grade or T-cell lymphomas.(47,49)
Since 2017, the WHO classification of lymphoid and hematopoietic tissue tumors, recognizes PCNSL as a separate entity.(50)
Despite existing sensitivity to both chemo- and radiotherapy, the survival of patients with PCNSL is inferior to that of lymphomas outside the brain. Although prognosis has improved over the last decades, relapse is still common and the 5-year survival rate is only at about 30 to 40%.(51)

1.4.2 Epidemiology

The PCNSL has an incidence of about 0.5/100 000/year and accounts for 4% of all primary CNS tumors and 4-6% of all extranodal lymphomas.(50,52) This malignancy is typically seen in patients of age 65 or older and has a male predominance (sex ratio male to female 1.35:1).(53,54) It is important to distinguish PCNSLs arising in immunocompetent patients from those occurring in immunosuppressed persons due to HIV/AIDS, chronic intake of immunosuppressive drugs or in organ transplant recipients.(55) It has been observed, that the overall incidence of PCNSL increased prior to 1995, whereafter it declined primarily in the younger age groups. This is presumably associated with the reduction of AIDS cases since then.(56) On the contrary, a rising incidence has been described in the elderly population, especially in those aged 60 years with the highest incidence in 70 to 79 year-old patients.(53) Nowadays, this age group compromises more than 50% of all patients with PCNSL. This constitutes a major challenge in regard to optimal treatment standards. (57)

1.4.3 Etiology

Most PCNSLs occur sporadically and the etiology of the CNS lymphomagenesis is still not clear. However, less than 10% of the patients harbor predisposing factors that compromise the immune system. Both acquired and primary immunodeficiency have been recognized as a significant risk factor for PCNSL.(58,59) The lifetime risk for PCNSL increases by 4% in patients with Wiskott-Aldrich syndrome, ataxia-telangiectasia and severe-combined or common-variable immunodeficiency.(60) Furthermore, patients undergoing renal

transplantation harbor a lifetime risk of 1 to 2% and patients receiving cardiac, lung or liver transplants have a 2 to 7% risk.(61) Furthermore, patients who have AIDS associated with a very low CD4 T-cell count have a far greater risk of developing PCNSL when compared with the general population.(62,63) Typically these immunosuppressive states have been characterized by the presence of an Epstein Barr Virus (EBV) infection. In contrast, immunocompetent patients with PCNSL rarely present with an EBV infection, suggesting it to represent an alternative immunobiological entity.(58)

1.4.4 Clinical presentation

The signs and symptoms of PCNSL depend on the location of the primary lesion and are summarized in Figure 3.(55) Most commonly, the symptoms are rather unspecific and progress rapidly.(53) The majority, about 70% of the patients present with focal neurological deficits and about 43% display nonspecific neuropsychiatric symptoms. Furthermore, 33% of all patients harbor signs and symptoms such as headache, nausea, vomiting and confusion, due to a rising intracranial pressure.(64) By contrast, seizures only occur in 14% of the patients because PCNSLs tend to spare the gray matter.(65) The typical B-symptoms (sweats, fever and weight loss) that are commonly observed in nodal lymphomas are rather uncommon in PCNSL. Patients with leptomeningeal involvement might present with meningism and radicular symptoms.(66) Sometimes the lymphoma infiltrates the peripheral nervous system (PNS), leading to peripheral neuropathy and radiculopathy, designated as neurolymphomatosis. Spinal cord involvement may lead to asymmetric motor and sensory deficits of arms and legs, as well as to bladder or bowel dysfunction. Additionally, the ocular involvement could clinically present as an intraocular inflammatory process, such as a chronic uveitis. These patients often complain about a blurry vision, floaters and less frequently about ocular pain and photophobia.(48,55)

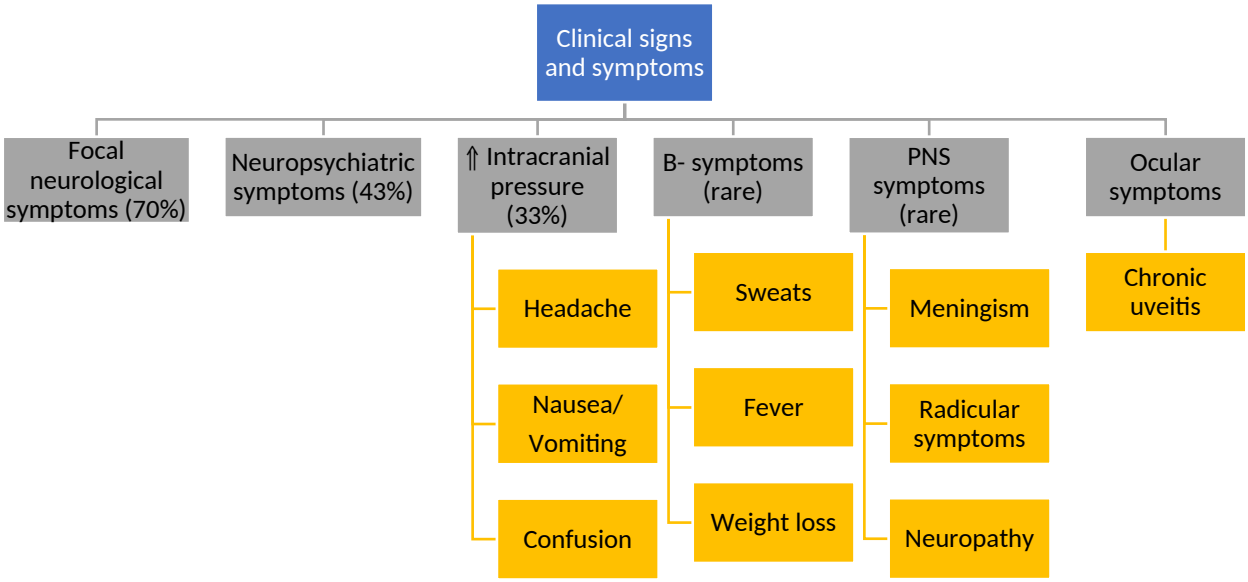


Figure 3 Graphic presentation of possible clinical manifestations in PCNSL(53)

1.4.5 Diagnosis

If a patient presents with the above mentioned symptoms, the diagnostic modality of choice is a contrast-enhanced magnetic resonance imaging (MRI).(67) The lesion can be multifocal or solitary. The tumor is frequently located in the periventricular region, predominantly within the frontal lobe but involves other lobes as well. It might affect the corpus callosum, basal ganglia, brainstem, cerebellum and less commonly the spinal canal. (55,68) The lesion often presents iso-to hypointense on T1-weighted and iso-to hypointense on T2 weighted MR images. Furthermore, it enhances homogeneously and shows a significant diffusion restriction due to a high cellularity.(55,64)

Nonetheless, the appearance on imaging cannot be distinguished from other cerebral processes. Besides, PCNSL lesions may contain atypical features such as a cyst, necrosis and hemorrhage and may not enhance on contrast imaging at all.

Thus, imaging-guided stereotactic needle biopsy is required to obtain a histopathologic confirmation.(53,69) Importantly, a treatment with corticosteroids should be avoided prior to biopsy because it has a lymphocytic effect, leading to a significant reduction in the sensitivity of the biopsy.(53,70)

After confirmation of the PCNSL diagnosis, a systemic disease should be excluded. This requires a thorough examination of peripheral lymph nodes and testes in men, including ultrasonography as well as a whole-body computed tomography or positron emission tomography and a bone marrow biopsy.(54,69,71)

Up to 20% of all patients with a PCNSL show an ocular involvement. Therefore, the eyes are examined using a slit lamp, ophthalmoscope and if needed, a vitreous biopsy in all patients during staging. When a vitreous biopsy is performed, it should always be combined with a sub-retinal aspirate or chorioretinal biopsy as the diagnostic failure rate of this diagnostic modality is up to 30%.(67)

Additionally, a leptomeningeal involvement can be verified through a positive cerebrospinal fluid cytology, flow-cytology and imaging techniques. The latter, showing enhancement of the cranial nerves, leptomeninges or the periventricular region.(72) This described chronology of diagnostic steps is graphically demonstrated in Figure 4.

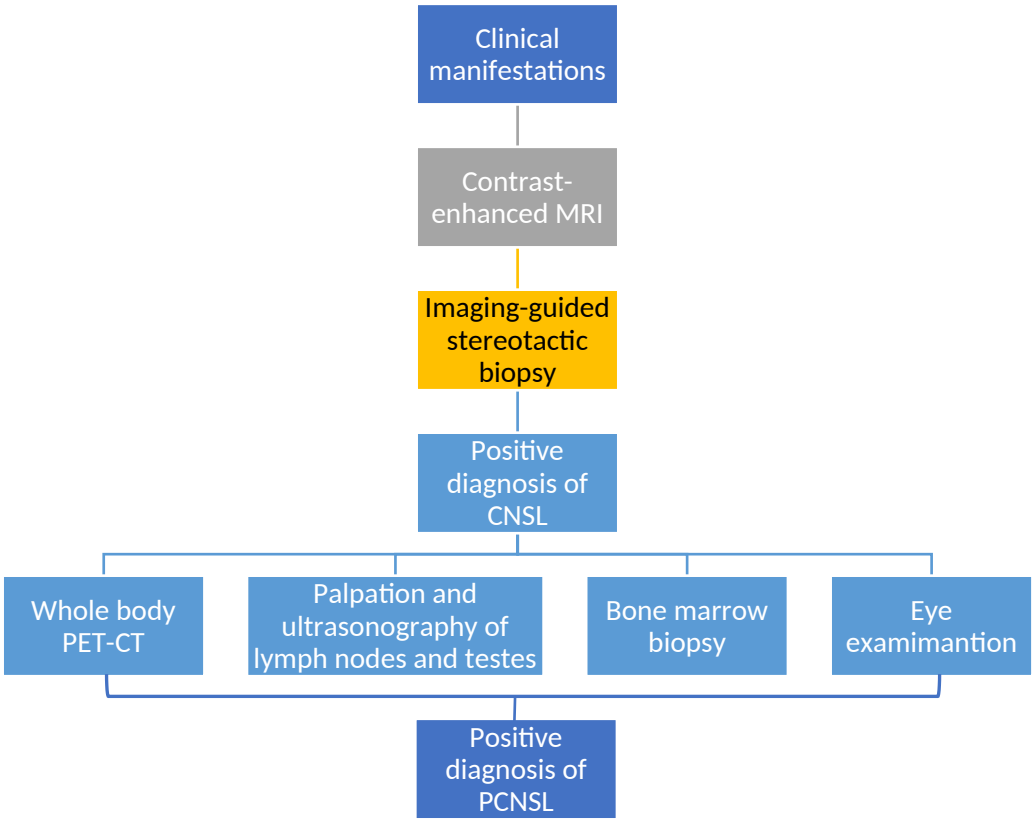


Figure 4 A graphic representation of a simplified version of the diagnostic procedure of PCNSL(53) CNSL: Central Nervous System Lymphoma

The performance of a stereotactic brain biopsy is potentially dangerous for the patient and technically demanding. Therefore, research has focused further attention on finding new diagnostic biomarkers.(73)

1.4.5.1 Serological Studies

Generally, serological studies cannot be used to diagnose PCNSL but should be performed in every patient. The evaluation of serum parameters can deliver important prognostic information and some parameters might support the diagnosis of the suspected underlying disease. First of all, the presence of an HIV infection should be tested as HIV-positive patients harbor an increased risk for PCNSL. One example of a prognostic serum parameter is lactate dehydrogenase (LDH). It is a marker of rapid cell turnover and can be elevated in non-Hodgkin lymphomas. However, in absence of an established diagnosis of lymphoma it is highly unspecific.(72) Furthermore, the liver as well as renal function should be monitored prior to treatment initiation as methotrexate, a commonly used drug in PCNSL might cause renal as well as hepatic toxicity.(69)

1.4.5.2 $ß_2$-Microglobulin

Another indicator for a high cell turnover is β_2-microglobulin. It is a HLA antigen which can be found on cell surfaces and if elevated, has been correlated with the presence of both systemic and CNS lymphoma. Especially in patients with CNS involvement, the β_2-microglobulin measured in the cerebrospinal fluid was found to be elevated. Nevertheless, its specificity is low as it may also be increased in other neurological diseases. High serum $ß_2$-microglobulin levels have proven to entail prognostic power in patients with PCNSL. It has been hypothesized, that increased levels correlate with high tumor burden. Nevertheless, the underlying mechanism of the negative impact on survival remains unclear.(74–76)

1.4.5.3 Cerebrospinal fluid (CSF) Studies

Aside from the above-mentioned CSF $ß_2$-micoglobulin-studies, research has focused further attention towards finding diagnostic CSF markers to facilitate early diagnosis of

PCNSL. If one day, the analysis of the spinal fluid could confirm the diagnosis of PCNSL, this diagnostic procedure would promise to cause lower complication rates, as compared to the cerebral biopsies.(77) More than 80% of the people diagnosed with CNS lymphoma have at least one abnormal CSF parameter. HIV-positive patients are screened for Epstein-Barr-Virus DNA because its presence makes PCNSL more likely.(78) Routinely investigated is the cell count, protein and glucose level, but those parameters cannot reliably confirm a CNS involvement.

In contrast, the CSF cytology studies examine the morphology of the cells and can detect neoplastic lymphocytes and provide diagnostic information. The CSF flow cytometry is used to identify the immunophenotype of lymphocytes and changes the diagnostic ability in high risk patients for the better.(72)

In addition, studies on interleukins, chemokines, receptors and miRNAs expressed in the CSF are ongoing. As for example, Interleukin-10 (IL-10), a growth factor for B-lymphocytes and Interleukin-6 (IL-6), responsible for lymphoid cell growth and immune regulation have been found to be increased in the presence of PCNSL and in comparison to other brain tumors.(79) Furthermore, osteopontin a proinflammatory cytokine, activating immune cells and causing migration as well as proliferation of B-cells has shown to be elevated in CSF samples of patients with CNS lymphoma and in contrast to inflammatory brain diseases.(80) Additionally, neopterin, a nonspecific T-helper cell-related marker which is often increased in inflammatory brain diseases was found to be significantly elevated in patients with PCNSL and in contrast to other brain tumors.(73)

Beyond that, there is a CXC chemokine ligand, named CXCL 13. This ligand is responsible for the migration of B-cells and has been identified to be highly specific for the diagnosis of PCNSL.(81) Micro-RNAs are suggested to play a key role in genetic pathways, controlling the differentiation, proliferation and apoptosis of cells. In CSF samples of PCNSL patients three microRNAs: miR-29, miR-19 and miR-92a were found to be present and demonstrated high specificity and sensitivity.(82)

To summarize, many of these biomarkers have demonstrated diagnostic power and may be helpful for diagnosis in patients where biopsy is not possible or inconclusive. As for now, diagnosis is still dependent on the biopsy result but further effort should be put on the identification of disease markers as this could accelerate the diagnostic procedure.(83)

1.4.6 Differential Diagnosis

The most significant differential diagnoses of PCNSLs are malignant gliomas, metastasis and multiple sclerosis, MRI studies cannot differentiate those lesions reliably. Nevertheless, imaging can give hints that might help to guide clinicians towards the diagnosis of PCNSL.

In general, if lesions involve both hemispheres some of the differentials that must be considered are gliomas, CNSLs or demyelinating diseases. Gliomas and metastasis show a higher degree of edema and mass effect as compared to PCNSL. In contrast, PCNSL shows a more restricted diffusion owed to a high cellularity. Tumefactive multiple sclerosis on the other hand, has a minimal mass effect regarding lesion size. Furthermore, it has smaller restricted diffusion rates compared to gliomas as well as CNSLs and presents with an incomplete rim enhancement.(65)
Despite the high sensitivity of MRI and the possibility to discriminate PCNSL from other tumors through diffusion-weighted imaging, the specificity of this technique remains moderate.(84) As imaging is important not only for diagnosis, but staging and response assessment and only a subset of MRI features are currently used in the daily practice, these are further investigated and continued to be optimized.(85) Meanwhile, new imaging modalities are under investigation, such as CXCR4-targeted PET imaging with ^{68}Ga-pentixafor. C-X-C chemokine receptor 4 (CXCR4), is a chemokine receptor with the sole C-X-C ligand 12 (CXCL12). This chemokine is important for survival, growth and dissemination of aggressive B-cell lymphomas. As PCNSL cells display high expression of CXCR4, this modality is proposed to be of additional value. It provides high contrast images and the initial uptake of CXCR4 could as well be correlated with treatment response.(84)

1.4.7 Histopathology and molecular pathophysiology

PCNSLs are mature B-cell lymphomas characterized by Pan-B-cell markers (CD19, CD20, CD22 and CD79a) as well as germinal center (BCL-6 and less commonly CD10) and late germinal center B-cell markers (MUM-1).(60,71)

Microscopically, the tumor cells are mainly composed of centroblasts, infiltrating the neural parenchyma with invasive, diffuse or perivascular growth patterns.(86) Typically, these cells have a high proliferation rate (Ki-67-Index >70%) and accumulate around cerebral blood vessels in rings, constituting the perivascular growth pattern. This pattern has been associated with a more rapid disease progression and inferior prognosis in some studies.(87)

In addition, a reactive perivascular T-cell infiltration (RPVI) could be observed in about 36% and was associated with a better outcome, as compared to RPVI-negative tumors. (71,88)

Several studies showed that most PCNSLs, when investigated by the immunohistochemistry-based Hans algorithm, exhibit the ABC-like phenotype, entailing $CD10^{-}$ $BCL\text{-}6^{+}$ $MUM1^{+}$, $CD10^{-}$ $BCL\text{-}6^{-}$ $MUM1^{+}$, and $CD10^{-}$ $BCL\text{-}6^{-}$ $MUM1^{-}$ cells. (34,89) Using gene expression profiling, the tumor cells most commonly resemble late germinal center B-cells, verified by the co-expression of MUM-1 and BCL-6.(86,90)

Frequently, genetic alterations of the B-cell-receptor pathway such as CD79B, toll-like-receptor, CARD11 and MYD88 and the NF-κB pathway are found. These somatic hypermutations trigger lymphocyte proliferation and prevent apoptosis, thus indicating them to play a major role in the development of PCNSL.(71,86)

1.4.8 Treatment

One of the major challenges in the treatment of PCNSL is the blood brain barrier (BBB), a physical barrier, protecting the brain from metabolites, microorganisms and chemicals. This allows only small molecular weight molecules with 400-600 Da to penetrate efficiently the BBB, resulting in a low delivery of therapeutic agents to the brain. On top of that, the brain constitutes an immune-privileged site, maintaining a tightly regulated immunological niche. Consequently, patients with PCNSL have a poorer prognosis as compared to patients with systemic DLBCL.(91–93) Furthermore, the low incidence of PCNSL and the requirement of stereotactic brain biopsy for the definite diagnosis have led to far fewer randomized clinical trials on PCNSL as compared to non-CNS lymphoma.(92) Moreover, the median age of patients diagnosed with PCNSL is 65 years and more than 20% of all patients are over 80 years old.(53) Older patients have physiological deficits, altered pharmacokinetics and pharmacodynamics, leading to an increased risk for drug

toxicity. Therefore, treatment is now based on age and performance status (PS), allowing elderly patients to receive optimal treatment. As for instance, fit elderly patients might tolerate normal treatment strategies and dosages.(53) However, finding the balance between prolonging survival and minimizing toxicity remains challenging. Besides, only few prospective studies are dedicated to elderly patients, thus optimal management continues to be poorly defined.

Since PCNSL is sensitive to both chemo- and radiotherapy, treatment strategies in the early 1970s mainly used whole brain radiotherapy (WBRT), steroids or a chemotherapeutic approach with a CHOP-like (cyclophosphamide, doxorubicin, vincristine and prednisone) regimen. As WBRT achieved total response rates of 90% the initial enthusiasm was great. However, the overall survival remained low and radiation-induced neurotoxicity with symptoms such as cognitive dysfunction, urinary incontinence and ataxia occurred in 19-83% of the patients. Especially elderly suffered from early and more severe neurotoxicity, which is why deferred radiotherapy and chemotherapy-only regimens were established. (57,92,94)

The initially applied CHOP-based chemotherapy, a standard treatment for systemic DLBCL showed to be ineffective in PCNSL patients. This is explained by the poor BBB penetration of these chemotherapeutic agents.

Nowadays, high dose methotrexate (HD-MTX), a drug that is able to cross the BBB, forms the backbone of chemotherapy regimens for CNSL.(95) Even higher response rates were observed in retrospective analyses, when adding rituximab, targeting the CD20 B-cell marker to MTX.(96)

Irrespective of these advances, recurrence of disease remains common and survival, poor. Thus, addition of a variety of other chemotherapeutic agents, including procarbazine and vincristine were studied.(97,98) However, these studies could not show superiority of one specific treatment regimen. Therefore, the choice of the treatment regimen these days largely depends on the preference of the institution.(99,100)

Many patients presenting with acute symptoms of PCNSL receive steroid therapy. It rapidly improves the symptoms as it reduces the tumor-associated edema and leads to a temporary regression of the tumor on radiography in about 40%.(53,101,102) Evidently, an initial response to steroid therapy has shown to be associated with superior survival.(70). But as a consequence, steroid therapy might interfere with the histopathological diagnosis and should therefore, if possible be refrained from prior to biopsy in case PCNSL is

suspected. Furthermore, the majority of patients relapse rapidly after initial response. Steroids are still used when life-threatening symptoms occur, preventing cerebral herniation but should be given for only a short period of time and with a low dose to prevent adverse side effects.(53,102)

Currently, two treatment phases can be distinguished. The first therapeutic phase is the induction therapy, aiming on complete radiographic response. Followed by consolidation therapy to eradicate residual disease as well as prolongation of overall survival (OS) as demonstrated in Figure 5.(103)

Principally, the type of induction therapy is chosen depending on the fitness (age and performance status) of the patient at the time of diagnosis.(53) The National Comprehensive Cancer Network (NCCN) guidelines 2020 recommend WBRT for newly diagnosed unfit patients.(104) Alternative options are novel agents such as Lenalidomide or Ibrutinib or a MTX based therapy. On the contrary, fit patients receive systemic HD-MTX as backbone combined with either rituximab and temozolomide (TMZ) or a reduced dose of MTX is administered together with rituximab, vincristine, procarbazine (R-MVP) and WBRT.

After complete remission (CR) or unconfirmed CR (CRu), the consolidation therapy with either autologous stem cell transplantation (ASCT), low dose WBRT or monthly MTX administration can be performed. For patients who do not achieve CR/Cru, WBRT should be considered. Both ASCT and WBRT have shown to be effective in PCNSL patients, but considering the risk of neurotoxicity after radiotherapy, ASCT is preferred in younger patients with a good performance status. Whereas in unfit patients 65 years of age, showing contraindications to high dose (HD) chemotherapy, a low dose WBRT is favored.

