Aus der Klinik für Hämatologie, Onkologie und klinische Immunologie
der Heinrich-Heine-Universität Düsseldorf
Kommissarischer Direktor: Prof. Dr. Ulrich Germing

Clinical Spectrum of Primary Adrenal Lymphoma

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

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2022

Angabe der Gutachter/innen

Als Inauguraldissertation gedruckt mit Genehmigung der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

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Dekan: Prof. Dr. med. Nikolaj Klöcker
Erstgutachter: Prof. Dr. med. Norbert Gattermann
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Teile dieser Arbeit wurden veröffentlicht:

Majidi, F., S. Martino, M. Kondakci, C. Antke, M. Haase, V. Chortis, W. Arlt, C. L. Ronchi, M. Fassnacht, C. Laurent, J. M. Petit, O. Casasnovas, M. A. Habra, A. Kanji, R. Salvatori, A. T. N. Ho, A. Spyroglou, F. Beuschlein, D. Villa, W. Limvorapitak, B. E. Wahlin, O. Gimm, M. Rudelius, M. Schott, U. Germing, R. Haas, and N. Gattermann. 2020. "Clinical spectrum of primary adrenal lymphoma: results of a multicenter cohort study." *Eur J Endocrinol* 183 (4): 453-462.

Zusammenfassung

Etwa 30% der Non-Hodgkin-Lymphome und 1% der Hodgkin-Lymphome weisen einen extranodalen Befall auf, entweder als Primärmanifestation oder sekundär im Rahmen einer hämatogenen Ausbreitung, ausgehend von einer nodalen oder anderen extranodalen Lokalisation. Extranodale Lymphome (ENL) können jedes Organ betreffen, die häufigsten Entstehungsorte sind jedoch der Magen-Darm-Trakt (43%) und die Kopf-Hals-Region (14%) (Reginelli et al. 2020). Neben dem histopathologischen Subtyp wurde auch die Lokalisation eines ENL als prognostischer Marker beschrieben. Zum Beispiel sind Hirn- und Hodenbeteiligung im Allgemeinen mit einer schlechteren Prognose verbunden als andere ENL-Lokalisationen. Die organabhängige Prognose von ENL impliziert, dass für diese Entitäten unterschiedliche Behandlungsansätze erforderlich sein könnten (Vannata und Zucca 2015).

Das primäre Nebennierenlymphom (PAL) ist ein seltenes ENL, mit nicht mehr als 300 in PubMed auffindbaren Fällen. Um das klinische Spektrum von PAL besser zu verstehen, haben wir Entlassungsbriefe, Laborergebnisse, Pathologieberichte sowie radiologische Bilder und Befunde aus 14 Zentren in den USA, Europa und Kanada gesammelt und ausgewertet. Neben einer möglichst umfassenden Beschreibung der klinischen Merkmale zeigen wir Defizite in der Diagnose und Behandlung von PAL auf, in der Hoffnung, damit zur verbesserten zukünftigen Behandlung dieser seltenen Entität beizutragen.

Unsere retrospektive Analyse von 97 Patienten aus 14 Zentren ist die bisher größte Untersuchung zu PAL. Wir konnten erstmals zeigen, dass es sich bei PAL um eine heterogene Erkrankung handelt, die sowohl Fälle mit isolierter Beteiligung des Nebennierengewebes (iPAL) als auch Fälle mit zusätzlichen extraadrenalen Organmanifestationen (PAL+) umfasst. Bei Patienten mit iPAL fanden wir unerwartet ein häufigeres Vorkommen bei Frauen, weniger B-Symptome und ein signifikant schlechteres klinisches Ergebnis als bei Patienten mit Beteiligung zusätzlicher extraadrenaler Lokalisationen. Dies sollte Anlass zu weiteren Untersuchungen sein, um festzustellen, ob die klinische Unterscheidung zwischen iPAL und PAL+ sich durch Unterschiede in der Molekularbiologie dieser Lymphome bestätigen lässt.