Maintenance therapy might serve elderly patients, who do not tolerate consolidation therapy as an alternative. Chemotherapeutic as well as targeted agents such as TMZ, procarbazine, lenalidomide or ibrutinib may be used as an alternative to WBRT after CR or partial remission (PR) in order to prolong remission and delay relapse.(53,105)

Advances in understanding the pathogenesis of PCNSL fostered the development of new therapeutic approaches. Research has focused attention on targeted therapies and immune checkpoint inhibitors which might become attractive for frail and elderly patients who don’t tolerate a HD-MTX therapy in future.(106) They mainly focus on the signaling

pathways of B-cells, the microenvironment, immune checkpoints and the permeability of the BBB.

In the ABC subtype of DLBCL, mutations targeting CD79B and the Toll-like receptor protein MYD88, activate the BCR signaling and promote cell survival.

Ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK) blocks the NF-κB activation of the BCR signaling, downstream. It showed high clinical activity, especially in those tumors harboring both mutations. The downside of this therapy is, that it inhibits the anti-infectious functions of B-cells, resulting in failure of protection against pathogens such as Aspergillus fumigatus.(92,107)

Lenalidomide, a second-generation immunomodulatory agent, downregulates IRF4 expression, reduces CARD11 pro-survival signaling, stimulates natural killer as well as T-cell expansion and promotes the antibody-dependent cell-mediated cytotoxicity of rituximab.(108,109) This agent was administered as monotherapy or in combination with rituximab in induction and maintenance therapy in patients with PCNSL. Studies showed a prolonged progression free survival (PFS) and OS when used as maintenance therapy, being especially beneficial for elderly patients, who do not tolerate WBRT or HD chemotherapy. A downside of this targeted therapy is, that it causes several adverse events such as cytopenia.(110)

Another therapeutic approach is to increase the permeability of the BBB. A protein called NGR-hTNF recognizes CD13, which is widely expressed on tumor vessels. By binding CD13 it increases the leakage from the vessels into the tumor tissue, facilitating antitumoral effects. The results of a study that used NGR-hTNF prior to R-CHOP regimen (rituximab-cyclophosphamide, doxorubicin, vincristine and prednisone) could show fast and prominent tumor regression.(92,111)

Additionally, chimeric antigen receptor (CAR) T cells which could successfully be utilized in B-cell leukemia and lymphoma as well as checkpoint inhibitors, including anti-programmed cell death protein (PD)-1 and -2 antibodies could show therapeutic potential in PCNSLs in some studies but need further investigations.(92)

In summary, the therapy of PCNSL is far behind if compared to that of non-CNS DLBCL. Further studies are needed to establish optimal treatment regimens, especially considering the elderly and addressing the adverse effects of targeted therapies.(53,92) Furthermore, about 10-15% of the patients are refractory after the initial treatment and about 35-60%

relapse within the first years. Here as well, treatment regimens continue to remain unclear and need to be further explored.(53)

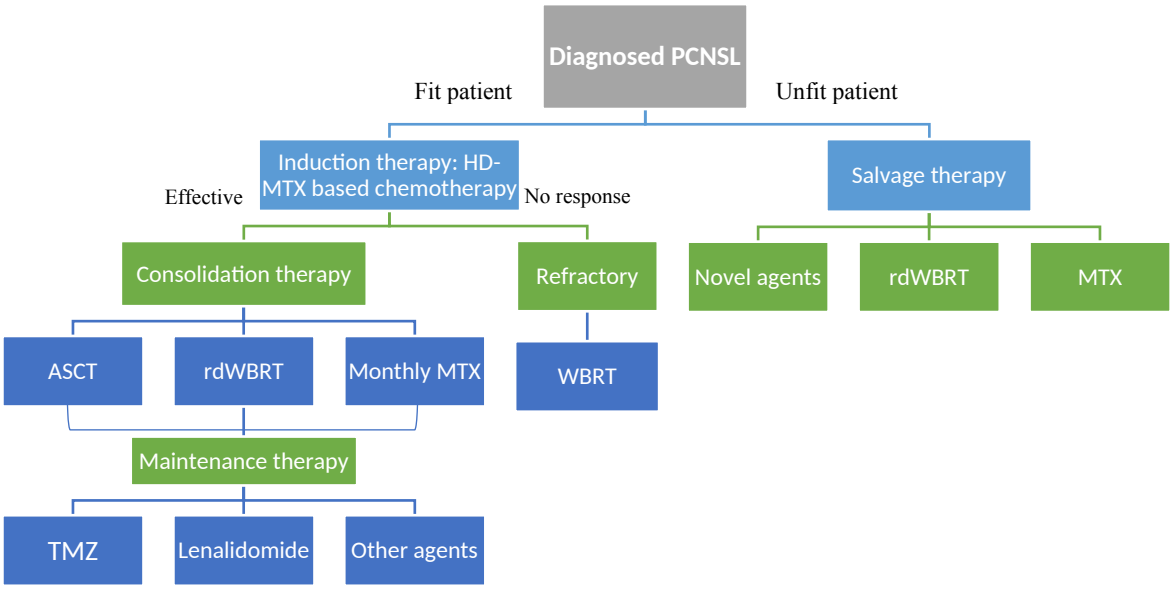


Figure 5 Graphic representation of treatment options for PCNSL patients (53) TMZ: Temozolomide; rdWBRT: reduced-dose Whole-Brain Radiotherapy

1.4.9 Prognosis

Despite the improvement of treatment regimens and overall survival rates, the prognosis of patients with PCNSL, especially of the elderly remains poor. It has been reported that the overall survival increased from 12-18 months to 16.3-66 months since the implementation of HD-MTX-based chemotherapies. Nevertheless, this improvement was restricted to younger patients. Persons aged 60 years and older still have a one-year progression-free survival of about 40%, stressing the fact that this is the age group with the highest incidence.(53,112)

Thus, the relevance of prognostic scoring and stratification of patients increases. Reliable prognostic scores improve the prediction of clinical outcomes, the identification of vulnerable patients and adaptation of therapy aggressiveness, accordingly.(55,112,113)

Up until now, several prognostic models have been developed for PCNSL, but the risk stratification continues to remain unsatisfactory.(53)

Despite the great heterogeneity of DLBCL, three main scoring systems have been developed: the International Prognostic Index (IPI), revised IPI (R-IPI) and National Cancer Network IPI (NCCN-IPI).(114)

The IPI, a prognostic model for aggressive NHL was introduced in 1993 and includes the following risk factors: age (≤60 vs. >60 years), tumor stage (Ann Arbor stage I or II vs. stage III or IV), the number of extranodal sites of disease (≤1 vs. 1), performance status (0 or 1 vs. 2) and serum LDH level (≤1 times normal vs. >1 times normal), shown in Table 1. The patients are stratified into four risk groups that are linked to a 5-year overall survival, ranging from 26 to 73%.(115)

Table 1 IPI Prognostic scoring system(106)

Risk factor	**0 Point**	**1 Point**
Age	≤ 60	> 60
Ann Arbor stage	I or II	III or IV
Serum LDH level	≤1 times normal	>1 times normal
No. of extranodal sites involved	≤ 1	>1
ECOG performance status	0-1	2

Low risk group (0-1 points), Low-intermediate (2 points), High-intermediate (3), High risk group (4-5 points)

With the addition of rituximab to the CHOP chemotherapy, the survival rates of patients improved significantly, thus demanding an update of the previously established IPI. Therefore, the revised IPI (R-IPI) was introduced, redistributing the prognostic factors. Instead of categorizing patients into four, three main prognostic groups were distinguished, allowing for a better risk stratification as shown in Table 2.(116)

Table 2 Revised IPI- risk group stratification

Risk group	**Points**
Very good	0
Good	1-2
Poor	3-5

Another attempt to refine the IPI was made by the NCCN database, using five predictors as shown in Table 3: age (40-60; 60-75; >75years), LDH, extranodal involvement (CNS, bone marrow, liver/GI tract or lung), Ann Arbor stage (III-IV) and ECOG performance status (2). This model identifies four groups: high (6), high-intermediate (4-5), low-

intermediate (2-3) and low risk (0-1) with a better risk discrimination compared to the IPI. (117)

Table 3 NCCN-IPI scoring system

Risk factor	**1 Point**	**2 Points**	**3 Points**
Age	40-60 years	60-75 years	>75 years
ECOG Performance status	2		
LDH	≤ 3x upper limit of normal	>3 x upper limit of normal	
Ann Arbor stage	III-IV		
Extranodal sites	1		

Low risk (0-1 points), Low-intermediate (2-3 points), High-intermediate (4-5 points), High (6 points)

However, these scores are not specifically designed for central nervous system disease and have shown to be less predictive in patients with PCNSL.(118)

Thus, specified scores have been developed. The most frequently incorporated variables are age and performance status.(53)

In order to determine the patient's fitness, the performance status is evaluated using one of two scales. The Karnofsky index and/or the Eastern Cooperative Oncology Group (ECOG) criteria displayed in Table 4.(69,119)

Table 4 Performance status: ECOG vs. Karnofsky index(120)

Karnofsky Status	Karnofsky Grade	ECOG Grade	ECOG status
Normal, no complaints	100	0	Fully active, able to carry on all pre-disease performance without restriction.
Able to carry on normal activities. Minor signs or symptoms of disease.	90	0	
Normal activity with effort.	80	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
Care for self. Unable to carry on normal activity or to do active work.	70	1	
Requires occasional assistance, but able to care for most of his needs.	60	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
Requires considerable assistance and frequent medical care.	50	2	
Disabled. Requires special care and assistance.	40	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
Severly disabled. Hospitalisation indicated though death nonimminent.	30	3	
Very sick. Hospitalisation necessary. Active supportive treatment necessary.	20	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
Moribund	10	4	
Dead	0	5	Dead

The two most commonly utilized prognostic models for PCNSL are the International Extranodal Lymphoma Study Group experience (IELSG) and the Memorial Sloan-Kettering Cancer Center (MSKCC) score.

The IELSG is composed of five variables as shown in Table 5: age (>60 years), ECOG score (>1), elevated serum LDH, high CSF protein concentration and deep brain involvement. Deep brain involvement is defined as involvement of the periventricular region, brainstem, basal ganglia, cerebellum and brainstem.(121) The final score is achieved by assigning a value with either 0, if it is favorable or 1 point, if it is unfavorable

and adding these numbers. Three groups are distinguishable: low risk (0-1 points), intermediate risk (2-3 points) and high risk (4-5 points). They are associated with a two-year survival rate of 80%, 48% or 15%, respectively.(55,121)

Table 5 IELSG score (122)

Variables	**Favorable Feature (Value=0)**	**Unfavorable Feature (Value=1)**
Age (years)	≤60	>60
Involvement of deep brain structures	No	Yes
LDH serum level	Normal	Elevated
CSF Protein level	Normal	Elevated
ECOG score	0-1	>1

Low risk (0-1 points); Intermediate risk (2-3 points); High risk (4-5 points)

The major drawback of this scoring system is that some of its variables are not uniformly obtained in everyday clinical practice and therefore cannot be utilized for all patients. Considering that PCNSL is a space-occupying lesion within the brain, patients often show an increased intracranial pressure. Consequently, a lumbar puncture is not performed prior to treatment, leading to missing CSF protein values and ultimately, incomplete IELSG scores.(123) In addition, only 105 patients were included in the development of this model and the follow-up time was merely 24 months.(124)

On the contrary, the MSKCC model solely uses two variables as shown in Table 6: age (<50 years vs. 50 years) and the Karnofsky performance status (KPS 70% vs. <70%). It stratifies the patients into three groups: good (1) (patients <50 years), intermediate (2) (patients 50 years, KPS 70%) and high (3) risk (patients 50 years, KPS<70%).(125) They are related to a median overall survival of 8.5, 3.2 and 1.1 years, respectively.(55)

Table 6 MSKCC Prognostic model

Variables	**Good risk (1)**	**Intermediate risk (2)**	**High risk (3)**
Age	<50	50	50
Karnofsky performance status		70	<70

The major disadvantage of this score is, that it was a single-institution study, generating an intrinsic selection bias and that it included a relatively small cohort of 338 patients.(124)

Another proposed model is the Nottingham/Barcelona prediction scoring system. It encompasses three parameters: age (60 years), ECOG (>1) and multifocal disease or meningeal involvement. Each unfavorable variable is given 1 point, stratifying the patients into four groups (0-3) and a median survival of 55, 41, 32 and 1 month, respectively.(126) Unfortunately, the utility of this model is limited as it was developed, using old chemotherapeutic regimens. Apart from that, the study group was restricted to 77 patients.

Another, more recently developed prognostic score is the Taipei Score, using age (>80 years), deep brain involvement and ECOG (2), forming four distinct risk groups (0-3 points). Interestingly, the chosen cut-off value for age was 80 years, as they received a more significant stratification of their cohort. The study group proposed that the higher cut-off value for age could have been influenced by the overall increased life expectancy of patients with PCNSL nowadays.(127)
A further approach to adapt the prognostic stratification to new treatment strategies was implemented by the National Cancer Center Singapore-National Neuroscience Institute (NCCS-NNI) in 2022. They developed a new NCCS-NNI prognostic model, using age (>70 years) and two new variables: pre-and post-steroid (both pre-chemoimmunotherapy) Neutrophil-to-Lymphocyte Ratio (NLR). The patients were stratified into three risk groups with a 2-year mortality of 5% in the low, 38% in the intermediate and 73% in the high-risk group. They took into consideration, that corticosteroids changed the neutrophil-to-lymphocyte balance and investigated whether the response to steroid treatment correlated with survival. Indeed, they found higher NLR levels post-steroid treatment to be associated with a better survival. Nevertheless, this study has several limitations but the observation of NLR dynamics in response to corticosteroids constitutes an interesting variable which should be further explored.(128)

In conformity with the aim of these last studies, it is important to constantly update and improve the existing models as therapy regimens have changed and the knowledge of the pathobiology continues to grow.(124,129) One attempt towards ameliorating prognostic validity, is the implementation of gene expression studies, such as GEP. As mentioned before, it recognizes where within the lymphoid maturation stages the tumor cells originated from, which has shown to play a key role in the tumor development and prediction of outcome. GEP studies showed that patients with PCNSL harboring the ABC DLBCL subtype had the worst prognosis. The five-year survival rate of patients with an

ABC DLBCL was 35% as compared to 60% in patients with the GCB subtype and 39% in patients with an unclassifiable DLBCL. This worse prognosis in patients with ABC DLBCL is in part explained by the difference in chromosomal alterations, activation of signaling pathways and sensitivity to certain drugs, as compared to the GCB subtype. Thus, the COO classification can be utilized for prognostic subgrouping of patients with DLBCL. However, GEP is expensive and not generally available and cannot be considered as a routine clinical test. (130–132) Therefore, Hans et al. created an alternative way of prognostic subgrouping, using immunohistochemical stains. They were able to predict survival in a similar manner. The GCB subtype had a 5-year OS of 76%, whereas the non-GCB group had a worse prognosis with a 5-year OS of only 34%.(34)

Another readily accessible way of prognostic classification is represented by the immunohistochemically determined co-expression of MYC and BCL-2. The expression of one or both double expressor features could be linked to a worse prognosis. Furthermore, one study demonstrated that the implementation of DEL biology could improve the prognostic power of the NCCN-IPI. Therefore, the determination of the DEL status might represent a potential extension of already developed prognostic models.(118)

Additionally, tumors harboring DHL or THL have shown to have a more aggressive course and to be chemorefractory, resulting in inferior outcomes compared to patients without MYC and BCL-2/BCL-6 rearrangements. Consequently, methods capturing these DHL and THL subtypes have become important for the prognosis of PCNSL patients, too. (41,42)

The downside of these molecular markers is the extend of cost, time, availability and laboratory effort that is needed. Therefore, routinely investigated, easily obtained and cost-effective alternative markers have to be established.(133)

Alternatively, the prognostic role of routinely investigated blood parameters was explored. One example of a serologic prognostic parameter, which is widely used, is the LDH level. It has been successfully integrated into the IELSG score for PCNSL patients. Moreover, a high LDH level could independently be associated with a poor prognosis and lower response rates.(121) Anemia is frequently observed in patients with cancer. *In vivo* studies showed that the cytokines Interleukin-1 (IL-1), Interleukin-6 (IL-6) as well as Interleukin-10 (IL-10) could inhibit the action of erythropoietin and are suggested to be involved in cancer-associated anemia. It was determined, that patients with DLBCL as well as PCNSL who had anemia prior to therapy onset, had a shorter OS as compared to patients without

anemia.(134,135) Another study could show high total bilirubin levels to have a negative influence upon OS and PFS in patients with PCNSL.(135)

The role of inflammation in tumor progression has been known for a long time. New insights on how the tumor microenvironment, which encompasses high concentrations of inflammatory cells, plays a role in proliferation, survival and migration of tumor cells have led to prognostic studies involving systemic inflammatory parameters.(136)

Accordingly, the absolute lymphocyte count (ALC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), neutrophil-to-monocyte ratio (NMR), neutrophil-to-lymphocyte ratio (NLR) and many more have been investigated.(124)

As a result, a low absolute lymphocyte count, a marker of host immunity was identified to be an indicator of poor prognosis in patients with PCNSL.(137,138)

Apart from examining distinct parameters, ratios based on cell counts and inflammatory parameters were developed. For instance, the lymphocyte-to-monocyte ratio (LMR) represents an inflammatory marker that can easily be obtained. Investigations showed, that a low LMR was associated with a significantly worse OS in patients with PCNSL and therefore represents a possible biomarker of prognostic value.(135,139)

The neutrophil-to-lymphocyte ratio was also discovered to be an indicator of systemic inflammatory response. Studies suggested, that a high NLR has a negative impact on the prognosis of patients with PCNSL.(128,140,141) A similar prognostic value is possessed by the derived NLR (dNLR). In order to obtain the dNLR, the neutrophil count is divided by (leukocytes-neutrophiles) and was established as the ALC was not always documented in clinical trials.(142,143)

However, some studies failed to verify the prognostic value of inflammation markers and ratios. Therefore, new ratios were established combining inflammatory markers with tumor burden-associated markers such as LDH. LDH is an enzyme, catalyzing the reaction of pyruvate to lactate, enabling glycolysis and with that, promoting tumor initiation and metastasis. Previous studies could demonstrate a significant association between high serum LDH levels and poor prognosis in PCNSL patients.(124,144) Therefore, LDH has been incorporated in several prognostic ratios. One example is the LMR to LDH ratio, which has been identified to be a poor prognostic parameter in patients with PCNSL.(124)

Another ratio that has demonstrated prognostic value in a variety of cancers including PCNSL, is the platelet-to-lymphocyte ratio (PLR).(145) Platelets are essential for hemostasis, angiogenesis, inflammation, vascular integrity, innate immunity, wound

healing and cancer biology. Cancer cell interactions with platelets might facilitate cancer cell survival and spread. Thus, platelets are suggested to play a critical role in tumor metastasis.(146) Consequently, a high PLR in patients with PCNSL prior to treatment could be identified as a poor prognostic marker and has already been integrated in a new prognostic model.(145)

This new prognostic model, the complete blood count score model (CBC score) was established in 2021. It includes the above mentioned NLR, PLR and two new additional indexes, namely the systemic immune inflammation index (SII) and systemic inflammation response index (SIRI). SII is perceived by calculating the following formula: platelet count x neutrophil count/ lymphocyte count and has already demonstrated prognostic power in other cancers.(147) SIRI can be achieved by the following formula: neutrophil count x monocyte count/ lymphocyte count and evaluates the relationship between anti-tumor immune effects and inflammation.(148) The CBC score stratifies patients into three prognostic groups: low risk (no high expression of CBC's), intermediate risk (1-2 high expressions) and high risk (3-4 high expressions), showing significantly different OS and PFS rates. It might present a promising, cost-effective and easily available prognostic score for PCNSL patients. Nevertheless, this study presents several limitations and is in need of further validation.(147)

Apart from these serological and histological parameters, a clinical and easily accessible factor, the Mini Mental State Examination (MMSE)-score has been proposed to be an independent prognostic parameter for OS and PFS, distinguishing patients with a MMSE-score ≤27 having a worse OS, as compared to patients with a MMSE >27. (149)

1.5 Aim of this study

The aim of this thesis was to comprehensively evaluate the prognostic relevance of clinical and patient derived data, such as blood parameters as well as histopathological features and phenotypes. All data were collected at the time of diagnosis and were mainly correlated to the overall survival by performing univariate statistical tests. We included already established prognostic parameters and scores to validate their clinical significance. Beyond that, we aimed on identifying new potential biomarkers of prognostic power. Hence, this thesis represents a potential basis for the development of a new prognostic model to improve the risk stratification of patients with PCNSL.

2 Material und Methods

We performed a retrospective monocentric study, including 74 patients who were diagnosed with PCNSL according to the World Health Organization criteria at the division of Hematology at the Medical University of Graz in Austria, between November 2004 and February 2021. The baseline characteristics of our study group are demonstrated in Table 7.

Table 7 Baseline characteristics of patients with PCNSL

Characteristic	Total number of patients = 74	Characteristic	Total number of patients = 74
Sex (%)		ECOG (%)	
Male	59.5% (n=44)	0-1	58.1% (n=43)
Female	40.5% (n=30)	>1	41.9% (n=31)
Age (y)		Relapse to therapy (%)	
Mean	63	YES	45.95% (n=34)
Range	23-88	NO	54.05% (n=40)
>60 y (%)	56.76% (n=42)		
Primary tumor localisation (%)		Involvement of deep brain structure (%)	
Brain	82.43% (n=61)	Yes	55.41% (n=41)
Eye	17.57% (n=13)	No	44.59% (n=33)
First-line treatment (%)		Response to therapy (%)	
HD-MTX based chemotherapy	86.49% (n=64)	Complete remission (CR)	47.29% (n=35)
MTX with Rituximab	37.5% (n=24)	Progressive disease (PD)	20.27% (n=15)
MTX without Rituximab	62.5% (n=40)	Partial response (PR)	18.92% (n=14)
Other	13.51% (n=10)	Very good partial remission (vgPR)	4.05% (n=3)
		Missing	9.46% (n=7)
BMI (%)			
<18.5 Underweight	(n=0)		
18.5-24.9 Normal	32.4 % (n=24)		
25-29.9 Overweight	45.9 % (n=34)		
30-34.9 Class 1 Obesity	20.3 % (n=15)		
35-39.9 Class 2 Obesity	1.4 % (n=1)		
≥40 Class 3 Obesity	(n=0)		

The clinical and serological data (as described in Tables 7 and 9) were collected from electronic medical records obtained by the division of hematology of the Medical University of Graz at the time of diagnosis. The pathological parameters of the primary diagnostic sample (as described in Figure 9) were received from the Institute of Pathology of the Medical University of Graz. All listed parameters in Table 8 were collected prior to treatment initiation.

Table 8 Collected clicinal, serological and histopathological Parameters

Clinical parameters	Histopathological parameters	Serological parameters
Age	Diagnosis of DLBCL	Leukocyte count
Sex	COO (using the Hans algorithm)	Absolute neutrophil count
BMI	DEL status	Lymphocyte count
Primary manifestation site	p53	Neutrophil-to-lymphocyte ratio
Involvement of deep brain	CD3	derived NLR
structures	CD5	Hemoglobin
Observation time	CD10	Thrombocyte count
Date of death	CD30	Platelet-to-lymphocyte ratio
Applied therapy regimen	CD79A	C-reactive protein
Cycles of therapy	FOXP1	Systemic immune inflammation
Response to therapy	BCL-2	index
Date of relapse	BCL-6	Lactade dehydrogenase
IPI	MUM1	LDH-to-lymphocyte ratio
R-IPI	GCET1	Gamma-GT
NCCN-IPI	c-MYC	Uric acid
Karnofsky index	MIB-1/k67	Creatinine
ECOG	PD-L1	ß2 microglobulin
MSKCC	PD-L2	Albumin
Diagnosis of additional		Total protein
neoplasia		Bilirubin

Due to the relatively small pathologic samples that were received through a stereotactic brain biopsy, the institute of pathology was not able to perform all histopathological investigations on all samples, which leads to missing values. Consequently, we refrained from statistically analyzing those histopathologic and serologic parameters in case more than 50% of the values were missing. Hence, we could not investigate the impact of these parameters upon OS.

Patients were mainly treated with HD-MTX-based regimens with or without the addition of rituximab. Post-treatment surveillance included routine clinical and laboratory examinations. We used MRI as standard imaging technique and the follow-up evaluations were performed every three months during the first year. Thereafter the time intervals were stretched according to the individual's condition. Patients were excluded from this study in case of seropositivity for HIV and secondary CNS lymphoma. Dates of death were

obtained from the hospitals in case patients died during a hospital stay or through an electronic data query from authorized health insurance companies. The overall survival was defined as time in years from the date of diagnosis to the date of death due to any cause within the follow-up period. Disease-free survival was defined as the time in months from the date of diagnosis to the date of radiologically or histologically confirmed recurrent disease. Disease-free survival was censored at the time of death or at the last date of follow up, if patients remained to be free from tumor.

This study was approved by the local ethical committee of the Medical University of Graz (28-517 ex15/16 and 28−496 ex 15/16).

2.1 Statistical analyses

The primary endpoint of our study was overall survival. We used univariate analyses to assess the serologic, pathologic and clinical parameters that are significantly associated with OS. Furthermore, we applied the Fisher's exact test, using contingency tables to determine the statistical association between the variables and OS. We chose the significance level $p<0.05$. For the graphic representation of our results, we used Kaplan-Meier survival curves, bar charts and box blots. All statistical analysis were performed using Statistical Package for Social Sciences (SPSS Inc. version 26, Chicago, IL, USA) and GraphPad Prism (GraphPad Software, Inc. version 9.3.1).

3 Results

3.1 Patient cohort

Our patient cohort included 78 patients with newly diagnosed PCNSL by stereotactic brain biopsy. Four patients were excluded during the study as the diagnosed CNSL was due to a systemic DLBCL with secondary spread to the CNS. Thus, the final cohort consists of 74 patients as demonstrated in Figure 6.

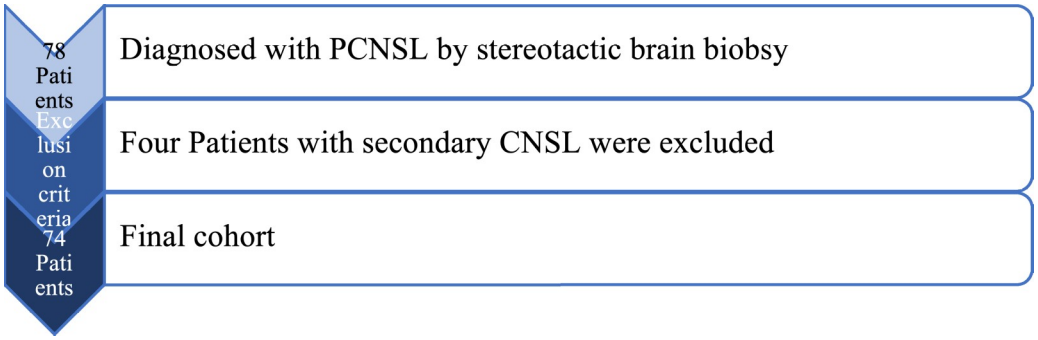


Figure 6 Patient selection

The median age of our patients was 64 years, ranging from 23 to 88 years as demonstrated in Figure 7. 59.5% of our patients were male and 40.5% were female. 13 patients (17.57%) were diagnosed with a primary intraocular lymphoma and 61 patients (82.43%) had a primary cerebral manifestation. Of those with a primary brain involvement, 41 patients had a lesion affecting deep brain structures. The mean follow-up time for all patients was 3.21 years, ranging from 0.01 to 17.4 years. At the time of our study, 25 patients (33.78%) were still alive.

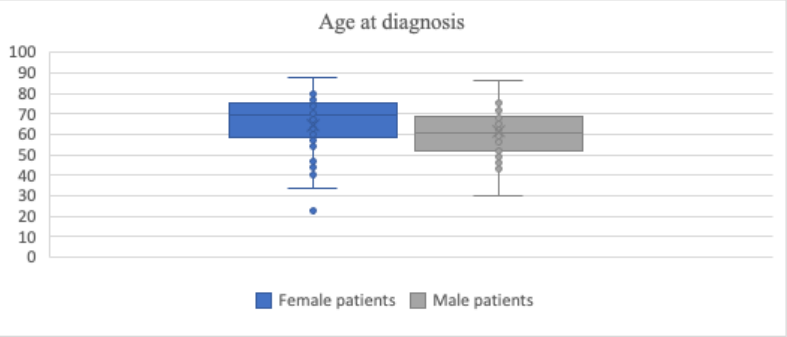


Figure 7 Distribution of age at diagnosis

The majority, namely 64 patients received a HD-MTX based chemotherapy, wherefrom 24 patients received Rituximab additionally. 27 patients received an autologous stem cell transplantation. Ten patients were treated with a therapy other than MTX. Five of those did not receive therapy at all, one patient received R-CHOP, one patient a palliative chemotherapy with TMZ and two of them got an intravitreal therapy with bevacizumab. As many as 35 patients experienced a complete remission (CR), 14 patients had a partial remission (PR), three patients had a very good partial remission (vgPR) and 15 patients had progressive disease after treatment initiation. Within the follow-up period 34 patients (45.95%) relapsed.

The performance status was evaluated at the time of diagnosis, using the Karnofsky index and the ECOG performance score. 41.89% of our patients had a poor baseline status (Karnofsky <70% and ECOG >1) at the time of diagnosis. Despite the performance status we also evaluated the distribution of body fat in our cohort by using the Body Mass Index (BMI) and found 67.57% of our patients to be overweight.

Apart from clear cut-off values for standard serological parameters predetermined by our laboratory, there is no established reference range for the following ratios: NLR, LLR, SII and PLR. Therefore, we calculated the median as well as the interquartile ranges of those ratios within our patient collective and used them as cut-off values. This enabled us to stratify our cohort into two groups, thus allowing for statistical analyses of survival differences between high- and low-level groups. The investigated serologic parameters are described in Table 9.

Table 9 Serologic parameters

Variable	Total number of patients=74	Variable	Total number of patients= 74
Total Protein (%)		Leucocytes (%)	
<6.6 g/dL	37.84% (n=28)	<4.4 G/L	4.05% (n=3)
6.6-8.3 g/dL	60.81% (n=45)	4.4-11.3 G/L	64.86% (n=48)
>8.3 g/dL	1.35% (n=1)	>11.3 G/L	31.08 % (n=23)
Albumin (%)		Creatinin (%)	
<3.5 g/dL	13.51% (n=10)	<0.7 mg/dL	16.22% (n=12)
3.5-5.5 g/dL	82.43% (n=61)	0.7-1.2 mg/dL	75.68% (n=56)
>5.5 g/dL	(n=0)	>1.2 mg/dL	6.76% (n=5)
Missing values	4.06% (n=3)	Missing values	1.35% (n=1)
Thrombocytes (%)		Neutrophils (%)	
<140 G/L	5.41% (n=4)	<1.8 G/L	(n=0)
140-440 G/L	91.89% (n=68)	1.8-7.7 G/L	52.70% (n=39)
>440 G/L	2.70% (n=2)	>7.7 G/L	47.29% (n=35)
Uric acid (%)		Beta2 MIG (%)	
<3.4 mg/dL	17.57% (n=13)	<1.3 mg/L	(n=0)
3.4-7.0 mg/dL	77.03% (n=57)	1.3-5.5 mg/L	20.27% (n=15)
>7 mg/dL	4.05% (n=3)	>5.5 mg/L	2.70% (n=2)
Missing values	1.35% (n=1)	Missing values	77.03% (n=57)
GGT (%)		Platelets to Lymphocytes Ratio 3rd Quartile (%)	
>55 U/L	33.78% (n=25)	>226.33	25.68% (n=19)
≤55 U/L	64.86% (n=48)	≤226.33	74.32% (n=55)
Missing values	1.35% (n=1)		
LDH (%)		LDH to lymphocyte ratio Median (%)	
<120 U/L	1.35% (n=1)	>149.71	50% (n=37)
120-240 U/L	77.03% (n=57)	≤149.71	50% (n=37)
>240 U/L	21.62% (n=16)		
CRP (%)		Neutrophil-to-lymphocyte Ratio Median (%)	
≤5 mg/L	75.68 % (n=56)	>4.94	50% (n=37)
>5 mg/L	24.32 % (n=18)	≤4.94	50% (n=37)
Hemoglobin gender-adapted (%)		SII Median (%)	
<12 g/dl for female or <14 g/dl for male patients	33.78% (n=25)	>1249.43	50% (n=37)
Normal range Hb	64.86% (n=48)	≤1249.43	50% (n=37)
>16 g/dl for female or >18g/dl for male patients	1.35% (n=1)		

Interestingly, a large proportion of our patients, ranging from 61% to 92% showed to have normal serologic parameters. Consequently, only a minority showed pathologic values at the time of diagnosis. Leukocytosis was determined in 31.08% of our patients at the time of diagnosis. Neutrophilia was observed in 47.29% of our cohort. We implemented gender-specific ranges for hemoglobin. 33.78% of our cohort were diagnosed with anemia prior to treatment.

Additionally, we intended to validate the clinical usefulness of prognostic models designed for systemic DLBCL namely IPI, R-IPI and NCCN-IPI in patients with PCNSL. The IPI stratified our cohort into four groups. 22.97% of our patients were assigned to the low, 33.78% to the low-intermediate, 31.08% to the high-intermediate and 12.16% to the high risk group.

Applying the R-IPI, 22.97% had a very good, 63.51% a good and 13.51% a poor prognosis. Last but not least, the NCCN-IPI assigned 1.4% to the low, 50% to the low-intermediate, 44.6% to the high-intermediate and 4.1% to the high-risk group.

Moreover, we used the MSKCC score, a prognostic model specifically designed for patients with PCNSL. It stratified our patients into three risk groups. 14.9% belonged to low risk, 47.3% to intermediate and 37.8% to high risk group. We planned on using the IELSG score but lacked CSF protein values. Therefore, we didn't calculate the IELSG score. The patient stratification obtained by the utilization of the IPI, R-IPI, NCCN-IPI and MSKCC score are demonstrated in Figure 8.

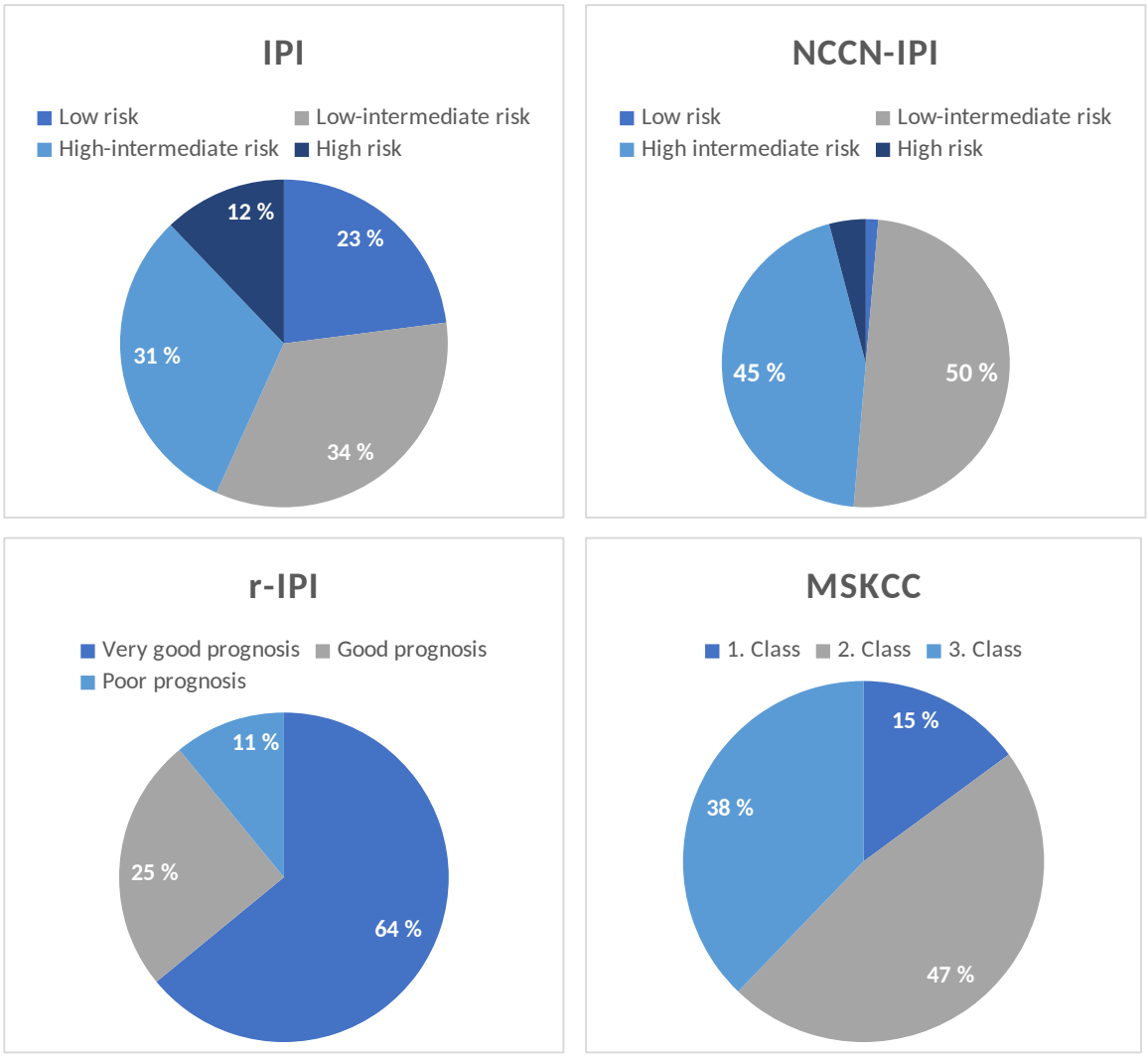


Figure 8 Graphic demonstration of the patient cohort stratification by the following prognostic models: IPI, R-IPI,NCCN-IPI and MSKCC

Using the Hans algorithm, five of our patients (6.76%) were identified to have a GCB-like DLBCL, whereas 62 patients (83.78%) had a non-GCB like DLBCL. The tissue biopsies of seven patients could not be classified as the tumor samples were too small.

Immunohistochemistry revealed 18 patients (24.32%) to exhibit a co-expression of MYC and BCL-2. 28 patients (37.83%) exhibited one of the double-expressor features and eight patients (10.81%) did neither show the expression of MYC nor BCL-2. As demonstrated in Table 10, the tumor was assigned to express one DEL feature (DEL1) in case ≥30% of the cells were positive for MYC and less then 70% of the cells were positive for BCL-2 or vice versa <30% were positive for MYC and ≥70% were positive for BCL-2. Lymphoma tissue which expressed MYC in ≥30% and BCL-2 in ≥70% of the cells was determined as DEL2.

Table 10 Definition of DEL status(118)

DEL status	**MYC positivity**	**BCL-2 positivity**
DEL 1 or	≥30%	<70%
DEL 1	<30%	≥70%
DEL 2	≥30%	≥70%

The median Ki-67 labeling index was 85% in our study cohort. Furthermore, protein expression of CD3, CD5, CD10, CD30 and CD79A was found in 41.89%; 16.22%; 18.92%; 16.22% and 47.29%, respectively. Bcl-2, Bcl-6, MUM1, GCET-1, FOX-P1 and kappa and lambda light chain expression were present in 70.27%; 62.16%; 64.86%; 17.57%; 47.29%, respectively. Furthermore, we found the expression of p53, programmed death 1 (PD-1) and its ligand (PD-L1), as well as programmed death 2 (PD-2) in 39.18%; 2.7%; 13.51% and 22.97%, respectively. Meanwhile, it is worth mentioning that the rather small samples of tumor tissue made it impossible for the pathologist to apply and subsequently interpret some of the immunohistochemical stains. Consequently, we missed one and up to 39 of 74 values according to the evaluated histopathological parameter. This implicates, that the actual distribution of the cell characteristics could in fact be different to that showed in Figure 9.

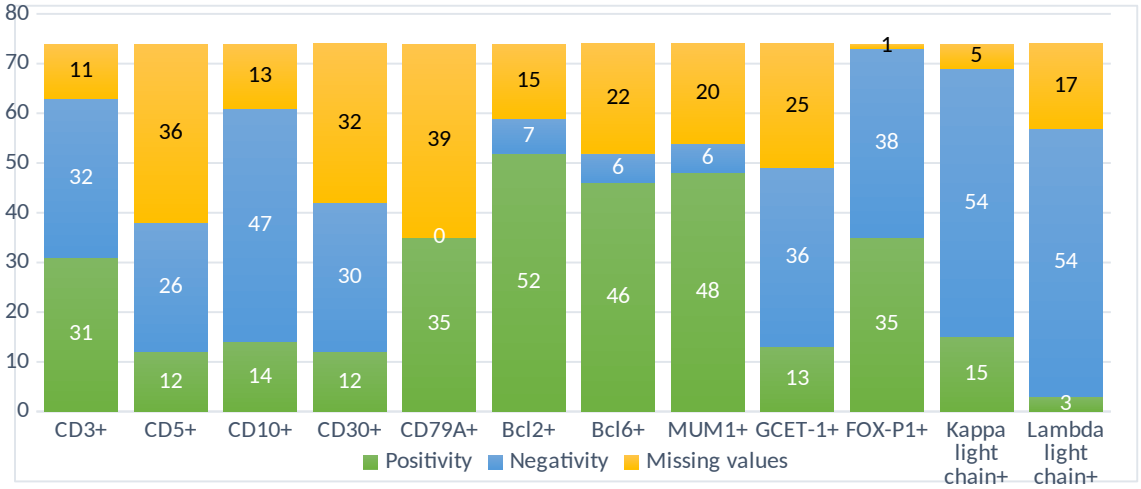


Figure 9 Immunohistochemical characteristics of our patient cohort

3.2 Prognostic models

We evaluated the following prognostic scores: MSKCC, IPI, R-IPI, NCCN-IPI and Tapei score as listed in Table 11.

Table 11 Overview of the evaluated Prognostic scores

Score	Groups	n	Median survival (years)	P-value
MSKCC	Low	11	NR	0.0003
	Intermediate	35	5.7	
	High	28	0.6	
IPI	Low	1	6.63	0.0087
	Low-intermediate	37	5.48	
	High intermediate	33	0.84	
	High	3	0.35	
R-IPI	Very good	17	6.63	0.0012
	Good	47	2.31	
	Poor	10	0.68	
NCCN-IPI	Low	1	2.23	0.0012
	Low-intermediate	37	5.48	
	High-intermediate	33	2.29	
	High	3	0.21	
Tapei score	Low	18	3.25	0.1387
	Low-intermediate	39	4.05	
	High-intermediate	16	0.84	
	High	1		

The MSKCC score assigned 11 patients (14.9%) to the low risk, 35 patients (47.3%) to the intermediate risk and as many as 28 patients (37.8%) to the high risk group. As expected, the statistical tests identified three prognostic groups with clearly different survival outcomes. We could herewith prove the clinical usefulness of this prognostic model (p=0.0003). The Kaplan-Meier survival curve is shown Figure 10 and the survival data collected in Table 12 show the prognostic impact of the MSKCC score upon OS of our patients.

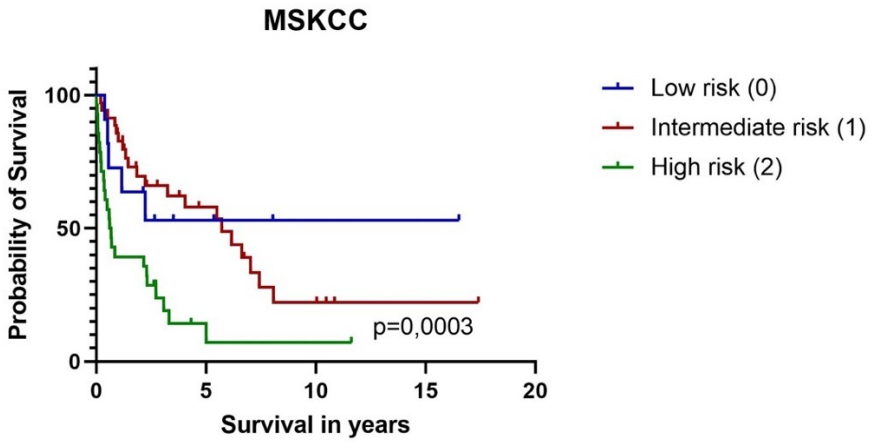


Figure 10 MSKCC: Kaplan-Meier survival curve

Table 12 MSKCC: Survival data

	Low risk	Intermediate risk	High risk
Median survival (years)	NR	5.7	0.6

Similar to other study groups, our patient collective largely lacked CSF samples, needed for the evaluation of the IESLG score. Because of this, we were not able to clinically utilize the IELSG for our patient cohort. Thus, we could not analyze the prognostic significance of this prognostic score.

The IPI, R-IPI and NCCN-IPI are prognostic scores which have been developed for DLBCL and are not specific for patients with PCNSL.(114,117) Nevertheless, all these scores could successfully stratify our patients into distinct prognostic groups. We assigned all patients with PCNSL to the Ann Arbor stage I_E (involvement of a single extralymphatic organ or site).(150)

The IPI stratified our cohort into four groups: low risk (22.97%), low intermediate (33.78%), high-intermediate (31.08%) and high risk (12.16%) groups with a median

survival of 6.63, 5.48, 0.84 and 0.35 years, respectively (p=0.0087). The corresponding survival data and Kaplan-Meier survival curve are illustrated in Figure 11 and Table 13.