Summary

About 30% of Non-Hodgkin lymphomas and 1% of HLs involve extranodal sites, either primarily or secondary to hemotogenous spread from another nodal or extranodal location. Extranodal lymphomas (ENL) can originate from any organ, but the most common sites of origin are the gastrointestinal tract (43%) and the head and neck region (14%) (Reginelli et al. 2020). In addition to its histopathologic subtype, the location of an ENL has also been described as a prognostic marker. For example, brain and testicular involvement are generally associated with a worse prognosis than other locations of ENL. The site-dependent prognosis of ENLs implies that site-adapted treatment approaches may be necessary for these entities (Vannata and Zucca 2015).

Primary adrenal lymphoma (PAL) is a rare ENL with no more than 300 reported cases in PubMed. In order to improve our knowledge of the clinical spectrum of PAL, we collected information from discharge letters, laboratory results, pathology reports, and radiology reports and images from 14 centers across the USA, Europe, and Canada. Besides aiming at a more comprehensive description of clinical features, we tried to identify current deficits in the diagnosis and treatment of PAL, in the hope of facilitating future improvements in the management of this rare entity.

Our retrospective analysis of 97 patients from fourteen centers is the largest analysis of PAL so far. We showed for the first time that PAL is a heterogeneous disease, which comprises cases with isolated involvement of adrenal tissue (iPAL) and cases with additional extra-adrenal organ manifestations (PAL+). Unexpectedly, patients with iPAL showed an unusual male/female ratio, less B symptoms and significantly worse clinical outcome than those with involvement of additional, extra-adrenal sites. This finding should stimulate further investigation to see whether the clinical distinction between iPAL and PAL+ is corroborated by differences in molecular biology.

Abbreviations

ABC	activated B-cell
ABC-DLBCL	activated B-cell like diffuse large B-cell lymphoma
ACTH	adrenocorticotropic hormone
AI	adrenal insufficiency
AITL	angioimmunoblastic T-cell lymphoma
ALCL	anaplastic large cell lymphoma
ALK	anaplastic lymphoma kinase
allo-SCT	allogeneic stem cell transplantation
ATLL	adult T-cell leukemia/lymphoma
auto-SCT	autologous stem cell transplantation
BCL2	B-cell lymphoma 2
BCL6	B-cell lymphoma 6
BL	Burkitt lymphoma
BTK	Bruton tyrosine kinase
CAR-T-cell	chimeric antigen receptor T-cell
CD20	cluster of differentiation 20
CD79B	cluster of differentiation 79B
CDKN2A	cyclin-dependent kinase inhibitor 2A
cHL	classical Hodgkin lymphoma
CLP	common lymphocyte progenitor
CT	computer tomography
DEL	double expressing lymphoma
DHL	double hit lymphoma
DLBCL	diffuse large B-cell lymphoma
DN	double negative
DP	double positive
EMA	European Medicines Agency
ENKTCL	extranodal NK/T-cell lymphoma
ENL	extranodal lymphoma
ENSAT	European network for the study of adrenal tumors
ETV6	E-26 transforming variant 6

FDA	Food and Drug Administration
FDG	fluorodeoxyglucose
FL	follicular lymphoma
GC	germinal center
HGBL	high grade B-cell lymphoma
HL	Hodgkin lymphoma
HSC	hematopoietic stem cell
iPAL	isolated primary adrenal lymphoma
JAK	Janus kinase
mAB	monoclonal antibody
MALT	mucosa-associated lymphoid tissue
MCL	mantle cell lymphoma
MHC	major histocompatibility complex
MMAE	monomethyl auristatin E
MYC	myelocytomatosis
MYD88	myeloid differentiation primary response 88
NFKBIZ	NF-κB inhibitor zeta
NF-κB	nuclear factor kappa light chain enhancer of activated B cells
NHL	non-Hodgkin lymphoma
NK	natural killer
NKT	natural killer T cell
NOTCH	neurogenic locus notch homolog protein
PAI	primary adrenal insufficiency
PAL	primary adrenal lymphoma
PAL+	primary adrenal lymphoma with extraadrenal manifestation
PCNL	primary central nervous lymphoma
PD-L1	programmed death-ligand 1
PD-L2	programmed death-ligand 2
PET	positron emission tomography
PI3K	phosphoinositide-3-kinase
PIM1	proviral integration site for Moloney murine leukemia virus 1
PMBL	primary mediastinal B-cell lymphoma
PTCL-NOS	peripheral T-cell lymphoma, not otherwise specified