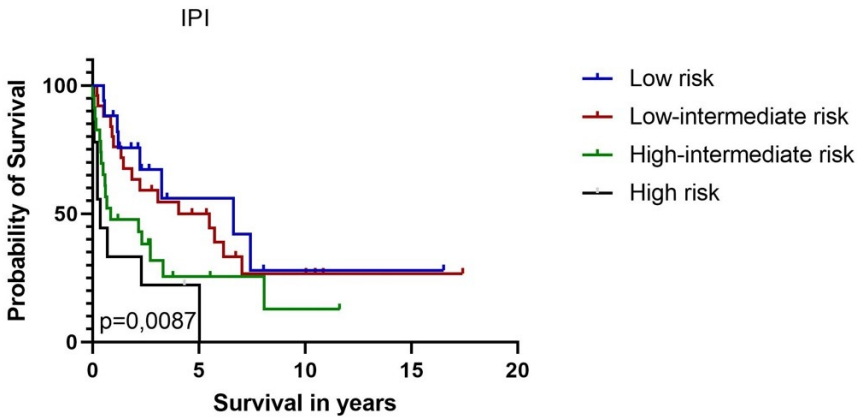


Figure 11 IPI: Kaplan-Meier survival curve

Table 13 IPI: Survival data

	Low risk	Low-intermediate risk	High-intermediate risk	High risk
Median survival (years)	6.63	5.48	0.84	0.35

The R-IPI on the other hand classifies the patients into three risk groups: very good (22.97%), good (63.51%) and poor (13.51%) with a median survival of 6.6, 2.3 and 0.7 years, respectively (p=0.0112). The survival of these three risk groups over time is demonstrated in Table 14 and Figure 12.

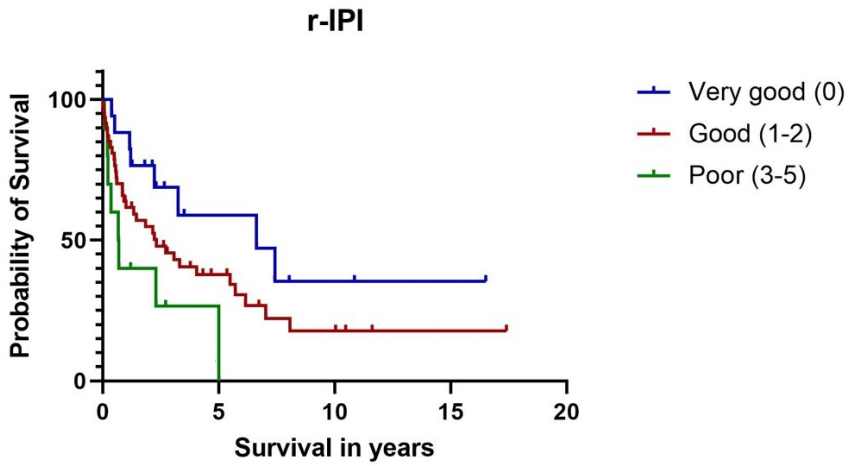


Figure 12 R-IP:I Kaplan-Meier survival curve

Table 14 R-IPI: Survival data

	Very good	Good	Poor
Median survival (years)	6.6	2.3	0.7

Finally, the NCCN-IPI assigned one patient (1.4%) to the low risk group, 37 patients (50%) to the low-intermediate, 33 patients (44.6%) to the high-intermediate and three patients (4.1%) to the high risk group with subsequent median survival rates of 2.23, 5.48, 2.29 and 0.21 years (p=0.0012). The survival differences of these groups are shown in the Kaplan Meier curve in Figure 13 and Table 15.

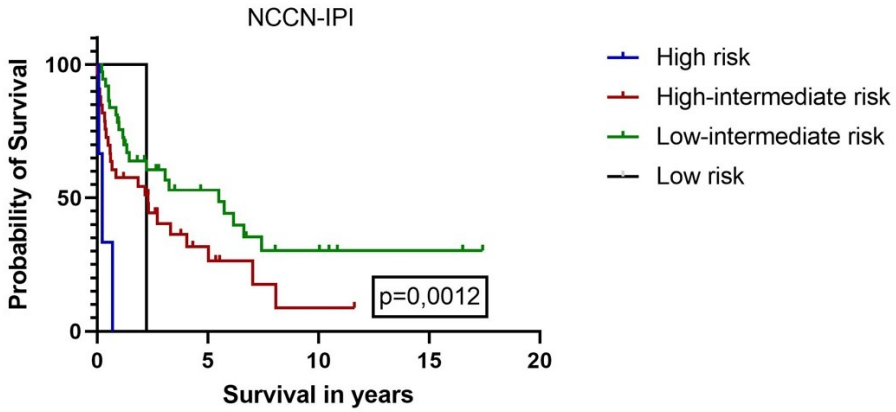


Figure 13 NCCN-IPI: Kaplan-Meier survival curve

Table 15 NCCN-IPI: Survival data

	Low risk	Low-intermediate risk	High-intermediate risk	High risk
Median survival (years)	2.23	5.48	2.29	0.21

As only a few patients were grouped into the low and high risk groups, we decided to merge the low with the low-intermediate (36 patients) and high-intermediate with high risk groups (38 patients) to receive more meaningful results. The median survival of the high risk group was 1.8 years and that of the low risk group was 5.5 years (p=0.0287; HR:1.8). The associated survival rates are demonstrated in Table 16 and Figure 14.

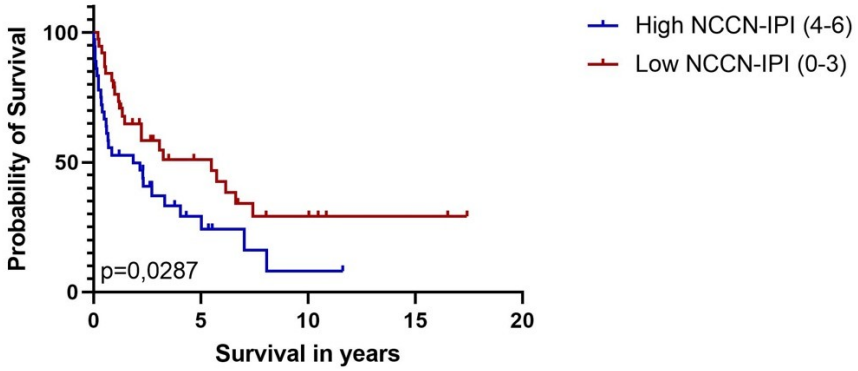


Figure 14 Adapted NCCN-IPI: Kaplan-Meier survival curve

Table 16 NCCN-IPI: Survival data

	Low NCCN-IPI (0-3)	High NCCN-IPI (4-6)
Median survival (years)	5.5	1.8
Hazard ratio	1.8	1 (reference)

Based on univariate analysis, the Kaplan-Meier survival curves showed a good discrimination of survival outcomes of the different risk groups for all the above-mentioned scores.

3.3 Clinical markers

The clinical markers we investigated in this study were the primary manifestation site, age, Karnofsky index, ECOG performance status, POD24, involvement of deep brain structures, sex, BMI and ASCT as shown in Table 17.

Table 17 Overview of the evaluated clinical parameters

Clinical parameter	**Groups**	**n**	**Median survival (years)**	**p-value**	**Hazard ratio**
Primary manifestation site	Cerebral	61	2.1	0.013	2.8
	Eye	13	7.0		1 (reference)
Age	≤60 years	32	5.7	0.0087	1 (reference)
	>60 years	42	1.3		2.14
Karnofsky index	≤70	31	0.69	0.0045	2.2
	>70	43	5.72		1 (reference)
ECOG	0-1	43	5.72	0.0045	1 (reference)
	>1	31	0.69		2.2
POD24	Yes	37	0.67	<0.0001	6.6
	No	36	7.42		1 (reference)
Involvement of deep brain structure	Yes	41	3.3	0.3732	1 (reference)
	No	33	1.45		1.28
Sex	Male	44	3.25	0.3722	1.29
	Female	30	1.27		1 (reference)
BMI	High ≥25	50	2.71	0.9524	1.01
	Normal/Low <25	24	2.15		1 (reference)
ASCT	Yes	23	7.42	0.0002	1 (reference)
	No	46	1.45		4.1

Primary intraocular lymphoma (PIOL) is a subset of PCNSL.(151) An increasing incidence of ocular involvement has been observed over the last years, affecting about 15-25% of PCNSL cases.(48) It has been postulated before, that patients with this rather rare subtype harbor a better prognosis as compared to patients with cerebral involvement.(152) Our cohort included 13 patients (17.57%) with PIOL and 61 patients (82.43%) with primary brain involvement. Patients with a primary brain manifestation had a significantly reduced median survival of 2.1years as compared to patients with PIOL with a median survival of 7.0 years (p=0.013; HR: 2.8). The Kaplan Meier curve, comparing the survival rates of these two groups is shown in Figure 15.

Figure 15 Primary manifestation of the PCSNL: Kaplan-Meier survival curve

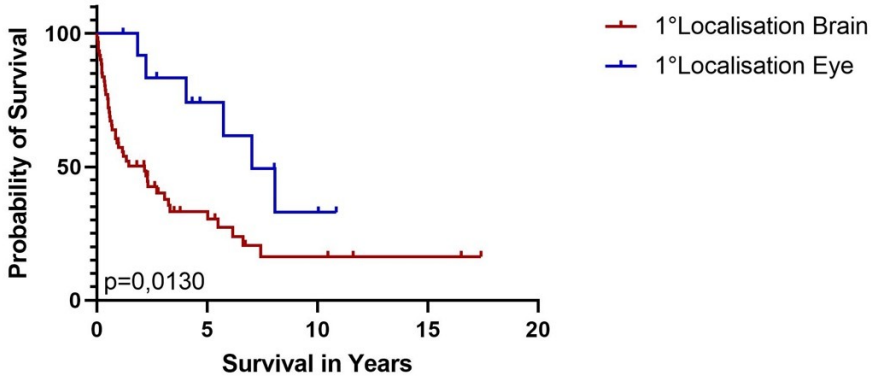


Table 18 1° Localization: Survival data

	1° Localization Brain	1°Localization Eye
Median survival (years)	2.1	7.0
Hazard ratio	2.8	1 (reference)

Epidemiologic studies have reported that the incidence of PCNSL in elderly has increased over the last decades and this is likely to continue, in regard to the demographic change.(153) Although the age cutoffs vary throughout the established prognostic models, being 50 years in MSKCC, 60 years in IELSG and 80 years in the Taipei score, we chose the traditional and most often utilized cutoff value of 60 years for our analysis.(127) In our cohort too, the majority namely 42 out of 74 patients (56.76%) were aged over 60 years at the time of diagnosis. Our statistical investigation showed a median survival of 2.1 years for patients aged over 60 years and a median survival of 7.0 years within the younger patient group, aged ≤60years (p=0.009; HR: 2.14). The corresponding Kaplan Meier curve is shown in Figure 16.

Figure 16 Age: Kaplan-Meier survival curve

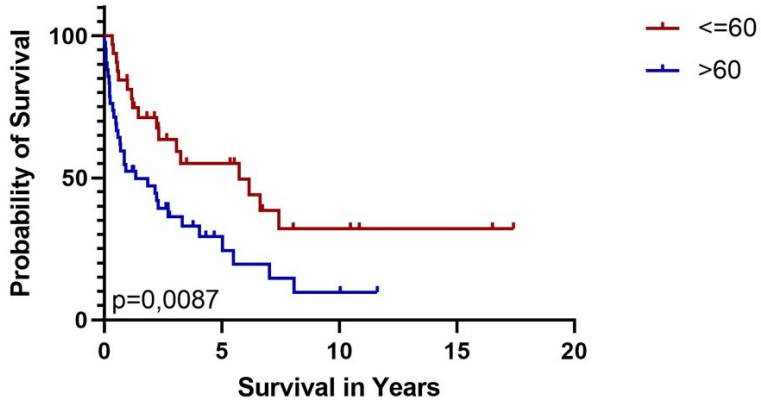


Table 19 Age: Survival data

	Age ≤60 years	**Age >60 years**
Median survival (years)	7.0	2.1
Hazard ratio	2.14	1 (reference)

Besides age, the performance status is one of the most consistent independent prognostic factors for PCNSL patients and is incorporated in all established prognostic models.(125) The Karnofksy performance status ranges from 0 (dead) to 100 (normal activity) and describes the ability of a patient to perform ordinary tasks. The higher the score is, the better is the patients capability to carry out day-to-day tasks.(154) It is an integrated part of the MSKCC score with a chosen cut-off value of ≤70. Patients showing a score of >70 have a better overall survival as compared to those ≤70. In conformity with the MSKCC study, the 31 patients (41.89%) with a Karnofsky index of ≤70 had a significantly shorter median survival of 0.7 years as compared to the 43 patients (58.11%) with a Karnofsky index of > 70, showing a median survival of 5.7 years in our study group (p=0.0045; HR 2.4 (95% CI of ratio: 1.2-3.4)). In Figure 17 the associated Kaplan Meier curve is illustrated.

Figure 17 Karnofsky index: Kaplan-Meier survival curve

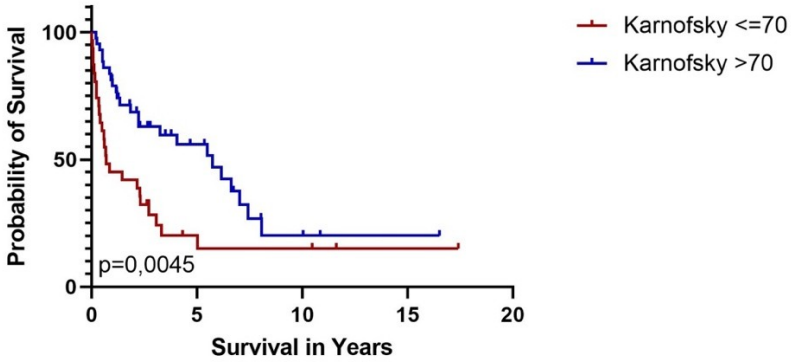


Table 20 Karnofsky index: Survival data

	Karnofsky index ≤70%	**Karnofsky index >70%**
Median survival (years)	0.7	5.7
Hazard ratio	2.4	1 (reference)

In 1960 the Eastern Co-operative Oncology Group (ECOG) developed a simplified version of the Karnofsky status, ranging from 0 (normal activity) to 5 (dead).(155) Since then, the ECOG status has been used to develop several prognostic models for patients with DLBCL, such as the IPI, R-IPI, NCCN-IPI. Moreover, it is incorporated into prognostic models for patients with PCNSL as well, namely the IELSG, Nottingham/Barcelona and the Taipei score, separating patients into two groups: 0-1 and >1.43 of our patients (58.11%) had an ECOG score of 0-1 and 31 patients (41.89%) showed to have an ECOG score of >1 at the time of diagnosis. Patients within the latter group had a median survival of 0.69 years compared to 5.73 years within the patient group with an ECOG score of 0-1 (p=0.005). The Kaplan Meier curve in Figure 18 shows the survival differences of these groups.

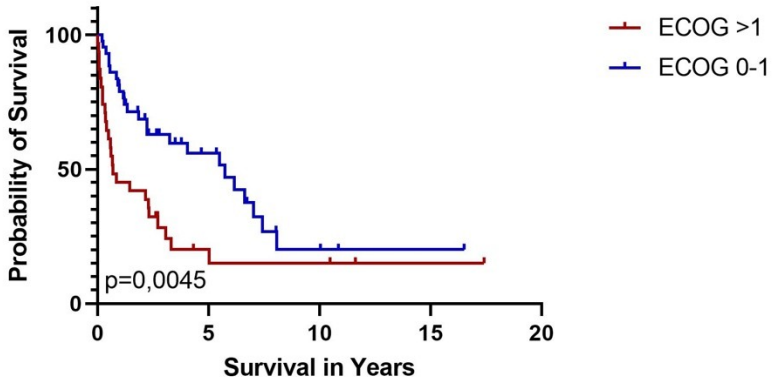


Figure 18 ECOG: Kaplan-Meier survival curve

Table 21 ECOG: Survival data

	ECOG 0-1	ECOG >1
Median survival (years)	0.69	5.73
Hazard ratio	2.19	1 (reference)

A new prognostic marker was found, as a subset of patients with follicular lymphoma with early disease progression within the first 24 months showed to have an inferior survival compared to those without early disease progression.(156) Inspirated by this discovery, we investigated the influence of early progression of disease within the first 12 months (POD12) and 24 months (POD24) upon the overall survival of patients with PCNSL. The starting point was based on the date of definite histologic diagnosis and progression was defined as clinically verified progression of disease, relapse or no response to therapy within the first 12 or 24 months.

In total, 44.59% (33 patients) had POD12, 20.27% (15 patients) relapsed after 12 months and 33.78% (25 patients) did not relapse at all. Moreover, 50% (37 patients) of our cohort showed to have POD24 and as many as 14.86% (11 patients) relapsed after 24 months and 33.78% (25 patients) did not experience relapse. We missed data from one patient in both groups (1.35%). The median survival of patients within the POD12 group was 0.6 years. Patients who relapsed after 12 months had a median survival of 5.72 years and the group with patients who did not relapse, did not reach the median survival ($p<0.0001$, HR 6.9 (95% CI of ratio: 3.5-13.6)). The survival rates of the above-mentioned groups are represented graphically in Figure 19.

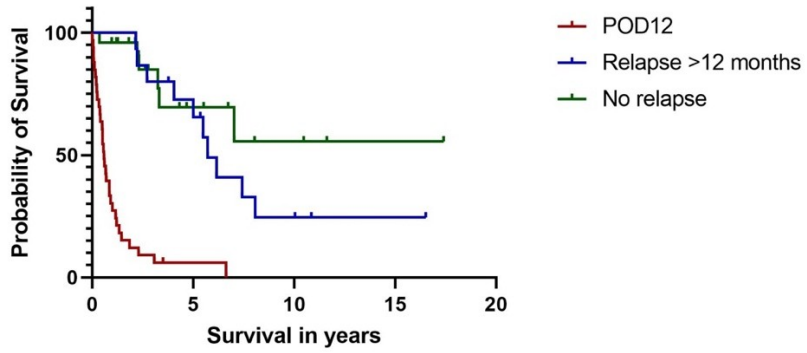


Figure 19 POD12: Kaplan Meier survival curve

Table 22 POD12: Survival data

	POD 12	Relapse >12m	No relapse
Median survival (years)	0.6	5.7	NR

The POD24 group had a median survival of 0.7 years (HR: 6.6 (95% CI of ratio: 3.5-12.34)). Patients who relapsed after 24 months had a median survival of 7.42 years and patients who never relapsed did not reach the median survival.

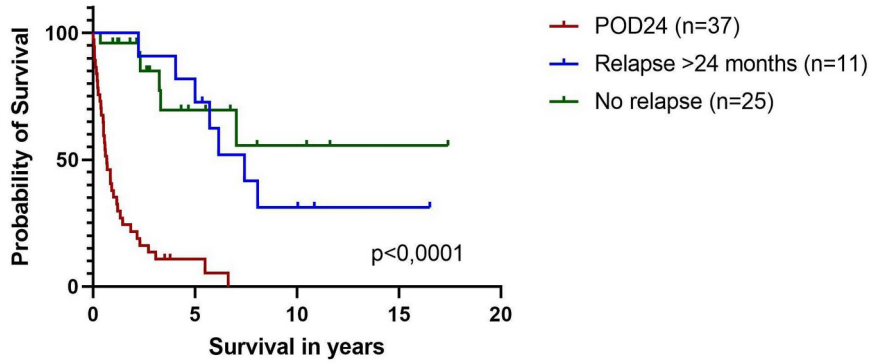
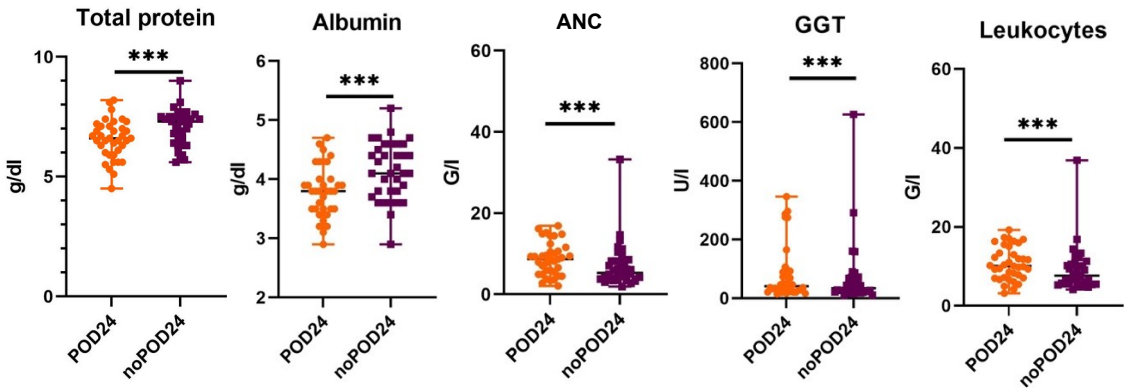


Figure 20 POD 24: Kaplan-Meier survival curve

Table 23 POD 24: Survival data

	POD 24	Relapse > 24 months	NO relapse
Median survival (years)	0.67	7.42	NR

Since POD24 possessed a strong association with poor survival and has never been investigated in PCNSL so far, we further investigated potential differences between the groups POD24 and noPOD24, using the Mann-Whitney U test. Here we observed significant differences in the leukocyte count, the neutrophil count, GGT, total protein and albumin levels as displayed in Figure 21.



*** = statistically significant (p=0.0001 - 0.001)

Figure 21 Scatter plots- total protein, albumin, ANC, GGT and leukocyte levels in POD24 vs. noPOD24 groups (the horizontal line indicates the median value and the error bars the range of values)

Subsequently we created contingency tables and performed the fisher exact test upon these variables because the size of the groups were relatively small.

Table 24 Contingency table: POD24& leukocytosis

n	**POD24**	**NoPOD24**	**p-value**
Leukocytosis	18	29	0.037
No Leukocytosis	15	7	
Total	33	36	

Table 25 Contingency table: POD12& ANC

N	**POD12**	**noPOD12**	**p-value**
ANC normal	20	36	0.031
ANC high	9	4	
Total	29	40	

We could observe that the number of patients with leukocytosis and/or neutrophilia at the time of diagnosis was significantly higher within the POD12/ POD24 group compared to patients without POD12/POD24 as shown in Table 24 and 25.

3.4 Serologic markers

Serological markers have been increasingly investigated upon their influence on the prognosis of patients with PCNSL. We analyzed available serum markers prior to treatment and in timely proximity to the date of diagnosis in an univariate fashion. All serological parameters that were investigated are displayed in Table 26.

Table 26 Overview of the evaluated serologic parameters

Serological Parameter	**Groups**	**Cut-off values**	**n**	**Median Survival (years)**	**p-value**	**Hazard ratio**
Leukocyte count	Leukocytosis	>11.3G/L	23	1.2	0.0131	2.0
	No Leukocytosis	≤11.3 G/L	51	3.3		1 (reference)
Gamma-GT	High	>55 U/L	25	1.45	0.0372	1.8
	Low	≤55 U/L	48	3.07		1 (reference)
Neutrophil count	Neutrophilia	>7.7G/L	35	1.2	0.0175	2.1
	No Neutrophilia	≤7.7 G/L	39	3.3		1 (reference)
LDH-to-Lymphocyte ratio (LLR)	High	>149.71	37	2.23	0.0475	1.75
	Low	≤149.71	37	3.31		1 (reference)
Neutrophil-to-Lymphocyte ratio (NLR)	High	>4.94	37	0.99	0.0015	2.44
	Low	≤4.94	37	6.15		1 (reference)
Derived NLR (dNLR)	High	>7.09	19	0.85	0.0009	2.58
	Low	≤7.09	55	5.01		1 (reference)
Systemic immune inflammation index (SII)	High	>1249.43	37	1.2	0.0044	2.2
	Low	≤ 1249.43	37	5.7		1 (reference)
Platelet-to-Lymphocyte Ratio (PLR)	High	>226.33	19	1.45	0.0586	1.78
	Low	≤226.33	55	3.31		1 (reference)
Anemia	Yes	(f<12; m<14 g/dl)	25	5.01	0.8487	1 (reference)
	No		49	2.23		1.06
CRP	High	>5 mg/L	18			
	Low	≤5 mg/L	56			
Fibrinogen	High	>400 mg/dl	10	3.89	0.9362	1 (reference)
	Low	≤400 mg/dl	37	2.31		1.03
Creatinine	High	>1.2 mg/dl	5	4.05	0.6944	1.22
	Low	≤1.2 mg/dl	68	2.3		0.82
Bilirubin	High	>1.20 mg/dl	2	2.23	0.9405	1 (reference)
	Low	≤ 1.20 mg/dl	71	2.72		1.08
Uric acid	High	>7.0	3	Undefined	0.6481	1 (reference)
	Low	≤7.0	70	2.31		1.577
Albumin	Normal	3.5-5.5	60	3.25	0.0048	1 (reference)
	Low	<3.5	10	0.4		2.82
Total protein	High	□6.6 g/dl	46	5.72	<0.0001	1 (reference)
	Low	<6.6 g/dl	28	0.77		2.91
LDH	High	>240	10	5.01	0.9660	1 (reference)
	Low	≤240	45	3.31		1.02

31.1% (n=23) of our patients presented with leukocytosis at the time of diagnosis, which could demonstrate to have a detrimental influence on the prognosis of PCNSL patients. The leukocytosis group (leukocyte count >11.3 G/L) had a median survival of 1.2 years, whereas the no-leukocytosis group had a median survival of 3.3 years (p=0.013, HR: 2.0 (95% CI of ratio: 1.1-3.8)).

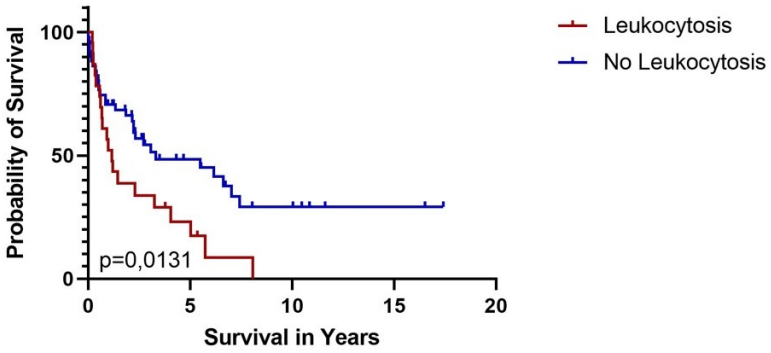


Figure 22 Leukocytosis: Kaplan-Meier survival curve

Table 27 Leukocytosis: Survival data

	Leukocytosis	**No Leukocytosis**
Median survival (years)	1.2	3.3
Hazard ratio	1 (reference)	2.0

Gamma Glutamyl Transferase is a membrane-bound enzyme. It is involved in the synthesis, degradation and transport of glutathione, an antioxidant that contributes to the protection against oxidative stress. GGT is used as a hepatobiliary biomarker and has shown to be an adverse prognostic parameter in a variety of life-threatening diseases and malignant neoplasms. To our knowledge, no association has been found between high GGT levels and DLBCL or PCNSL up until now. However, 33.8% of our patient cohort presented elevated GGT levels at the time of diagnosis. These patients had a median survival of 1.45 years, whereas patients with normal GGT levels had a median survival of 3.07 years (p=0.037). The corresponding survival curve and data are shown in Figure 23 and Table 28.

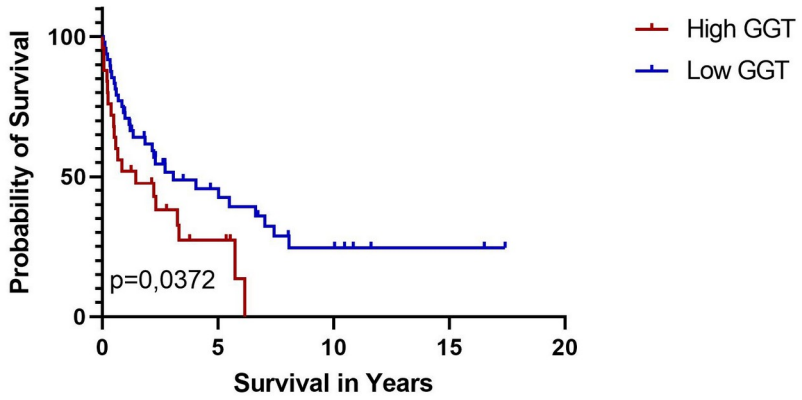


Figure 23 GGT: Kaplan-Meier survival curve

Table 28 GGT: Survival data

	High GGT (>55 U/L)	**Low GGT (≤55 U/L)**
Median survival (years)	1.45	3.07
Hazard ratio	1.80	1 (reference)

Hypoalbuminemia is a frequent observation in hospitalized patients and is caused by many different diseases such as cirrhosis, malnutrition, sepsis or the nephrotic syndrome.(157) 13.51% of our patient cohort showed to have low albumin levels (<3.5 g/dl) and had a statistically significant shorter OS as compared to the patients with normal albumin values (p=0.0048) as graphically illustrated in Figure 24 .

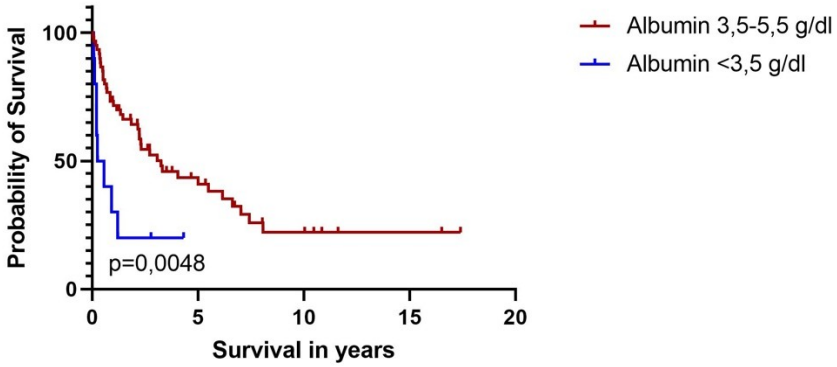


Figure 24 Albumin: Kaplan-Meier survival curve

Table 29 Albumin: Survival data

	Normal Albumin (3.5-5.5 g/dl)	**Low Albumin (<3.5g/dl)**
Median survival (years)	3.25	0.4
Hazard ratio	1 (reference)	2.82

As albumin compromises 50 to 60% of the total protein, hypoproteinemia is often associated with hypoalbuminemia.(158) Proteins are major components in the build-up of body tissues and are involved in many cellular activities.(159) The pathogenesis of hypoproteinemia is very variable and is frequently observed in patients with malignancies. (160) In our cohort 37.84% of all patients showed low protein values (<6.6 g/dl). These patients were associated with a shorter OS as compared to patients with normal protein levels (p<0.0001). The matching survival curve and data are demonstrated in Figure 25 and Table 30.

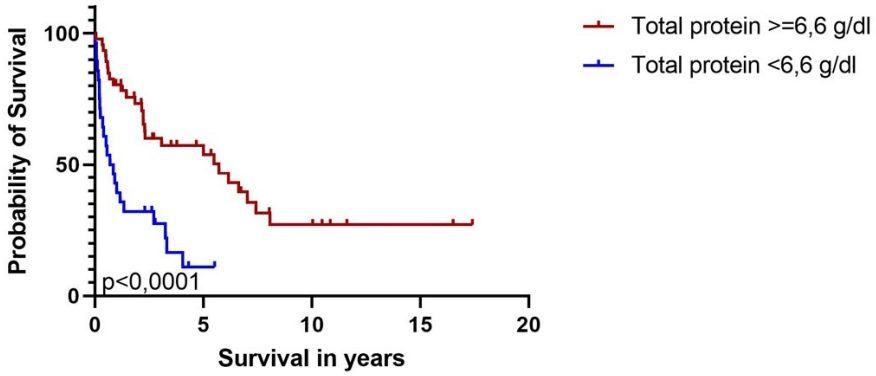


Figure 25 Total protein: Kaplan-Meier survival curve

Table 30 Total protein: survival data

	Normal total protein (6.6-8.3 g/dl)	**Low total protein (<6.6 g/dl)**
Median survival (years)	5.72	0.77
Hazard ratio	1 (reference)	2.91

Inflammation has shown to participate in tumor progression by influencing the microenvironment and promoting the survival of cancer cells. Many markers of systemic inflammation have already been identified as prognostic factors in lymphoma.

The absolute neutrophil count (ANC) represents the innate immune system and is one of the first effectors within an acute inflammatory response and affects the development of tumors.(136,145) Based hereon, we studied the influence of high pre-treatment ANC levels upon the outcome of patients with CNSL. The cut-off value for ANC was 7.7 G/L and 47.3% of all patients had values above this cut-off. These patients showed a significantly reduced OS as compared to the study group with values equal to or below the cut-off value

(p=0.0175; HR: 2.1 (95% CI of ratio: 0.9-4.9)). These results are represented in Figure 26 and Table 31.

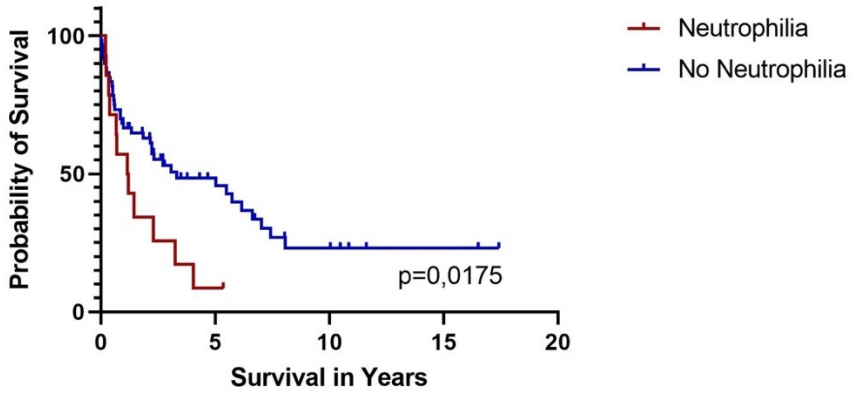


Figure 26 Neutrophilia: Kaplan-Meier survival curve

Table 31 Neutrophilia: Survival data

	Neutrophilia (>7.7 G/L)	**No Neutrophilia (≤7.7 G/L)**
Median survival (years)	1.2	3.3
Hazard ratio	2.1	1 (reference)

Elevated tumor-burden markers are poor prognostic factors. Thus, the idea evolved to combine inflammation markers and tumor-burden markers in a ratio to further improve the prediction of the clinical outcome of patients.(161)

The lactate dehydrogenase-to-lymphocyte ratio is such a combined prognostic marker. (124)

We calculated the ratio, by dividing the LDH level in U/L by the lymphocyte count in G/L for each patient. Hereafter, we calculated the median and interquartile ranges of the LLR values. Survival analyses showed the median value of 149.71 to be the best cut-off value. Subsequently, we divided our cohort into two groups. Patients were either assigned to group 1, if their LLR was ≤ 149.71 or to group 2, if their value was higher than the median LLR value of our study group. 37 patients (50%) were included in group 1 and 37 patients (50%) within group 2. Patients in group 2 had a significantly inferior median survival (2.2 years) as compared to patients within group 1, who had a median survival of 3.3 years (p=0.048; HR: 1.7 (95% CI of ratio: 1.0-3.1)). The survival difference of these groups is depicted graphically in Figure 27 with the associated survival data collected in Table 32.

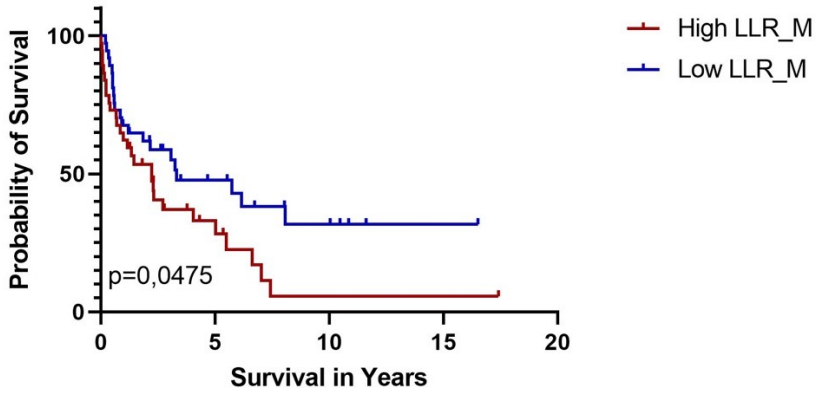


Figure 27 LLR: Kaplan-Meier survival curve

Table 32 LLR_M: Survival data

	Low LLR_M	**High LLR_M**
Median survival (years)	3.3	2.2
Hazard ratio	1 (reference)	1.7

The neutrophil-to-lymphocyte ratio has been identified as a prognostic marker for a wide variety of solid tumors and represents an inexpensive and easily accessible biomarker. (162) More recently, it has been discovered to be of prognostic significance in PCNSL as well. We calculated the median value of the NLR in our cohort and established it as the cut-off value as it showed to stratify our patients the best. In compliance with other studies, we could show that a high NLR value prior to treatment resembled a poor prognostic marker for patients with PCNSL.(145) 37 out of our 74 patients (50%) had values higher than the chosen cut off value 4.94. This patient group had a significantly shorter median survival of 0.98 years compared to 6.15 years in the group with lower NLR values (p=0.0015, HR:2.44 (95% CI of ratio 1.4-4.3)). The associated survival curve and data are presented in Figure 28 and Table 33.

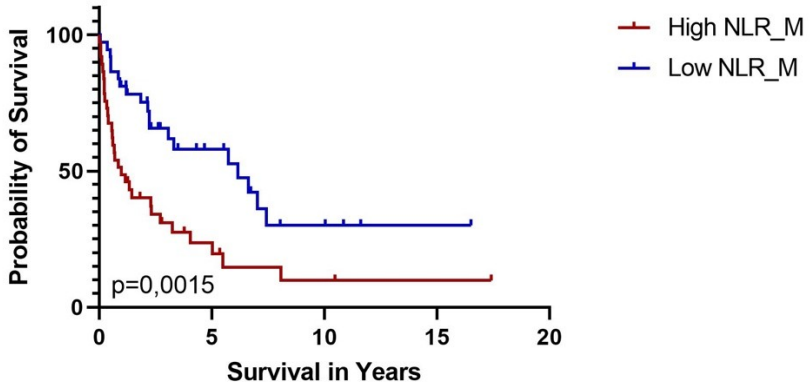


Figure 28 NLR: Kaplan-Meier survival curve

Table 33 NLR_M: Survival data

	Low NLR_M	High NLT_M
Median survival	6.15	0.98
Hazard ratio	1 (reference)	2.44

The derived NLR (dNLR) is a similar ratio to that of the NLR. We obtained the values by calculating the following formula: neutrophil count / (leukocyte count- neutrophil count). This ratio could in previous studies show to have prognostic power upon the survival of PCNSL patients, too.(124) Using this ratio, the third quartile value of 7.09 could stratify our patients the best. Patients with values of above 7.09 had a median survival of 0.9 years. In contrast, patients who had values equal to or below this cut-off showed to have a median survival of five years (p=0.0009; HR: 2.6). The corresponding Kaplan-Meier curve and survival data are illustrated in Figure 29 and Table 34.

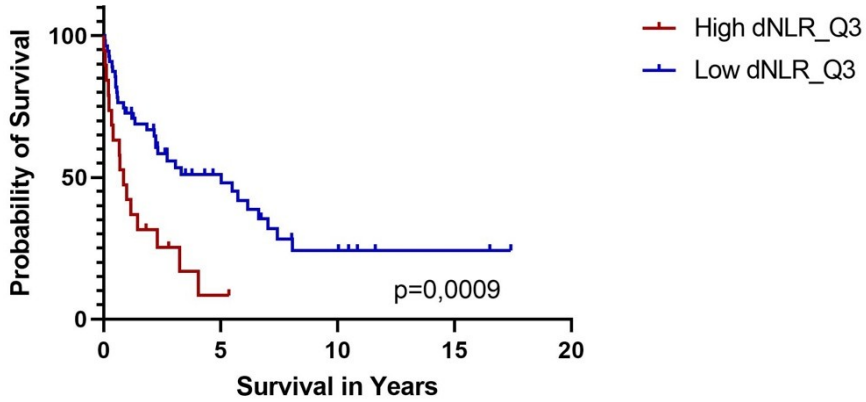


Figure 29 dNLR: Kaplan-Meier survival curve

Table 34 dNLR_Q3: Survival data

	Low dNLR_Q3	High dNLR_Q3
Median survival (years)	5	0.9
Hazard ratio	1 (reference)	2.6

The SII is an integral part in one of the most recently developed prognostic models for PCNSL, the CBC model and could be significantly associated with the prognosis of survival of patients.(147) We obtained values of SII by multiplying the neutrophil count with the thrombocyte count and divided this value by the lymphocyte count. Subsequently, we utilized the median value of 1249.43 as a cut-off. The ones with high SII values (>cutoff) showed significantly inferior survival rates compared to the 37 patients harboring values below or equal to the cutoff (p=0.0044; HR 2.2) as demonstrated in Figure 30 and Table 35.

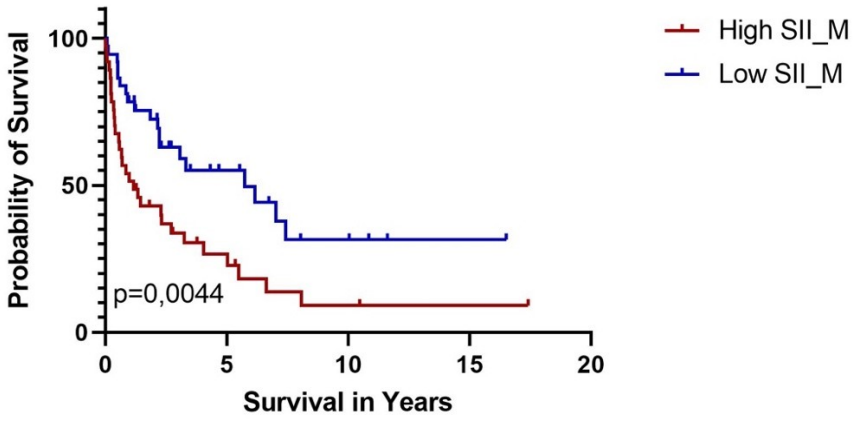


Figure 30 SII: Kaplan-Meier survival curve

Table 35 SII: Survival data

	High SII (>1249.43)	Low SII (≤1249.43)
Median survival (years)	1.2	5.7
Hazard ratio	2.2	1 (reference)

3.4.1 Pathologic Markers

We investigated multiple immunohistochemical parameters (COO, DEL status, CD30, CD10, P53, MIB, CD3, CD5, CD79a, MUM, GCET-1, c-MYC, PD-L1, PD-L2 and FOX-P1) as summarized in Table 36. We missed more than 50% of the values for c-MYC, PD-

L1, PD-L2 and FOX-P1. Therefore, we decided to exclude those parameters from our statistical test.

Table 36 Overview of the evaluated histopathological parameters

Histopathologic parameters	**Groups**	**n**	**Median survival**	**p-value**	**Hazard ratio**
COO	GCB	5	NR	0.0463	5.79
	NGCB	62	2.15		1 (reference)
DEL status	BCL-2& MYC	18	0.79	0.0033	
	BCL-2/ MYC	28	3.31		
	Neither	8	6.15		
CD30	Positivity	12	12.02	0.0135	1 (reference)
	Negativity	30	1.15		3.09
GCET-1	Positivity	13	7.42	0.0386	1 (reference)
	Negativity	25	0.9		2.69
CD10	Positivity	14	3.31	0.4174	1 (reference)
	Negativity	47	2.15		1.37
P53	Positivity	29	2.23	0.4614	1 (reference)
	Negativity	10	1.45		1.327
MIB	≥85	25	2.15	0.5752	1.19
	<85	28	1.21		1 (reference)
CD3	Positivity	31	3.31	0.1285	1 (reference)
	Negativity	32	2.03		1.63
CD5	Positivity	12	2.15	0.8885	1 (reference)
	Negativity	26	2.3		1.06
CD79a	Positivity	35	3.25	0.7681	1.09
	Negativity	39	2.31		1 (reference)
MUM-1	Positivity	48	0.99	0.0995	3.07
	Negativity	6	5.72		1 (reference)

As immunohistochemistry-based analyses are cost-effective and readily available, they have been widely incorporated into the clinical practice.(132) The classification of DLBCL into GCB and non-GCB subtypes via Hans algorithm has initially revealed the GCB-subtype to be a good prognostic marker in systemic DLBCL.(163) But the predictive value

of IHC has more recently been questioned as it showed to have poor concordance with GEP based results.(164–166)

In our study group the vast majority, in total 62 patients (83.78%) were identified to harbor the non-GCB subtype. This is in accordance with the distribution of subtypes within other study groups. Patients with the non-GCB-subtype had a median survival of 2.15 years, whereas the group harboring the GCB-subtype did not reach the median survival as demonstrated in Figure 31 and Table 37 (p=0.0463; HR:5.8).

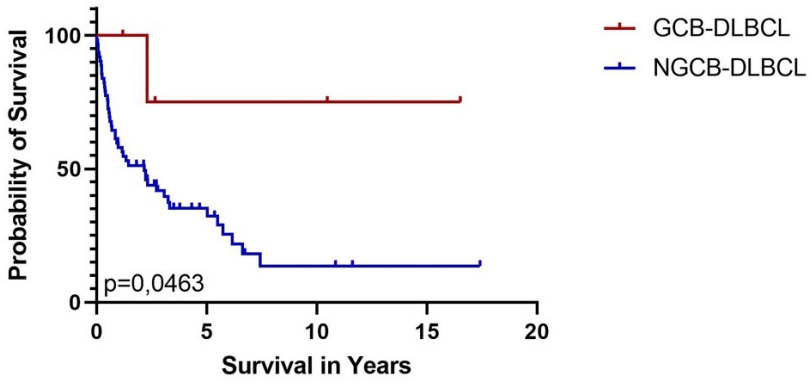


Figure 31 COO: Kaplan-Meier survival curve

Table 37 COO: Survival data

COO	**NGCB-DLBCL**	**GCB-DLBCL**
Hazard ratio	5.79	1 (reference)
Median survival (years)	2.15	NR

Patients with PCNSL can show an overexpression of BCL-2 and/or c-MYC, which are defining features of the double-expressor lymphoma (DEL). They can both be absent or if present they occur either separately or together and are detected by immunohistochemistry. (43,118) Overall, 10.81% of our patient collective did not show any of these double expressor features and had a median survival of 6.15 years. 37.83% expressed one DEL feature and had a median survival of 3.31 years and finally 24.32% expressed both BCL-2 and c-MYC and had a median survival of 0.79 years (p=0.003). The survival differences of these groups are displayed in Figure 32.