PTL	primary testicular lymphoma
R-CHOP	Rituximab + cyclophosphamide + hydroxydaunorubicin (doxorubicin) + Oncovin (vincristine) + prednisolone
R-EPOCH	Rituximab + etoposide + prednisolone + Oncovin (vincristine) + cyclophosphamide + hydroxydaunorubicin (doxorubicin)
SAI	secondary adrenal insufficiency
SKY	spleen tyrosine kinase
SMZL	splenic marginal zone lymphoma
STAT	signal transducer and activator of transcription
TAI	tertiary adrenal insufficiency
TCR	T-cell receptor
TFH	T follicular helper cell
Th cell	T helper cell
Treg cell	regulatory T cell
UPP	ubiquitin proteasome pathway

Content

Introduction

Lymphoma

Lymphomas, the seventh most common malignancies worldwide, comprise a heterogeneous group of malignant disorders of the immune system that show overlapping clinical and molecular features. Age, male gender, environmental exposure to pesticides, infections, immunosuppression, autoimmune diseases, radiation, smoking, and a familial predisposition are factors increasing the risk of lymphoma (Lewis, Lilly, and Jones 2020). The accumulation of genetic lesions, such as chromosomal rearrangements and somatic mutations, which can affect proto-oncogenes and tumor-suppressor genes during the developmental process of lymphocytes, as well as chronic antigenic stimulation through a self-antigen or an invading pathogen, can play important roles in lymphomagenesis (Malcolm et al. 2016).

Hodgkin vs non-Hodgkin lymphoma

Malignant lymphomas are traditionally classified as Hodgkin (HL) and non-Hodgkin lymphoma (NHL). HL is a rare lymphatic neoplasm in the general population but one of the most common cancers in young adults (Momotow et al. 2021). HL is a B-cell malignancy characterized by the formation of Reed-Sternberg cells (Figure 1), which are giant multinucleated cells with prominent nucleoli. The affected lymph nodes usually show a pronounced secondary accumulation of a variety of reactive inflammatory cells (Piccaluga et al. 2011).

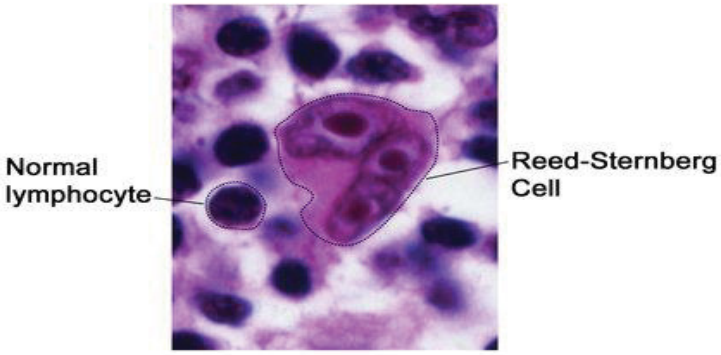

Figure 1: Adopted from: https://visuals.nci.nih.gov/details.cfm?imageid=7172

Non-Hodgkin lymphomas are the most common hematological malignancies. They derive from clonal expansion of B, T or NK cells and their precursors. Gene expression profiling and next generation DNA sequencing revealed biomarkers that support risk stratification and helped to develop new therapeutic strategies. A summary of the most important genomic alterations in lymphomas of B-cell, T-cell and NK-cell origin is given in Figures 2 and 3.

B-cell origin of lymphoma

B-cell development starts in the bone marrow. Naïve B-cells become activated after encountering a matching antigen and then develop transient structures within lymph node follicles, called germinal centers (GC). GC B-cells carry an elevated risk for developing malignancies due to attenuation of DNA repair and low activity of cell proliferation checkpoints. Follicular lymphoma (FL), Burkitt lymphoma (BL) and GC B-cell like diffuse large B-cell lymphoma (GCB-DLBCL) arise in germinal centers, whereas activated B-cell like diffuse large B-cell lymphoma (ABC-DLBCL), primary mediastinal B-cell lymphoma (PMBL) and classical Hodgkin lymphoma (cHL) originate from post-GC B cells. Mantle cell lymphoma (MCL) is derived from the mantle zone of lymph nodes (Sun, Medeiros, and Young 2016; Malcolm et al. 2016; Mlynarczyk, Fontán, and Melnick 2019).