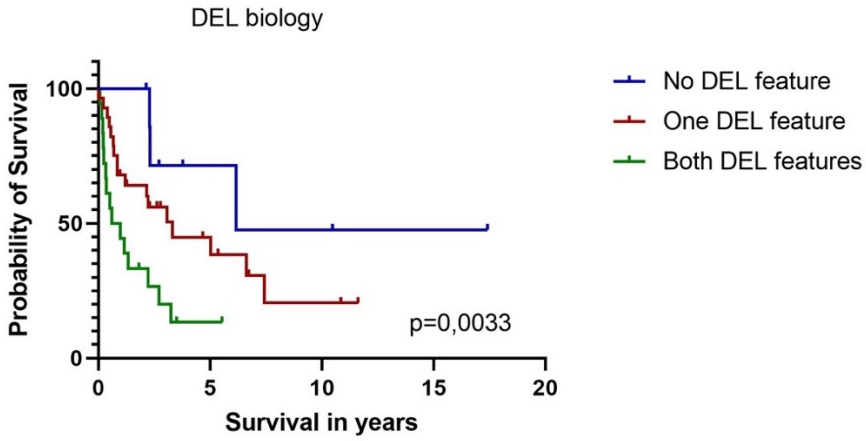


Figure 32 DEL status: Kaplan-Meier survival curve

Table 38 DEL status: Survival data

	No DEL feature	**Bcl-2 or MYC**	**Bcl-2 and MYC**
Median survival (years)	6.15	3.31	0.79

CD30 belongs to the family of tumor necrosis factor receptors and was originally discovered to be a cell-surface marker of Hodgkin and Reed-Sternberg cells. However, despite its expression in Hodgkin lymphomas, several non-Hodgkin lymphomas as for example DLBCL variably express CD30 as well. Studies have shown that about 14-25% of all DLBCL express CD30. However, its implication on prognosis is still not clear. As many as 12 patients (16.22%) showed to express CD30 in our cohort and had a median survival of 12.2 years compared to those without CD30 expression who had a median survival of 1.15 years (p=0.0135) as illustrated in Figure 33.

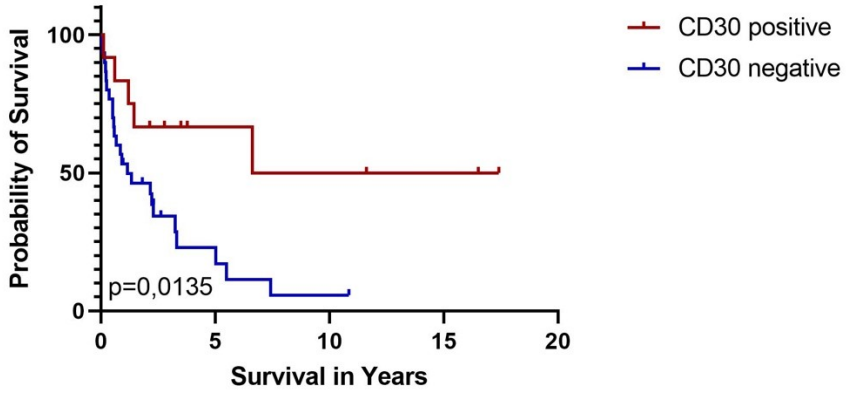


Figure 33 CD30- Kaplan-Meier survival curves

Table 39 CD30 expression: Survival data

	CD 30^{+}	**CD30^{-}**
Median survival (years)	12.02	1.15
Hazard ratio	1 (reference)	3.09

The GCET-1 (germinal center B-cell-expressed transcript-1) gene encodes for a serpin, which is expressed in GC B-cells. Therefore, GCET-1 is a marker that is highly restricted to germinal center-derived lymphomas. It has been incorporated into several immunohistochemistry-based algorithms, using different phenotypic markers such as Choi (based on GCET-1, BCL-6, MUM1, CD10 and FOXP1) or Tally (comparing the expression of GCET1 and CD10 vs. FOXP1 and MUM1).(167,168) GCET-1 is commonly expressed in follicular lymphoma and DLBCL with a GC B-cell differentiation.(169) We missed GCET-1 related data from 36 patients in our cohort. Out of the 38 patients remaining, 13 patients showed an expression of GCET-1. These patients had a median survival of 7.42 years. The 25 patients without expression of GCET-1 had a median survival of 0.9 years (p=0.0386, HR: 2.69) as illustrated in Figure 34 and Table 40.

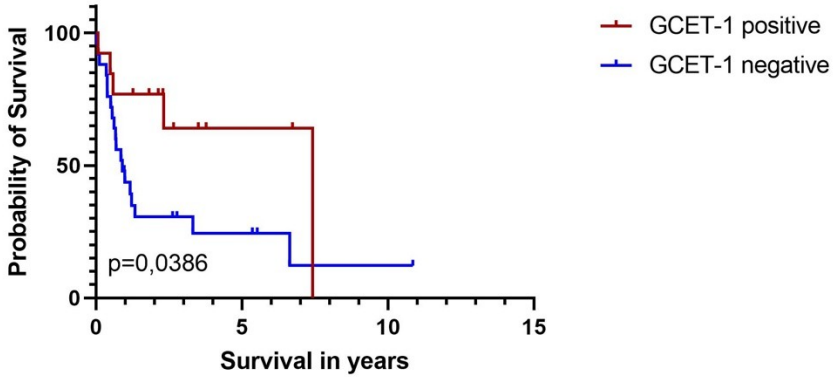


Figure 34 GCET-1 expression: Kaplan-Meier survival curve

Table 40 GCET-1 expression: Survival data

	GCET-1 positive	**GCET-1 negative**
Median survival (years)	7.42	0.9
Hazard ratio	1 (reference)	2.69

4 Discussion

PCNSLs harbor both a molecular and genetic diversity, resulting in distinct clinical presentations and outcomes. Generally, they tend to be highly aggressive and quickly symptomatic and are thus in need for a rapid treatment initiation. In order to facilitate the individual's treatment decision-making, well-developed and easily available prognostic scores are required.(170,171) Several studies have been dedicated to improve the prediction of outcome of patients with PCNSL. Nevertheless, most studies showed to have major limitations. This has led to contradicting results. Consequently, the prognosis of patients with PCNSL remains challenging. Therefore, this study constitutes an attempt to verify the validity and clinical utility of already established prognostic models and parameters as well as to identify new biomarkers for patients with PCNSL. In this study, we solely included patients diagnosed by stereotactic brain biopsy. The majority, namely 86.49% of our patients were treated with a HD-MTX based chemotherapy. Furthermore, our cohort exhibited representative characteristics regarding age and sex for PCNSL patients. The baseline clinical status was evenly distributed throughout the cohort. 41.89% had a poor baseline status (Karnofsky index <70%) and 58.11% showed to have a good baseline clinical status (Karnofsky ≥70%).

The most widely accepted prognostic markers of PCNSL are age and performance status. (172) Age has been known as the strongest prognostic factor for patients with PCNSL. In conformity with previous findings, age above 60 years was found to be a strong prognostic factor for inferior survival in our study, too. Not only is age an independent poor prognostic factor, leading to an overall inferior prognosis due to accumulating comorbidities, but it also predisposes the patients to be more severely affected by treatment-related iatrogenic toxicity. Nevertheless, the exact age for determining prognosis has not been established yet, varying between 50 and 80 years. Thus, further studies are required to determine the best cut-off to enable a unified and reliable prognostic stratification. (53,125,153)

As age by itself doesn't capture the fitness of a patient, the performance status has been widely used and accepted as amendment in therapy risk assessment and as a predictor of OS.(53) Subsequently, we were able to demonstrate an equally significant prognostic power for both the ECOG performance status and the Karnofsky index. Particularly when it comes to the management of elderly patients with PCNSL, we should consider a wider

range of assessment tools in regard to treatment selection. Studies have started off, using easy practicable geriatric, cognitive function and chemotherapy risk assessment scales to improve the evaluation of the individuals functional reserve and overall health status. (53,173–175) These might further ease the identification of high-risk patients and treatment decision-making for elderly patients. The MSKCC score which incorporates these two variables: age and ECOG performance status, is one of the most widely utilized prognostic models for PCNSL and could be strongly associated with OS in our study group, too.(134) Unfortunately, we were not able to use the IELSG score for our statistical analysis, because CSF samples were not uniformly collected from our patients. Liquor analyses are often missing in PCNSL patients, consequently this prognostic model is frequently inapplicable, highlighting the importance of more easily available prognostic markers when establishing new prognostic scoring systems.

The IPI, R-IPI and NCCN-IPI could successfully distribute our patients into separate risk groups with subsequent different survival outcomes. It is noteworthy, that the NCCN-IPI could stratify the patient cohort the best. This might be owed to the fact, that the NCCN-IPI was established to update the previously developed prognostic models IPI and R-IPI by the time rituximab was introduced. Therewith the NCCN-IPI score displays a more up-to-date approach for risk stratification of DLBCL patients.(114) These scores are not specifically developed for PCNSL patients and except for NCCN-IPI, no other study could confirm the prognostic impact of these prognostic models upon the survival of PCNSL patients. It was postulated, that this lack of prognostic power might be due to the variables "number of extranodal sides" and "stage of disease", which are invariable in CNSL patients.(113) Vice versa, we suppose that, as those two variables are constant in our study group, the remaining variables age, ECOC PS and LDH, which were strong independent prognostic markers within our study group, performed well enough to stratify our cohort into distinct prognostic risk groups.

In addition, the primary lesion site (brain vs. eye) impacts on the survival rates of our PCNSL patients. Within our cohort, patients with a primary intraocular lymphoma had superior survival outcomes as compared to the group with primary brain involvement. The median age within the PIOL subgroup was 66 years. Despite the older age distribution in this group, only two out of 13 patients had a poor baseline performance status (ECOG >1). The observation, that patients with PIOL have a generally better performance status in contrast with patients harboring cerebral involvement was made before and might explain the better OS within this subgroup. Furthermore, it was postulated that the overall tumor

burden is smaller in patients with a primary intraocular involvement, which might play a role as well.(152)

In our cohort, we found leukocytosis to be associated with a significant inferior OS. Brandt et al retrospectively investigated bone marrow samples of PCNSL patients and reported, that a significant proportion of PCNSL patients possessed a monoclonal B-cell lymphocytosis.(170) This discovery might in part explain the increased leukocyte levels in the 23 of our 74 patients. Especially when considering, that a monoclonal B-cell lymphocytosis is a common finding in elderly and the mean age of our cohort was 63 years.(170) Consequently, investigations of the extracted bone marrow biopsies should be considered to support this suggestion. Another possible reason for the leukocytosis in PCNSL has been described in a study, addressing the pathophysiology of leukocytosis in solid tumors. They discovered that the tumor or its environment augments the secretion of hematopoietic growth factors, fostering myelopoiesis and the expansion of myeloid-derived suppressor cells (MDSCs). These MDSCs are known to suppress the lymphocyte proliferation and present as neutrophils in the CBC, thus contributing to a high NLR.(176) Finally, leukocytes are cellular effectors of inflammation and play an important part in the tumor microenvironment.(177) The cancer-related inflammation is presumed to promote invasion, metastasis and tumor cell proliferation and could equally plausible explain the inferior survival in PCNSL patients with leukocytosis.(178) Summarizing, there are many possible explanations upon why leukocytosis might negatively influence the OS of patients with PCNSL. However, the exact underlying mechanism is yet to be explored.

Furthermore, we could identify patients with low albumin and total protein levels to have a significantly inferior OS. Irrespective of the cause, hypoalbuminemia is a strong predictor of mortality and morbidity. It might reflect a poor nutritional status or an inflammatory reaction to disease and is commonly found in elderly patients. A low albumin level has already been identified as a risk factor for patients with cancer, including those with DLBCL and has been incorporated into prognostic models.(157,179)

Moreover, we could demonstrate that high NLR (>4.94) and dNLR levels (>7.09) have a statistically significant association with a shorter OS. As both lymphocytes and neutrophils are systemic inflammatory markers, this might further underline the close relationship of systemic inflammation and cancer progression. It has been suggested that neutrophils contribute to the tumor development by releasing pro-angiogenic factors, chemokines and cytokines. Consequently, they are believed to promote a favorable environment for tumor growth, genomic instability and lymphangiogenesis, which might explain the shorter

survival of patients with elevated neutrophil levels.(136,145) In concordance with our findings, several previous studies could show a worse OS for patients with PCNSL, showing high pre-treatment NLR as well as dNLR levels. However, multivariate analysis failed to show prognostic significance and similar contradictive results have been observed in DLBCL.(145) Furthermore, we did not distinguish if patients received corticosteroid treatment prior to blood sampling or not. It has been described that corticosteroids cause neutrophilia as well as lymphopenia, thus explaining its possible influence on our results.(128) Therefore, in order to bypass the potential impact of corticosteroids on blood cell counts and hence strengthen the prognostic validity of both neutrophilia, NLR and dNLR on OS in future, statistical analyses of blood samples should be initiated prior to corticosteroid administration. Furthermore, a published study in 2022 has for the first time researched the dynamics of NLR in response to corticosteroids and its correlation with survival.(128) This represents a promising approach and should be further validated.

As distinct inflammatory biomarkers could not be proven to be significant prognostic markers in several studies, new prognostic ratios were developed combining makers of inflammation with tumor-related markers. LLR has been incorporated in a new prognostic model for extranodal natural killer/ T-cell lymphoma. It was also identified to be an independent prognostic factor in some other types of cancers such as in DLBCL. In 2021, the negative prognostic role of a high pre-treatment LLR could be demonstrated for PCNSL patients as well.(124) Our univariate analyses could confirm this association, as patients with a high ratio (>108.18) had a significantly shorter OS. LDH represents a marker of tumor burden in DLBCL and has the highest prognostic impact among the IPI risk factors. A high LDH level is an indirect marker of metastasis development, neovascularization, impaired host immune response amongst others and is an important marker of poor prognosis. A low absolute lymphocyte count is thought to lead to an insufficient production of anti-neoplastic chemokines and to increase the risk of treatment-associated side effects. Thus, this ratio represents a promising prognostic tool and has successfully been used to refine the prognostic capability of the MSKCC in previous studies, allowing for a better stratification of the low and intermediate risk group. Thus, the LLR further ameliorates the stratification of patients with PCNSL.(124,137)

The SII ratio including platelet, neutrophil and lymphocyte counts showed to have superior prognostic validity compared to that of NLR, LMR and PLR in patients with classic Hodgkin lymphoma.(180) Moreover, SII has been used to develop a new prognostic model for PCNSL patients.(147) We could confirm the prognostic validity of SII in our cohort.

This ratio was created as both platelets and neutrophils are suggested to play an important role in cancer cell proliferation, invasion, metastasis and immune evasion. Lymphopenia on the contrary, reflects an inefficient immune system, facilitating the spread of cancer cells.(181) This index represents an easy available and early prognostic marker for PCNSL patients by reflecting the balance between the host immune system and the inflammatory condition.(182)

Interestingly, we found a new prognostic marker for PCNSL patients, namely GGT. High levels could show to be significantly associated with a poorer prognosis of patients in our cohort. To the best of our knowledge, no other study has described this correlation up until now. A high GGT level is typically a sign of hepatobiliary dysfunction or excessive alcohol intake.(183) More recently, elevated GGT levels have been associated with the risk of cardiovascular events, the metabolic syndrome, diabetes II, hypertension, renal failure and cancer. Therefore, it is now speculated that other diseased tissues apart from the liver might influence the serum GGT activity, which might explain the negative predictive value of this parameter. Several studies investigated the association of GGT levels upon the risk of cancer incidence and found it to be associated with malignant neoplasms, for example lymphoid and hematopoietic cancers. Furthermore, GGT-derived pro-oxidants may influence the oxidative stress observed in cancers and might modulate processes that may play a role in tumor progression. The antioxidant activity of GGT on the other hand, increases the cells tolerability of oxidative stress, contributing to a resistance to pro-oxidant drugs.(184) Considering all these potential functions of GGT, the exact underlying mechanism of it on the survival of PCNSL patients remains a matter of debate.

We further identified groups of patients who had early progression of disease within the first 12 (POD12) and/or 24 months (POD24) to have a highly significant poorer prognosis. The association of POD12 and/or POD24 with OS was found in many different lymphoma entities but has not been observed in PCNSL so far.(156,185–189) Consequently, we compared the groups with or without POD24 and found lower total protein and albumin levels, higher leukocyte and neutrophil counts as well as higher GGT values within the POD24 group. The influence of high GGT, low protein and albumin levels and leukocytosis on survival were already discussed. As mentioned before, neutrophilia represents an independent poor prognostic factor and has been described to promote tumor progression.(145) Thus, the higher neutrophil values in the POD24 group might have influenced the early progression of lymphoma in these patients.

The prognostic significance of early progression has been explored in follicular lymphoma and meanwhile represents a robust prognostic factor for this lymphoma subtype. It was postulated that these patients harbor specific molecular drivers and complex biological interactions within the tumor microenvironment. Investigations aiming on identifying those, are ongoing.(156,185) In contrast, the association of POD12 or POD24 with OS in PCNSL has to our best knowledge, not been investigated before. In addition to the above-mentioned assumptions, a similar mechanism as postulated in follicular lymphoma could explain the shorter OS in early progressive PCNSLs. As a summary, we could demonstrate the highly significant influence of POD24 upon OS of PCNSL patients. The underlying mechanism is not known and is hence in need of further validation and investigation.

Furthermore, we analyzed the impact of the COO upon the survival of our patients. We could hereby find a significant association between the non-GCB subtype and inferior survival in our cohort, demonstrating the prognostic power of this IHC-based classification. Nevertheless, only five patients of our cohort harbored the GCB-subtype, thus the interpretation of this survival difference should be treated with caution. Considering that, the prognostic value of the Hans classification has been debated more recently, we should focus our attention towards the newly developed antibodies and digital gene expression tests to improve the reproducibility of GEP results.(164)

The prognostic role of the MYC and BCL-2 expression in PCNSL has been determined in several previous studies.(118,190–192) Similar to these studies, we could again verify the dismal outcome in patients expressing one or both double expressor features. We were able to distinguish three groups with clear survival differences and could further support the prognostic impact of the DEL status upon survival. The coexpression of MYC and BCL-2 has been associated with a high-risk gene expression signature in DLBCLs which might explain the corresponing molecular mechanism of inferior survival in these patients.(193) While high MYC protein levels have shown to imply an accelerated proliferation, BCL-2 has been identified as an antiapoptotic protein.(43)

According to our analyses, CD30 positive PCNSLs were associated with a better OS. CD30 is usually expressed in classical Hodgkin lymphomas and anaplastic large cell lymphomas but has also been discovered in other types of lymphomas such as DLBCLs.(194) A published study in 2013 reported CD30 expression to be a favorable prognostic factor in DLBCL. It was proposed that CD30 harbors an antiproliferative function, but the underlying molecular mechanism remains unclear. This study group further investigated these $CD30^{+}$ cases, using GEP. They found gene upregulations for negative regulators of

the nuclear factor κB activation and lymphocyte survival. Additionally, downregulation of B-cell receptor signaling could be observed, indicating these cells to harbor a distinct molecular mechanism.(194,195) These discoveries in combination with our findings, could explain the positive influence of CD30 expression in PCNSL patients and should therefore be further investigated.

Another newly identified significant prognostic marker in our patient cohort is the expression of GCET-1. Patients with GCET-1-positive tumor tissue had a significantly better OS as compared to patients without GCET-1 expression. As far as we know, no other study has found an association between the expression of GCET-1 and survival of PCNSL patients up until now. GCET-1 protein expression is restricted to GCB derived neoplasms. According to another study, GCET-1 was preferentially expressed in GCB-DLBCLs as compared with non-GCB-DLBCLs determined with the Hans algorithm. As the exact role of GCET-1 in lymphoma is still unclear, further studies are needed to identify the influence of its expression upon the clinical course and biologic features of PCNSL. Here as well, our results should be treated with caution as only 13 patients were identified to express GCET-1. Thus, independent studies should be performed to verify this association.(196)

In contrast with previous studies, we failed to associate deep brain involvement, bilirubin, PLR, anemia and the Taipei score with OS.(127,134,135,147) These discrepancies might be caused by the generally small study groups, the retrospective nature of studies upon PCNSL as well as the lack of external validation of study results. Moreover, the Taipei score was developed within an Asian population, whereas the other prognostic scores were developed in western countries.(113)

There are several limitations within our study that should be discussed. Considering the generally low incidence of PCNSL we chose to perform a retrospective monocentric study, harboring inevitable election and information biases. Moreover, our study cohort was relatively small, encompassing 74 patients only. Furthermore, 10 patients received a therapy, other than HD-MTX and we did not take into consideration whether patients had received glucocorticoid treatment prior to baseline blood parameters, which could have influenced the results, especially considering the neutrophil count. Additionally, the DEL status was determined by immunohistochemistry. As the related interpretation is highly dependent on the observer, results may differ from the gold standard tests.

In conclusion, we could demonstrate and validate the prognostic power of already established prognostic parameters (MSKCC, NCCN-IPI, Age, PS, LLR, NLR, dNLR, SII, COO and DEL biology). New potential prognostic markers were found as CD30 as well as GCET-1 expression, GGT and POD12/24 could show to have powerful prognostic impact on survival in patients with PCNSL in our analyses. These markers are routinely determined, easily obtained and promising tools for the prediction of prognosis in patients with PCNSL and might be used to further reform prognostic stratification and facilitate therapeutic decision-making.

Finally, to verify the prognostic relevance of these parameters, our results are in need of validation through an independent external cohort and a multivariate study in near future.

5 References

1. Sapkota S, Shaikh H. Non-Hodgkin Lymphoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [zitiert 26. Juli 2022]. Verfügbar unter: http://www.ncbi.nlm.nih.gov/books/NBK559328/
2. Matasar MJ, Zelenetz AD. Overview of lymphoma diagnosis and management. Radiol Clin North Am. März 2008;46(2):175–98, vii.
3. Mugnaini EN, Ghosh N. Lymphoma. Prim Care. Dezember 2016;43(4):661–75.
4. Shanbhag S, Ambinder R. Hodgkin Lymphoma: a review and update on recent progress. CA Cancer J Clin. März 2018;68(2):116–32.
5. Bröckelmann PJ, Böll B. Moving things forward in Hodgkin lymphoma. F1000Research. 13. November 2018;7:F1000 Faculty Rev-1786.
6. Ansell SM. Hodgkin lymphoma: A 2020 update on diagnosis, risk-stratification, and management. Am J Hematol. 2020;95(8):978–89.
7. GmbH DMS. DocCheck Flexikon. [zitiert 20. August 2022]. Hodgkin-Lymphom. Verfügbar unter: https://flexikon.doccheck.com/de/Hodgkin-Lymphom
8. Momotow J, Borchmann S, Eichenauer DA, Engert A, Sasse S. Hodgkin Lymphoma—Review on Pathogenesis, Diagnosis, Current and Future Treatment Approaches for Adult Patients. J Clin Med. 8. März 2021;10(5):1125.
9. Non-Hodgkin Lymphoma [Internet]. Lymphoma Research Foundation. [zitiert 6. Dezember 2021]. Verfügbar unter: https://lymphoma.org/aboutlymphoma/nhl/
10. Ansell SM. Non-Hodgkin Lymphoma: Diagnosis and Treatment. Mayo Clin Proc. 1. August 2015;90(8):1152–63.
11. Lenz G, Staudt LM. Aggressive Lymphomas. N Engl J Med. 15. April 2010;362(15):1417–29.
12. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 1. September 2008;112(5):1570–80.
13. Hoffman W, Lakkis FG, Chalasani G. B Cells, Antibodies, and More. Clin J Am Soc Nephrol CJASN. 7. Januar 2016;11(1):137–54.
14. B Cells | British Society for Immunology [Internet]. [zitiert 14. August 2022]. Verfügbar unter: https://www.immunology.org/public-information/bitesized-immunology/cells/b-cells

15. Wishnie AJ, Chwat-Edelstein T, Attaway M, Vuong BQ. BCR Affinity Influences T-B Interactions and B Cell Development in Secondary Lymphoid Organs. Front Immunol [Internet]. 2021 [zitiert 10. April 2023];12. Verfügbar unter: https://www.frontiersin.org/articles/10.3389/fimmu.2021.703918

16. Schatz DG, Leu TMJ. rag-1 and rag-2: Biochemistry and Protein Interactions. In: Jessberger R, Lieber MR, Herausgeber. Molecular Analysis of DNA Rearrangements in the Immune System [Internet]. Berlin, Heidelberg: Springer; 1996 [zitiert 8. September 2022]. S. 11–29. (Current Topics in Microbiology and Immunology). Verfügbar unter: https://doi.org/10.1007/978-3-642-50140-1_2

17. Oettinger MA, Schatz DG, Gorka C, Baltimore D. RAG-1 and RAG-2, Adjacent Genes That Synergistically Activate V(D)J Recombination. Science [Internet]. 22. Juni 1990 [zitiert 8. September 2022]; Verfügbar unter: https://www.science.org/doi/10.1126/science.2360047

18. Justiz Vaillant AA, Jamal Z, Ramphul K. Immunoglobulin. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [zitiert 8. September 2022]. Verfügbar unter: http://www.ncbi.nlm.nih.gov/books/NBK513460/

19. B Cell Lymphoma. | Semantic Scholar [Internet]. [zitiert 8. September 2022]. Verfügbar unter: https://www.semanticscholar.org/paper/B%C2%A0Cell-Lymphoma.-Meng-Min/cd6c73c88ecd5de707b2519061fc3bd43bfe45f2

20. Karasuyama H, Takatsu K. B Lymphocyte Activation. In: Delves PJ, Herausgeber. Encyclopedia of Immunology (Second Edition) [Internet]. Oxford: Elsevier; 1998 [zitiert 8. September 2022]. S. 349–52. Verfügbar unter: https://www.sciencedirect.com/science/article/pii/B0122267656000967

21. Roth DB. V(D)J Recombination: Mechanism, Errors, and Fidelity. Microbiol Spectr. Dezember 2014;2(6):10.1128/microbiolspec.MDNA3-0041–2014.

22. Zhou Y, Zhang Y, Han J, Yang M, Zhu J, Jin T. Transitional B cells involved in autoimmunity and their impact on neuroimmunological diseases. J Transl Med. Dezember 2020;18(1):1–12.

23. Allen CD, Okada T, Cyster JG. Germinal Center Organization and Cellular Dynamics. Immunity. August 2007;27(2):190–202.

24. Schroeder HW, Cavacini L. Structure and Function of Immunoglobulins. J Allergy Clin Immunol. Februar 2010;125(2 0 2):S41–52.

25. Robinson MJ, Ding Z, Pitt C, Brodie EJ, Quast I, Tarlinton DM, u. a. The Amount of BCL6 in B Cells Shortly after Antigen Engagement Determines Their Representation in

Subsequent Germinal Centers. Cell Rep. Februar 2020;30(5):1530-1541.e4.
26. Gatto D, Brink R. The germinal center reaction. J Allergy Clin Immunol. November 2010;126(5):898–907; quiz 908–9.
27. Leeman-Neill RJ, Bhagat G. BCL6 as a therapeutic target for lymphoma. Expert Opin Ther Targets. Februar 2018;22(2):143–52.
28. Kim DH, Li S, Garces S, Xu J. Large B-cell lymphoma with IRF4 rearrangement and follicular Pattern: A differential diagnosis of follicular lymphoma. Hum Pathol Rep. 1. März 2022;27:300602.
29. Beham-Schmid C. Aggressive lymphoma 2016: revision of the WHO classification. Memo. 2017;10(4):248–54.
30. Pasqualucci L, Dalla-Favera R. Genetics of diffuse large B-cell lymphoma. Blood. 24. Mai 2018;131(21):2307–19.
31. Xie Y, Pittaluga S, Jaffe ES. The Histological Classification of Diffuse Large B-cell Lymphomas. Semin Hematol. April 2015;52(2):57–66.
32. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, u. a. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. Februar 2000;403(6769):503–11.
33. Lenz G. Insights into the Molecular Pathogenesis of Activated B-Cell-like Diffuse Large B-Cell Lymphoma and Its Therapeutic Implications. Cancers. 22. Mai 2015;7(2):811–22.
34. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, u. a. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 1. Januar 2004;103(1):275–82.
35. Read JA, Koff JL, Nastoupil LJ, Williams JN, Cohen JB, Flowers CR. Evaluating cell-of-origin subtype methods for predicting diffuse large B-cell lymphoma survival: A meta-analysis of gene expression profiling and immunohistochemistry algorithms. Clin Lymphoma Myeloma Leuk. Dezember 2014;14(6):460-467.e2.
36. Choi WWL, Weisenburger DD, Greiner TC, Piris MA, Banham AH, Delabie J, u. a. A New Immunostain Algorithm Classifies Diffuse Large B-Cell Lymphoma into Molecular Subtypes with High Accuracy. Clin Cancer Res Off J Am Assoc Cancer Res. 1. September 2009;15(17):5494–502.
37. Meyer PN, Fu K, Greiner TC, Smith LM, Delabie J, Gascoyne RD, u. a. Immunohistochemical Methods for Predicting Cell of Origin and Survival in Patients With Diffuse Large B-Cell Lymphoma Treated With Rituximab. J Clin Oncol. 10. Januar

2011;29(2):200–7.

38. Visco C, Li Y, Xu-Monette ZY, Miranda RN, Green TM, Li Y, u. a. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: A report from the International DLBCL Rituximab-CHOP Consortium Program Study. Leukemia. September 2012;26(9):2103–13.

39. Scott DW, Mottok A, Ennishi D, Wright GW, Farinha P, Ben-Neriah S, u. a. Prognostic Significance of Diffuse Large B-Cell Lymphoma Cell of Origin Determined by Digital Gene Expression in Formalin-Fixed Paraffin-Embedded Tissue Biopsies. J Clin Oncol. 10. September 2015;33(26):2848–56.

40. Dang CV, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F. The c-Myc target gene network. Semin Cancer Biol. 1. August 2006;16(4):253–64.

41. Susanibar-Adaniya S, Barta SK. 2021 Update on Diffuse large B cell lymphoma: A review of current data and potential applications on risk stratification and management. Am J Hematol. 1. Mai 2021;96(5):617–29.

42. Crombie JL, Armand P. Diffuse Large B-Cell Lymphoma's New Genomics: The Bridge and the Chasm. J Clin Oncol. 20. Oktober 2020;38(30):3565–74.

43. Green TM, Young KH, Visco C, Xu-Monette ZY, Orazi A, Go RS, u. a. Immunohistochemical Double-Hit Score Is a Strong Predictor of Outcome in Patients With Diffuse Large B-Cell Lymphoma Treated With Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone. J Clin Oncol [Internet]. 4. Juni 2012 [zitiert 26. August 2022]; Verfügbar unter: https://ascopubs.org/doi/10.1200/JCO.2011.41.4342

44. Boussios S, Zerdes I, Vassou A, Bareta E, Seraj E, Papoudou-Bai A, u. a. Extranodal diffuse large B-cell lymphomas: A retrospective case series and review of the literature. Hematol Rep. 3. April 2018;10(1):7070.

45. Vitolo U, Seymour JF, Martelli M, Illerhaus G, Illidge T, Zucca E, u. a. Extranodal diffuse large B-cell lymphoma (DLBCL) and primary mediastinal B-cell lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up †. Ann Oncol. 1. September 2016;27:v91–102.

46. Ollila TA, Olszewski AJ. Extranodal Diffuse Large B-Cell Lymphoma: Molecular Features, Prognosis, and Risk of Central Nervous System Recurrence. Curr Treat Options Oncol. 21. Juni 2018;19(8):38.

47. Grommes C, DeAngelis LM. Primary CNS Lymphoma. J Clin Oncol. 20. Juli 2017;35(21):2410.

48. Chan CC, Wallace DJ. Intraocular Lymphoma:Update on Diagnosis and Management. Cancer Control J Moffitt Cancer Cent. 2004;11(5):285–95.
49. Phillips EH, Fox CP, Cwynarski K. Primary CNS Lymphoma. Curr Hematol Malig Rep. 2014;9(3):243–53.
50. Calimeri T, Steffanoni S, Gagliardi F, Chiara A, Ferreri AJM. How we treat primary central nervous system lymphoma. ESMO Open [Internet]. August 2021 [zitiert 21. November 2021];6(4). Verfügbar unter: https://www-1ncbi-1nlm-1nih-1gov-10013b5cu0bd8.han.medunigraz.at/pmc/articles/PMC8287145/
51. Schaff LR, Grommes C. Primary Central Nervous System Lymphoma. Blood. 26. Oktober 2021;blood.2020008377.
52. Villano JL, Koshy M, Shaikh H, Dolecek TA, McCarthy BJ. Age, gender, and racial differences in incidence and survival in primary CNS lymphoma. Br J Cancer. 25. Oktober 2011;105(9):1414–8.
53. Liu Y, Yao Q, Zhang F. Diagnosis, prognosis and treatment of primary central nervous system lymphoma in the elderly population (Review). Int J Oncol. 1. Februar 2021;58(3):371–87.
54. von Baumgarten L, Illerhaus G, Korfel A, Schlegel U, Deckert M, Dreyling M. The Diagnosis and Treatment of Primary CNS Lymphoma. Dtsch Aerzteblatt Online [Internet]. 22. Juni 2018 [zitiert 21. November 2021]; Verfügbar unter: https://www.aerzteblatt.de/10.3238/arztebl.2018.0419
55. Grommes C, Rubenstein JL, DeAngelis LM, Ferreri AJM, Batchelor TT. Comprehensive approach to diagnosis and treatment of newly diagnosed primary CNS lymphoma. Neuro-Oncol. 19. Februar 2019;21(3):296–305.
56. O'Neill BP, Decker PA, Tieu C, Cerhan JR. The changing incidence of primary central nervous system lymphoma is driven primarily by the changing incidence in young and middle-aged men and differs from time trends in systemic diffuse large B-cell non-Hodgkin's lymphoma. Am J Hematol. Dezember 2013;88(12):997–1000.
57. Siegal T, Bairey O. Primary CNS Lymphoma in the Elderly: The Challenge. Acta Haematol. 2019;141(3):138–45.
58. Gandhi MK, Hoang T, Law SC, Brosda S, O'Rourke K, Tobin JWD, u. a. EBV-associated primary CNS lymphoma occurring after immunosuppression is a distinct immunobiological entity. Blood. 18. März 2021;137(11):1468–77.
59. Green K, Munakomi S, Hogg JP. Central Nervous System Lymphoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [zitiert 28. März

2023]. Verfügbar unter: http://www.ncbi.nlm.nih.gov/books/NBK545145/

60. Rubenstein JL, Gupta NK, Mannis GN, LaMarre AK, Treseler P. How I treat CNS lymphomas. Blood. 3. Oktober 2013;122(14):2318–30.

61. Schabet M. Epidemiology of Primary CNS Lymphoma. J Neurooncol. 1. Juli 1999;43(3):199–201.

62. Kasamon YL, Ambinder RF. AIDS-Related Primary Central Nervous System Lymphoma. Hematol Oncol Clin North Am. 1. August 2005;19(4):665–87.

63. Gijs PJ, Clerc O. Long-term remission of AIDS-related primary central nervous system lymphoma in a patient under antiretroviral therapy: a case report and review of the literature. AIDS Res Ther. 19. Oktober 2021;18:76.

64. Morell AA, Shah AH, Cavallo C, Eichberg DG, Sarkiss CA, Benveniste R, u. a. Diagnosis of primary central nervous system lymphoma: a systematic review of the utility of CSF screening and the role of early brain biopsy. Neuro-Oncol Pract. Dezember 2019;6(6):415–23.

65. Miller B, Sirotkin I, Martinez C. Review of Radiologic Considerations in an Immunocompetent Patient With Primary Central Nervous System Lymphoma. Fed Pract. August 2019;36(Suppl 5):S51–3.

66. Mullangi S, Lekkala MR. CNS Lymphoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [zitiert 20. August 2022]. Verfügbar unter: http://www.ncbi.nlm.nih.gov/books/NBK563302/

67. Fox CP, Phillips EH, Smith J, Linton K, Gallop-Evans E, Hemmaway C, u. a. Guidelines for the diagnosis and management of primary central nervous system diffuse large B-cell lymphoma. Br J Haematol. 2019;184(3):348–63.

68. Haldorsen IS, Espeland A, Larsson EM. Central Nervous System Lymphoma: Characteristic Findings on Traditional and Advanced Imaging. AJNR Am J Neuroradiol. 2011;32(6):984–92.

69. Abrey LE, Batchelor TT, Ferreri AJM, Gospodarowicz M, Pulczynski EJ, Zucca E, u. a. Report of an International Workshop to Standardize Baseline Evaluation and Response Criteria for Primary CNS Lymphoma. J Clin Oncol. August 2005;23(22):5034–43.

70. Mathew BS, Carson KA, Grossman SA. Initial response to glucocorticoids. Cancer. 15. Januar 2006;106(2):383–7.

71. Ärzteblatt DÄG Redaktion Deutsches. Deutsches Ärzteblatt. 2018 [zitiert 28. Juli 2022]. Diagnostik und Therapie primärer ZNS-Lymphome. Verfügbar unter:

https://www.aerzteblatt.de/archiv/198714/Diagnostik-und-Therapie-primaerer-ZNS-Lymphome

72. Scott BJ, Douglas VC, Tihan T, Rubenstein JL, Josephson SA. A Systematic Approach to the Diagnosis of Suspected Central Nervous System Lymphoma. JAMA Neurol. 1. März 2013;70(3):311–9.

73. Viaccoz A, Ducray F, Tholance Y, Barcelos GK, Thomas-Maisonneuve L, Ghesquières H, u. a. CSF neopterin level as a diagnostic marker in primary central nervous system lymphoma. Neuro-Oncol. November 2015;17(11):1497–503.

74. Scott BJ, Douglas VC, Tihan T, Rubenstein JL, Josephson SA. A Systematic Approach to the Diagnosis of Suspected Central Nervous System Lymphoma. JAMA Neurol. 1. März 2013;70(3):311–9.

75. Mavligit GM, Stuckey SE, Cabanillas FF, Keating MJ, Tourtellotte WW, Schold SC, u. a. Diagnosis of Leukemia or Lymphoma in the Central Nervous System by Beta2-Microglobulin Determination. N Engl J Med. 25. September 1980;303(13):718–22.

76. Hyung J, Hong JY, Kim S, Ryu JS, Huh J, Suh C. Beta-2 microglobulin as a prognostic factor of primary central nervous system lymphoma. Blood Res. Dezember 2019;54(4):285–8.

77. van Westrhenen A, Smidt LCA, Seute T, Nierkens S, Stork ACJ, Minnema MC, u. a. Diagnostic markers for CNS lymphoma in blood and cerebrospinal fluid: a systematic review. Br J Haematol. August 2018;182(3):384–403.

78. Brandsma D, Bromberg JEC. Primary CNS lymphoma in HIV infection. Handb Clin Neurol. 2018;152:177–86.

79. Sasayama T, Nakamizo S, Nishihara M, Kawamura A, Tanaka H, Mizukawa K, u. a. Cerebrospinal fluid interleukin-10 is a potentially useful biomarker in immunocompetent primary central nervous system lymphoma (PCNSL). Neuro-Oncol. März 2012;14(3):368–80.

80. Strehlow F, Bauer S, Martus P, Weller M, Roth P, Schlegel U, u. a. Osteopontin in cerebrospinal fluid as diagnostic biomarker for central nervous system lymphoma. J Neurooncol. 1. August 2016;129(1):165–71.

81. Rubenstein JL, Wong VS, Kadoch C, Gao HX, Barajas R, Chen L, u. a. CXCL13 plus interleukin 10 is highly specific for the diagnosis of CNS lymphoma. Blood. 6. Juni 2013;121(23):4740–8.

82. Baraniskin A, Kuhnhenn J, Schlegel U, Chan A, Deckert M, Gold R, u. a. Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large

B-cell lymphoma of the central nervous system. Blood. 17. März 2011;117(11):3140–6.
83. Ferreri AJM. Therapy of primary CNS lymphoma: role of intensity, radiation, and novel agents. Hematol Am Soc Hematol Educ Program. 8. Dezember 2017;2017(1):565–77.
84. Herhaus P, Lipkova J, Lammer F, Yakushev I, Vag T, Slotta-Huspenina J, u. a. CXCR4-Targeted PET Imaging of Central Nervous System B-Cell Lymphoma. J Nucl Med. 1. Dezember 2020;61(12):1765–71.
85. Barajas RF, Politi LS, Anzalone N, Schöder H, Fox CP, Boxerman JL, u. a. Consensus recommendations for MRI and PET imaging of primary central nervous system lymphoma: guideline statement from the International Primary CNS Lymphoma Collaborative Group (IPCG). Neuro-Oncol. 9. Februar 2021;23(7):1056–71.
86. Yang XL, Liu YB. Advances in Pathobiology of Primary Central Nervous System Lymphoma. Chin Med J (Engl). 20. August 2017;130(16):1973–9.
87. He M, Zuo C, Wang J, Liu J, Jiao B, Zheng J, u. a. Prognostic significance of the aggregative perivascular growth pattern of tumor cells in primary central nervous system diffuse large B-cell lymphoma. Neuro-Oncol. Juni 2013;15(6):727–34.
88. Ponzoni M, Berger F, Chassagne-Clement C, Tinguely M, Jouvet A, Ferreri AJM, u. a. Reactive perivascular T-cell infiltrate predicts survival in primary central nervous system B-cell lymphomas. Br J Haematol. 2007;138(3):316–23.
89. Camilleri-Broët S, Crinière E, Broët P, Delwail V, Mokhtari K, Moreau A, u. a. A uniform activated B-cell–like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. Blood. 1. Januar 2006;107(1):190–6.
90. Radke J, Ishaque N, Koll R, Gu Z, Schumann E, Sieverling L, u. a. The genomic and transcriptional landscape of primary central nervous system lymphoma. Nat Commun. 10. Mai 2022;13(1):2558.
91. El-Khouly FE, van Vuurden DG, Stroink T, Hulleman E, Kaspers GJL, Hendrikse NH, u. a. Effective Drug Delivery in Diffuse Intrinsic Pontine Glioma: A Theoretical Model to Identify Potential Candidates. Front Oncol. 30. Oktober 2017;7:254.
92. Yuan Y, Ding T, Wang S, Chen H, Mao Y, Chen T. Current and emerging therapies for primary central nervous system lymphoma. Biomark Res. 6. Mai 2021;9:32.
93. Benhar I, London A, Schwartz M. The privileged immunity of immune privileged organs: the case of the eye. Front Immunol. 21. September 2012;3:296.

94. Chamberlain MC, Johnston SK. High-dose methotrexate and rituximab with deferred radiotherapy for newly diagnosed primary B-cell CNS lymphoma. Neuro-Oncol. Juli 2010;12(7):736–44.
95. Batchelor T, Carson K, O'Neill A, Grossman SA, Alavi J, New P, u. a. Treatment of Primary CNS Lymphoma With Methotrexate and Deferred Radiotherapy: A Report of NABTT 96–07. J Clin Oncol. 15. März 2003;21(6):1044–9.
96. Holdhoff M, Ambady P, Abdelaziz A, Sarai G, Bonekamp D, Blakeley J, u. a. High-dose methotrexate with or without rituximab in newly diagnosed primary CNS lymphoma. Neurology. 15. Juli 2014;83(3):235–9.
97. Morris PG, Correa DD, Yahalom J, Raizer JJ, Schiff D, Grant B, u. a. Rituximab, Methotrexate, Procarbazine, and Vincristine Followed by Consolidation Reduced-Dose Whole-Brain Radiotherapy and Cytarabine in Newly Diagnosed Primary CNS Lymphoma: Final Results and Long-Term Outcome. J Clin Oncol. 11. November 2013;31(31):3971.
98. Ferreri AJM, Cwynarski K, Pulczynski E, Ponzoni M, Deckert M, Politi LS, u. a. Chemoimmunotherapy with methotrexate, cytarabine, thiotepa, and rituximab (MATRix regimen) in patients with primary CNS lymphoma: results of the first randomisation of the International Extranodal Lymphoma Study Group-32 (IELSG32) phase 2 trial. Lancet Haematol. Mai 2016;3(5):e217-227.
99. T Low J, B Peters K. Ibrutinib in primary central nervous system diffuse large B-cell lymphoma. CNS Oncol. 9(1):CNS51.
100. Graham MS, DeAngelis LM. Improving outcomes in primary CNS lymphoma. Best Pract Res Clin Haematol. 1. September 2018;31(3):262–9.
101. Binnahil M, Au K, Lu JQ, Wheatley BM, Sankar T. The Influence of Corticosteroids on Diagnostic Accuracy of Biopsy for Primary Central Nervous System Lymphoma. Can J Neurol Sci. September 2016;43(5):721–5.
102. Batchelor TT. Primary central nervous system lymphoma: A curable disease. Hematol Oncol. 2019;37(S1):15–8.
103. Han CH, Batchelor TT. Diagnosis and management of primary central nervous system lymphoma. Cancer. 2017;123(22):4314–24.
104. NCCN Guidelines for Patients Primary Central Nervous System Lymphoma. 2020;
105. Yuan Y, Ding T, Wang S, Chen H, Mao Y, Chen T. Current and emerging therapies for primary central nervous system lymphoma. Biomark Res. 6. Mai 2021;9:32.
106. Wirsching HG, Weller M, Balabanov S, Roth P. Targeted Therapies and Immune Checkpoint Inhibitors in Primary CNS Lymphoma. Cancers. 20. Juni 2021;13(12):3073.