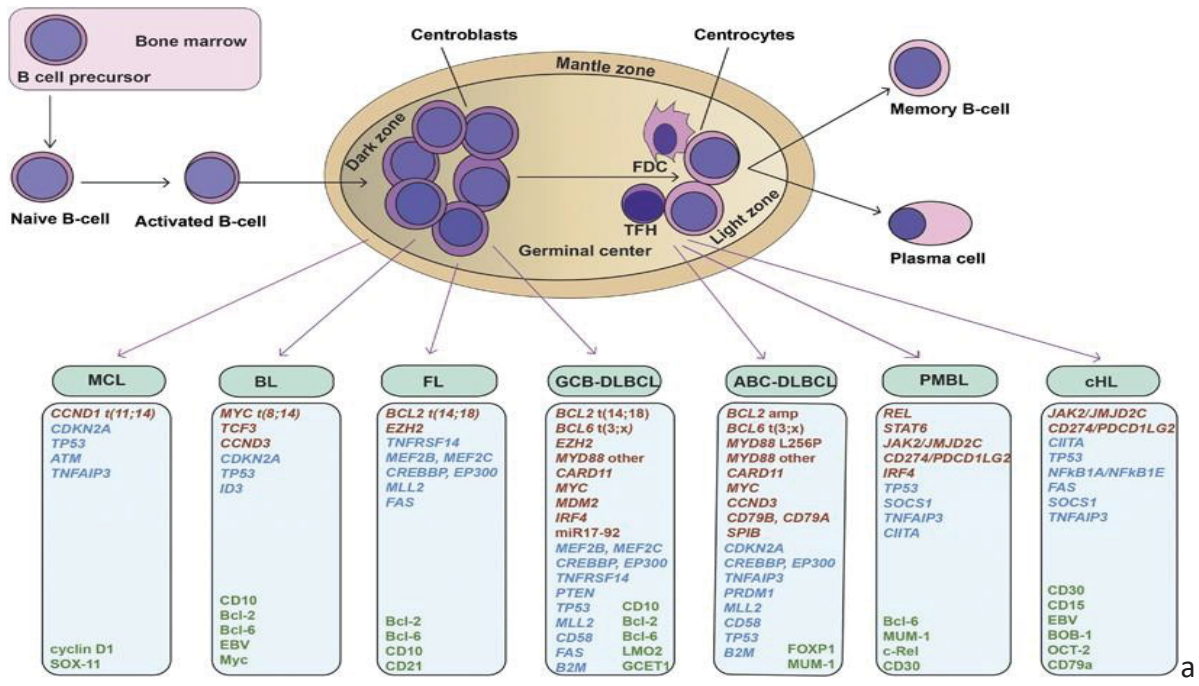

Figure 2. Graphic representation of origins and biomarkers of B-cell lymphomas
(adopted from Raifang Sun et al, 2016- Modern Pathology.
Abbreviations: ABC-DLBCL, activated B-cell-DLBCL; BL, Burkitt lymphoma; cHL, classical Hodgkin lymphoma; FDC, follicular dendritic cell; FL, follicular lymphoma; GCB-DLBCL, GC B-cell-like diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; TFH, T follicular helper cell.

T-cell and NK-cell origin of lymphoma

T lymphocytes are produced from differentiation of common lymphocyte progenitors (CLPs) in the bone marrow and migrate to the thymus for further development after undergoing positive and negative selection based on expression of relevant surface markers (Figure 3)(Sun, Medeiros, and Young 2016). T-cells surviving the selection process will differentiate into subsets including cytotoxic or helper (Th) T-cells, regulatory T-cells (Treg) and natural killer T-cells (NKT). T-cell and NK-cell lymphomas result from malignant transformation of tissue resident T-cells and tend to manifest mostly in extra-nodal sites (de Leval and Gaulard 2014; Jones et al. 2021).

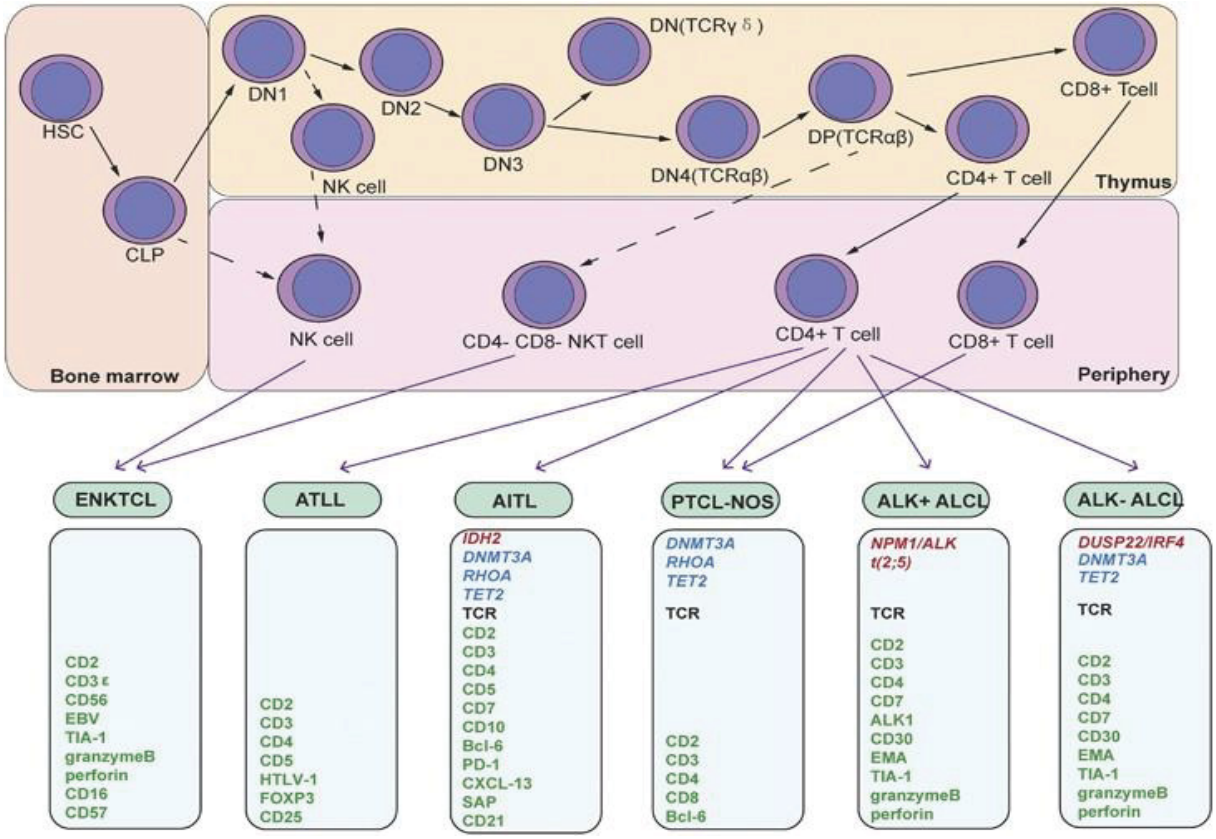

Figure 3. Schematic representation of origins and biomarkers of T-cell lymphomas (adopted from Raifang Sun et al, 2016, Modern Pathology).
Abbreviations: ALCL, anaplastic large cell lymphoma; AITL, angioimmunoblastic T-cell lymphoma; ALK, anaplastic lymphoma kinase; ATLL, adult T-cell leukemia/lymphoma; CLP, common lymphoid progenitors; DN, double negative; DP, double positive; ENKTCL, extranodal NK/T-cell lymphoma; HSC, hematopoietic stem cell; NK, natural killer; NKT, natural killer T-cell; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified.

Clinical manifestation of lymphoma

Depending on the type and stage of the disease, lymphomas show a wide spectrum of clinical presentations, ranging from asymptomatic enlarged lymph nodes to marked general symptoms such as fever, drenching night sweats, weight loss, pruritus, and fatigue. High-grade lymphomas can even lead to oncological emergencies like superior vena cava syndrome or spinal cord compression due to large tumors. In rare cases, lymphoma can present with a paraneoplastic syndrome (Armitage et al. 2017; Lewis, Lilly, and Jones 2020).