107. Lionakis MS, Dunleavy K, Roschewski M, Widemann BC, Butman JA, Schmitz R, u. a. Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary Central Nervous System Lymphoma. Cancer Cell. 12. Juni 2017;31(6):833.
108. Rubenstein JL, Geng H, Fraser EJ, Formaker P, Chen L, Sharma J, u. a. Phase 1 investigation of lenalidomide/rituximab plus outcomes of lenalidomide maintenance in relapsed CNS lymphoma. Blood Adv. 9. Juli 2018;2(13):1595–607.
109. Lopez-Girona A, Heintel D, Zhang LH, Mendy D, Gaidarova S, Brady H, u. a. Lenalidomide downregulates the cell survival factor, interferon regulatory factor-4, providing a potential mechanistic link for predicting response. Br J Haematol. 2011;154(3):325–36.
110. Vu K, Mannis G, Hwang J, Geng H, Rubenstein JL. Low-Dose Lenalidomide Maintenance after Induction Therapy in Older Patients with Primary Central Nervous System Lymphoma. Br J Haematol. Juli 2019;186(1):180–3.
111. Ferreri AJM, Calimeri T, Conte GM, Cattaneo D, Fallanca F, Ponzoni M, u. a. R-CHOP preceded by blood-brain barrier permeabilization with engineered tumor necrosis factor-α in primary CNS lymphoma. Blood. 18. Juli 2019;134(3):252–62.
112. Mendez JS, Ostrom QT, Gittleman H, Kruchko C, DeAngelis LM, Barnholtz-Sloan JS, u. a. The elderly left behind—changes in survival trends of primary central nervous system lymphoma over the past 4 decades. Neuro-Oncol. April 2018;20(5):687–94.
113. Jelicic J, Stauffer Larsen T, Bukumiric Z, Juul-Jensen K, Andjelic B. Prognostic models in primary central nervous system lymphoma patients: A systematic review. Crit Rev Oncol Hematol. 1. Mai 2021;161:103341.
114. Ruppert AS, Dixon JG, Salles G, Wall A, Cunningham D, Poeschel V, u. a. International prognostic indices in diffuse large B-cell lymphoma: a comparison of IPI, R-IPI, and NCCN-IPI. Blood. 4. Juni 2020;135(23):2041–8.
115. A Predictive Model for Aggressive Non-Hodgkin's Lymphoma | NEJM [Internet]. [zitiert 4. August 2022]. Verfügbar unter: https://www.nejm.org/doi/full/10.1056/NEJM199309303291402
116. Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, u. a. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 14. November 2006;109(5):1857–61.
117. Zhou Z, Sehn LH, Rademaker AW, Gordon LI, LaCasce AS, Crosby-Thompson A, u. a. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse

large B-cell lymphoma treated in the rituximab era. Blood. 6. Februar 2014;123(6):837–42.
118. Hatzl S, Posch F, Deutsch A, Beham-Schmid C, Stöger H, Greinix H, u. a. Immunohistochemistry for c-myc and bcl-2 overexpression improves risk stratification in primary central nervous system lymphoma. Hematol Oncol. August 2020;38(3):277–83.
119. ECOG Performance Status Scale [Internet]. ECOG-ACRIN Cancer Research Group. [zitiert 29. August 2022]. Verfügbar unter: https://ecog-acrin.org/resources/ecog-performance-status/
120. OncologyPRO. Performance Scales: Karnofsky & ECOG Scores [Internet]. [zitiert 16. September 2022]. Verfügbar unter: https://oncologypro.esmo.org/oncology-in-practice/practice-tools/performance-scales
121. Ferreri AJM, Blay JY, Reni M, Pasini F, Spina M, Ambrosetti A, u. a. Prognostic Scoring System for Primary CNS Lymphomas: The International Extranodal Lymphoma Study Group Experience. J Clin Oncol. 15. Januar 2003;21(2):266–72.
122. Cai Q, Fang Y, Young KH. Primary Central Nervous System Lymphoma: Molecular Pathogenesis and Advances in Treatment. Transl Oncol. 1. März 2019;12(3):523–38.
123. Jahr G, Broi MD, Holte H, Beiske K, Meling TR. Evaluation of Memorial Sloan-Kettering Cancer Center and International Extranodal Lymphoma Study Group prognostic scoring systems to predict Overall Survival in intracranial Primary CNS lymphoma. Brain Behav. 5. Februar 2018;8(3):e00928.
124. Gao Y, Wei L, Kim SJ, Wang L, He Y, Zheng Y, u. a. A Novel Prognostic Marker for Primary CNS Lymphoma: Lactate Dehydrogenase-to-Lymphocyte Ratio Improves Stratification of Patients Within the Low and Intermediate MSKCC Risk Groups. Front Oncol. 3. August 2021;11:696147.
125. Abrey LE, Ben-Porat L, Panageas KS, Yahalom J, Berkey B, Curran W, u. a. Primary Central Nervous System Lymphoma: The Memorial Sloan-Kettering Cancer Center Prognostic Model. J Clin Oncol. 20. Dezember 2006;24(36):5711–5.
126. Bessell EM, Graus F, Lopez-Guillermo A, Lewis SA, Villa S, Verger E, u. a. Primary non-Hodgkin's lymphoma of the CNS treated with CHOD/BVAM or BVAM chemotherapy before radiotherapy: long-term survival and prognostic factors. Int J Radiat Oncol Biol Phys. 1. Juni 2004;59(2):501–8.
127. Liu C, Lin S, Yang C, Yeh C, Kuan A, Wang H, u. a. A new prognostic score for disease progression and mortality in patients with newly diagnosed primary CNS lymphoma. Cancer Med. 3. Februar 2020;9(6):2134–45.

128. Lo YT, Lim VY, Ng M, Tan YH, Chiang J, Chang EWY, u. a. A Prognostic Model Using Post-Steroid Neutrophil-Lymphocyte Ratio Predicts Overall Survival in Primary Central Nervous System Lymphoma. Cancers. 3. April 2022;14(7):1818.
129. Kawaguchi A, Iwadate Y, Komohara Y, Sano M, Kajiwara K, Yajima N, u. a. Gene Expression Signature–Based Prognostic Risk Score in Patients with Primary Central Nervous System Lymphoma. Clin Cancer Res. 14. Oktober 2012;18(20):5672–81.
130. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, u. a. The Use of Molecular Profiling to Predict Survival after Chemotherapy for Diffuse Large-B-Cell Lymphoma. N Engl J Med. 20. Juni 2002;346(25):1937–47.
131. Jaffe ES. Diagnosis and Classification of Lymphoma: Impact of Technical Advances. Semin Hematol. Januar 2019;56(1):30–6.
132. Yoon N, Ahn S, Yoo HY, Kim SJ, Kim WS, Ko YH. Cell-of-origin of diffuse large B-cell lymphomas determined by the Lymph2Cx assay: better prognostic indicator than Hans algorithm. Oncotarget. 28. Februar 2017;8(13):22014–22.
133. Troppan KT, Schlick K, Deutsch A, Melchardt T, Egle A, Stojakovic T, u. a. C-reactive protein level is a prognostic indicator for survival and improves the predictive ability of the R-IPI score in diffuse large B-cell lymphoma patients. Br J Cancer. 1. Juli 2014;111(1):55–60.
134. Le M, Garcilazo Y, Ibáñez‐Juliá M, Younan N, Royer‐Perron L, Benazra M, u. a. Pretreatment Hemoglobin as an Independent Prognostic Factor in Primary Central Nervous System Lymphomas. The Oncologist. September 2019;24(9):e898–904.
135. Luo Q, Yang C, Fu C, Wu W, Wei Y, Zou L. Prognostic Role of Blood Markers in Primary Central Nervous System Lymphoma Patients Treated With High-Dose Methotrexate-Based Therapy. Front Oncol. 29. April 2021;11:639644.
136. Coussens LM, Werb Z. Inflammation and cancer. Nature. 19. Dezember 2002;420(6917):860–7.
137. Jang JE, Kim YR, Kim SJ, Cho H, Chung H, Lee JY, u. a. A new prognostic model using absolute lymphocyte count in patients with primary central nervous system lymphoma. Eur J Cancer. 1. April 2016;57:127–35.
138. Kim DH, Baek JH, Chae YS, Kim YK, Kim HJ, Park YH, u. a. Absolute lymphocyte counts predicts response to chemotherapy and survival in diffuse large B-cell lymphoma. Leukemia. Oktober 2007;21(10):2227–30.
139. Ji H, Niu X, Yin L, Wang Y, Huang L, Xuan Q, u. a. Ratio of Immune Response to Tumor Burden Predicts Survival Via Regulating Functions of Lymphocytes and

Monocytes in Diffuse Large B-Cell Lymphoma. Cell Physiol Biochem. 2018;45(3):951–61.

140. Pichler M, Hutterer GC, Stoeckigt C, Chromecki TF, Stojakovic T, Golbeck S, u. a. Validation of the pre-treatment neutrophil–lymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients. Br J Cancer. 5. März 2013;108(4):901–7.

141. Porrata LF, Ristow K, Habermann T, Inwards DJ, Micallef IN, Markovic SN. Predicting survival for diffuse large B-cell lymphoma patients using baseline neutrophil/lymphocyte ratio. Am J Hematol. 2010;85(11):896–9.

142. Proctor MJ, McMillan DC, Morrison DS, Fletcher CD, Horgan PG, Clarke SJ. A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. Br J Cancer. 7. August 2012;107(4):695–9.

143. Troppan K, Deutsch A, Gerger A, Stojakovic T, Beham-Schmid C, Wenzl K, u. a. The derived neutrophil to lymphocyte ratio is an independent prognostic factor in patients with diffuse large B-cell lymphoma. Br J Cancer. 21. Januar 2014;110(2):369–74.

144. Passardi A, Scarpi E, Tamberi S, Cavanna L, Tassinari D, Fontana A, u. a. Impact of Pre-Treatment Lactate Dehydrogenase Levels on Prognosis and Bevacizumab Efficacy in Patients with Metastatic Colorectal Cancer. PLoS ONE. 5. August 2015;10(8):e0134732.

145. Jung J, Lee H, Yun T, Lee E, Moon H, Joo J, u. a. Prognostic role of the neutrophil-to-lymphocyte ratio in patients with primary central nervous system lymphoma. Oncotarget. 24. August 2017;8(43):74975–86.

146. Menter DG, Tucker SC, Kopetz S, Sood AK, Crissman JD, Honn KV. Platelets and cancer: a casual or causal relationship: revisited. Cancer Metastasis Rev. März 2014;33(1):231–69.

147. Feng Y, Liu Y, Zhong M, Wang L. Complete Blood Count Score Model Predicts Inferior Prognosis in Primary Central Nervous System Lymphoma. Front Oncol. 26. März 2021;11:618694.

148. He Q, Li L, Ren Q. The Prognostic Value of Preoperative Systemic Inflammatory Response Index (SIRI) in Patients With High-Grade Glioma and the Establishment of a Nomogram. Front Oncol. 14. Mai 2021;11:671811.

149. van der Meulen M, Dirven L, Bakunina K, van den Bent MJ, Issa S, Doorduijn JK, u. a. MMSE is an independent prognostic factor for survival in primary central nervous system lymphoma. J Neurooncol. 2021;152(2):357–62.

150. Board PPTE. [Table], Table 4. Ann Arbor Staging Classification for Hodgkin Lymphomaa [Internet]. National Cancer Institute (US); 2022 [zitiert 17. Dezember 2022]. Verfügbar unter: https://www.ncbi.nlm.nih.gov/books/NBK65726/table/CDR0000062933__557/

151. Simakurthy S, Jena S, Tripathy K. Primary Intraocular Lymphoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [zitiert 21. August 2022]. Verfügbar unter: http://www.ncbi.nlm.nih.gov/books/NBK576390/

152. Hormigo A, Abrey L, Heinemann MH, DeAngelis LM. Ocular presentation of primary central nervous system lymphoma: diagnosis and treatment. Br J Haematol. 2004;126(2):202–8.

153. Houillier C, Soussain C, Ghesquières H, Soubeyran P, Chinot O, Taillandier L, u. a. Management and outcome of primary CNS lymphoma in the modern era. Neurology. 10. März 2020;94(10):e1027–39.

154. Definition of Karnofsky Performance Status - NCI Dictionary of Cancer Terms - NCI [Internet]. 2011 [zitiert 29. August 2022]. Verfügbar unter: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/karnofsky-performance-status

155. Medistat: ECOG [Internet]. [zitiert 29. August 2022]. Verfügbar unter: https://www.medistat.de/glossar/lebensqualitaet/ecog

156. Lipof JJ, Barr PM. Early Progression of Follicular Lymphoma: Biology and Treatment. Hematol Oncol Clin North Am. 1. August 2020;34(4):757–69.

157. Gatta A, Verardo A, Bolognesi M. Hypoalbuminemia. Intern Emerg Med. 1. Oktober 2012;7(3):193–9.

158. Gómez-Cantarino S, Agulló-Ortuño MT, de Dios-Aguado M, Ugarte-Gurrutxaga MI, Bouzas-Mosquera C. Prevalence of Hypoproteinemia and Hypoalbuminemia in Pregnant Women from Three Different Socioeconomic Populations. Int J Environ Res Public Health. Januar 2020;17(17):6275.

159. Ali AM, Kunugi H. Hypoproteinemia predicts disease severity and mortality in COVID-19: a call for action. Diagn Pathol. 13. April 2021;16:31.

160. Arends T, Coonrad EV, Rundles RW. Serum proteins in Hodgkin's disease and malignant lymphoma. Am J Med. 1. Juni 1954;16(6):833–41.

161. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, u. a. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature. 4. Mai 2017;545(7652):60–5.

162. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, u. a. Prognostic Role of Neutrophil-to-Lymphocyte Ratio in Solid Tumors: A Systematic Review and Meta-Analysis. JNCI J Natl Cancer Inst. 1. Juni 2014;106(6):dju124.
163. Liu J, Wang Y, Liu Y, Liu Z, Cui Q, Ji N, u. a. Immunohistochemical profile and prognostic significance in primary central nervous system lymphoma: Analysis of 89 cases. Oncol Lett. November 2017;14(5):5505–12.
164. Marcus C, Maragkos GA, Alterman RL, Uhlmann E, Pihan G, Varma H. GCB-type is a favorable prognostic factor in primary CNS diffuse large B-cell lymphomas. J Clin Neurosci. 1. Januar 2021;83:49–55.
165. Kreher S, Jöhrens K, Strehlow F, Martus P, Borowiec K, Radke J, u. a. Prognostic impact of B-cell lymphoma 6 in primary CNS lymphoma. Neuro-Oncol. Juli 2015;17(7):1016–21.
166. Raoux D, Duband S, Forest F, Trombert B, Chambonnière ML, Dumollard JM, u. a. Primary central nervous system lymphoma: Immunohistochemical profile and prognostic significance. Neuropathology. 2010;30(3):232–40.
167. College of American Pathologists [Internet]. [zitiert 30. Oktober 2022]. Molecular Subclassifications of DLBCL. Verfügbar unter: https://www.cap.org/member-resources/articles/molecular-subclassifications-of-dlbcl
168. GCET1 | NeoGenomics Laboratories [Internet]. [zitiert 30. Oktober 2022]. Verfügbar unter: https://neogenomics.com/test-menu/gcet1
169. Menter T, Gasser A, Juskevicius D, Dirnhofer S, Tzankov A. Diagnostic Utility of the Germinal Center–associated Markers GCET1, HGAL, and LMO2 in Hematolymphoid Neoplasms. Appl Immunohistochem Mol Morphol. August 2015;23(7):491–8.
170. Brandt A, Matschke J, Fehrle W, von Wenserski L, Bokemeyer C, Illerhaus G, u. a. A significant proportion of patients with primary central nervous system lymphoma harbor clonal bone marrow B-cells. Leuk Lymphoma. 28. Januar 2019;60(2):334–40.
171. Hernández-Verdin I, Kirasic E, Wienand K, Mokhtari K, Eimer S, Loiseau H, u. a. Molecular and clinical diversity in primary central nervous system lymphoma. Ann Oncol. 1. Februar 2023;34(2):186–99.
172. Corry J, Smith JG, Wirth A, Quong G, Liew KH. Primary central nervous system lymphoma: age and performance status are more important than treatment modality. Int J Radiat Oncol Biol Phys. 1. Juni 1998;41(3):615–20.
173. Chen T, Liu Y, Wang Y, Chang Q, Wu J, Wang Z, u. a. Evidence-based expert consensus on the management of primary central nervous system lymphoma in China. J

Hematol OncolJ Hematol Oncol. 29. September 2022;15:136.

174. Mohile SG, Dale W, Somerfield MR, Schonberg MA, Boyd CM, Burhenn PS, u. a. Practical Assessment and Management of Vulnerabilities in Older Patients Receiving Chemotherapy: ASCO Guideline for Geriatric Oncology. J Clin Oncol. 1. August 2018;36(22):2326–47.

175. Extermann M, Boler I, Reich RR, Lyman GH, Brown RH, DeFelice J, u. a. Predicting the risk of chemotherapy toxicity in older patients: The Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH) score. Cancer. 2012;118(13):3377–86.

176. Tavakkoli M, Wilkins CR, Mones JV, Mauro MJ. A Novel Paradigm Between Leukocytosis, G-CSF Secretion, Neutrophil-to-Lymphocyte Ratio, Myeloid-Derived Suppressor Cells, and Prognosis in Non-small Cell Lung Cancer. Front Oncol [Internet]. 2019 [zitiert 15. September 2022];9. Verfügbar unter: https://www.frontiersin.org/articles/10.3389/fonc.2019.00295

177. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. Juli 2008;454(7203):436–44.

178. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. Cell. 19. März 2010;140(6):883–99.

179. Wei X, Zheng J, Zhang Z, Liu Q, Zhan M, Huang W, u. a. Consecutive Hypoalbuminemia Predicts Inferior Outcome in Patients With Diffuse Large B-Cell Lymphoma. Front Oncol [Internet]. 2021 [zitiert 2. November 2022];10. Verfügbar unter: https://www.frontiersin.org/articles/10.3389/fonc.2020.610681

180. Systemic immune-inflammation index predicting survival outcome in patients with classical Hodgkin lymphoma | Biomarkers in Medicine [Internet]. [zitiert 9. September 2022]. Verfügbar unter: https://www.futuremedicine.com/doi/10.2217/bmm-2019-0303

181. Xie QK, Chen P, Hu WM, Sun P, He WZ, Jiang C, u. a. The systemic immune-inflammation index is an independent predictor of survival for metastatic colorectal cancer and its association with the lymphocytic response to the tumor. J Transl Med. 4. Oktober 2018;16:273.

182. Wang Q, Zhu D. The prognostic value of systemic immune-inflammation index (SII) in patients after radical operation for carcinoma of stomach in gastric cancer. J Gastrointest Oncol [Internet]. Oktober 2019 [zitiert 28. September 2022];10(5). Verfügbar unter: https://jgo.amegroups.com/article/view/29209

183. Coku V, Shkembi X. Serum Gamma-glutamyltransferase and Obesity: is there a Link? Med Arch. April 2018;72(2):112–5.

184. Corti A, Franzini M, Paolicchi A, Pompella A. Gamma-glutamyltransferase of Cancer Cells at the Crossroads of Tumor Progression, Drug Resistance and Drug Targeting. Anticancer Res. 1. April 2010;30(4):1169–81.
185. Nogueira DS, Lage LA de PC, Culler HF, Pereira J. Follicular Lymphoma: Refining Prognostic Models and Impact of Pod-24 in Clinical Outcomes. Clin Lymphoma Myeloma Leuk. 1. Februar 2022;22(2):67–75.
186. Luminari S, Merli M, Rattotti S, Tarantino V, Marcheselli L, Cavallo F, u. a. Early progression as a predictor of survival in marginal zone lymphomas: an analysis from the FIL-NF10 study. Blood. 5. September 2019;134(10):798–801.
187. Wang Y, Farooq U, Link BK, Hefazi M, Allmer C, Maurer MJ, u. a. Relapses after Achieving EFS24 in Patients with Diffuse Large B-Cell Lymphoma in the Rituximab Era. Blood. 29. November 2018;132(Supplement 1):454.
188. Wang Y, Larson MC, Castellino A, Maurer MJ, Feldman AL, Syrbu S, u. a. Event-Free Survival at 24 Months (EFS24) Becomes an Important Clinical Endpoint in Newly Diagnosed Mantle Cell Lymphoma in the New Era. Blood. 23. November 2021;138:2429.
189. Event-Free Survival at 24 Months Is a Robust End Point for Disease-Related Outcome in Diffuse Large B-Cell Lymphoma Treated With Immunochemotherapy | Journal of Clinical Oncology [Internet]. [zitiert 28. Oktober 2022]. Verfügbar unter: https://ascopubs.org/doi/10.1200/JCO.2013.51.5866
190. Kim S, Nam SJ, Kwon D, Kim H, Lee E, Kim TM, u. a. MYC and BCL2 overexpression is associated with a higher class of Memorial Sloan-Kettering Cancer Center prognostic model and poor clinical outcome in primary diffuse large B-cell lymphoma of the central nervous system. BMC Cancer. 10. Juni 2016;16:363.
191. Chen Y, Chen H, Chen L, Zheng X, Yang X, Zheng Z, u. a. Immunohistochemical overexpression of BCL-2 protein predicts an inferior survival in patients with primary central nervous system diffuse large B-cell lymphoma. Medicine (Baltimore). November 2019;98(45):e17827.
192. Makino K, Nakamura H, Shinojima N, Kuroda J ichiro, Yano S, Mikami Y, u. a. BCL2 expression is associated with a poor prognosis independent of cellular origin in primary central nervous system diffuse large B-cell lymphoma. J Neurooncol. 1. Oktober 2018;140(1):115–21.
193. Hu S, Xu-Monette ZY, Tzankov A, Green T, Wu L, Balasubramanyam A, u. a. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression

signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood. 16. Mai 2013;121(20):4021–31.

194. Pierce JMR, Mehta A. Diagnostic, prognostic and therapeutic role of CD30 in lymphoma. Expert Rev Hematol. 2. Januar 2017;10(1):29–37.

195. Hu S, Xu-Monette ZY, Balasubramanyam A, Manyam GC, Visco C, Tzankov A, u. a. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Blood. 4. April 2013;121(14):2715–24.

196. Montes-Moreno S, Roncador G, Maestre L, Martínez N, Sanchez-Verde L, Camacho FI, u. a. Gcet1 (centerin), a highly restricted marker for a subset of germinal center-derived lymphomas. Blood. 1. Januar 2008;111(1):351–8.