Diagnosis

Diagnosis of lymphoma is based on cytomorphology, histopathology and flow cytometric analysis of suspicious lymph node or extranodal tissue provided by incisional or excisional biopsy. Material acquired by fine needle biopsy is usually not adequate for reliable and precise diagnosis of lymphoma. Additional molecular genetic analysis is useful for the diagnosis of specific lymphoma entities, whose identification may generate therapeutic consequences. An example is “double hit” DLBCL with concomitant MYC and/or BCL2 and/or BCL6 rearrangement, which has a very poor prognosis in comparison with other DLBCL subtypes.

The location and extent of the disease should be assessed using state-of-the-art imaging techniques. Current guidelines recommend PET-CT for staging and pretreatment evaluation of lymphoma subtypes showing high avidity for FDG, including all histological types except chronic lymphocytic leukemia/small lymphocytic lymphoma, lymphoplasmacytic lymphoma, marginal zone lymphoma, and mycosis fungoides. (Cheson et al. 2014; Munakata et al. 2019). In contrast to previous belief, it is now well established that FDG avidity significantly depends on the histopathological lymphoma subtype. Nevertheless, in a cohort of 766 lymphoma patients with different histopathological subtypes, Weiler-Sagie et al. showed that most of the lymphoma subtypes are FDG-avid (Table 1) (Weiler-Sagie et al. 2010).

Histology	*n*	^{18}F-FDG–avid	Negative	% ^{18}F-FDG avidity
Hodgkin disease	233	233	0	100
Burkitt lymphoma	18	18	0	100
Mantle cell lymphoma	14	14	0	100
Anaplastic large T-cell lymphoma	14	14	0	100
Marginal zone lymphoma, nodal	8	8	0	100
Lymphoblastic lymphoma	6	6	0	100
Angioimmunoblastic T-cell lymphoma	4	4	0	100
Plasmacytoma	3	3	0	100
Natural killer/T-cell lymphoma	2	2	0	100
Diffuse large B-cell lymphoma	222	216	6	97
Follicular lymphoma	140	133	7	95
Peripheral T-cell lymphoma	10	9	1	90
Small lymphocytic lymphoma	29	24	5	83
Enteropathy-type T-cell lymphoma	3	2	1	67
Marginal zone lymphoma, splenic	3	2	1	67
MALT marginal zone lymphoma	50	27	23	54
Lymphomatoid papulosis	2	1	1	50
Primary cutaneous anaplastic large T-cell lymphoma	5	2	3	40
All	766	718	48	94

Table 1: 18F-FDG avidity of lymphoma according to the World Health Organization histopathologic classification (adopted from Weiler-Sagie et al, 2010, JNUMED)

Staging

A universally accepted staging system that reflects the location and extent of lymphoma, yields prognostic information, and provides a baseline for subsequent treatment monitoring is critical for standardized, high-quality management of patients. The Ann Arbor staging was the first globally accepted staging system for lymphomas, which was actually designed for Hodgkin lymphoma but was also widely used for the staging of non-Hodgkin lymphomas, despite the fact that the spreading pattern differs significantly between both types of lymphoma. In 2014, the Ann Arbor system was modified and the new Lugano classification has now become the standard staging system for lymphoma. The most important difference between the two systems is that the Lugano classification includes PET as a standard imaging technique for FDG-avid lymphomas. PET facilitates not only the assessment of lymph node involvement but also augments the evaluation of other organ involvement, including spleen and liver, and further eliminates the requirement of bone marrow biopsy for the routine staging of HL and most DLBCL (Munakata et al. 2019).

Stage	Involvement	Extranodal (E) Status
Limited		
I	One node or a group of adjacent nodes	Single extranodal lesions without nodal involvement
II	Two or more nodal groups on the same side of the diaphragm	Stage I or II by nodal extent with limited contiguous extranodal involvement
II bulky*	II as above with "bulky" disease	Not applicable
Advanced		
III	Nodes on both sides of the diaphragm; nodes above the diaphragm with spleen involvement	Not applicable
IV	Additional noncontiguous extralymphatic involvement	Not applicable

Table 2. Lugano Classification of Lymphoma (adopted from Chosen et al, 2014, JCO)

Treatment modalities

Lymphoma treatment comprises a wide range of options, including 'watch-and-wait' in asymptomatic indolent limited-stage lymphoma, treatment of a predisposing condition like H-pylori infection in mucosa-associated lymphoid tissue (MALT) lymphoma or hepatitis C infection in marginal zone lymphoma, as well as prompt immuno-chemotherapy and/or radiotherapy in aggressive fast growing lymphoma. Surgery may be indicated in rare conditions like splenic marginal zone lymphoma (SMZL).

Doxorubicin containing chemotherapy regimens, some of which are listed in Table 3, significantly improved the outcome of patients with both Hodgkin and non-Hodgkin lymphoma (Lewis, Lilly, and Jones 2020). However, despite many attempts, further advancement of lymphoma treatment was not achievable though modification of conventional chemotherapy.

Common Chemotherapy Regimens and Complications

Therapy	Regimen	Short-term complications	Long-term complications
Hodgkin lymphoma			
ABVD	Doxorubicin (**A**driamycin) **B**leomycin **V**inblastine (Velban) **D**acarbazine	Nausea/vomiting Alopecia Neutropenia Neuropathy Bleomycin-induced pulmonary toxicity	Cardiotoxicity (heart failure) Neuropathy Pulmonary fibrosis Increased risk of myocardial infarction
Stanford V	Doxorubicin Vinblastine Mechlorethamine Etoposide (Toposar) Vincristine Bleomycin Prednisone	Nausea/vomiting Fatigue Pulmonary toxicity Neuropathy	Neuropathy Pulmonary fibrosis Cardiotoxicity Rarely solid secondary malignancies of breast, lung, and skin
Escalated-BEACOPP	**B**leomycin **E**toposide Doxorubicin (**A**driamycin) **C**yclophosphamide Vincristine (**O**ncovin) **P**rocarbazine (Matulane) **P**rednisone	Anemia Leukopenia Thrombocytopenia Nausea/vomiting Infection Disulfiram reaction between ethanol and procarbazine	Acute myeloid leukemia Sterility/infertility
Non-Hodgkin lymphoma			
CHOP	**C**yclophosphamide Doxorubicin (**H**ydroxydaunorubicin) Vincristine (**O**ncovin) **P**rednisone	Heart failure Constipation Hyperglycemia Neuropathy	Cardiomyopathy Myelosuppression Neuropathy
R-CHOP	**R**ituximab (Rituxan) + **CHOP**	Reactivate hepatitis B infection	Progressive multifocal leukoencephalopathy

Table 3: Common chemotherapy regimens in lymphoma
(adopted from Lewis et al, 2016, AFP)

Targeted therapy

The development of monoclonal antibodies (mAbs) against cell surface antigens on lymphatic cells has revolutionized the treatment of lymphomas. Such antibodies can be unconjugated or drug-conjugated. The first game changing agent was rituximab, an anti-CD20 antibody widely used in the treatment of B-cell lymphoma. Another effective drug is Brentuximab-Vedotin, an antibody-drug conjugate that combines an anti-CD30 antibody with the drug monomethyl auristatin E (MMAE). This agent has significantly improved the outcome of both Hodgkin lymphoma and anaplastic large T-cell lymphoma. Developing mAbs against accessible surface antigens is a very dynamic research field that has yielded several drug approvals by the FDA and EMA (Wang et al. 2020).

Certain signaling pathways play a significant role in lymphoma progression and have thus attracted attention in the field of targeted therapy. The most important ones include spleen tyrosine kinase (SYK), Bruton tyrosine kinase (BTK), Janus kinase transducer and activator of transcription (JAK-STAT), NOTCH, NF-κB, the ubiquitin proteasome pathway (UPP) and the phosphoinositide-3-kinase (PI3K) pathway, as shown in figure 4 (Wang et al. 2020). Numerous clinical trials have evaluated the efficacy of inhibition of these pathways. Meanwhile, the BTK inhibitor ibrutinib, for example, is among the most important agents approved for the treatment of lymphoma.

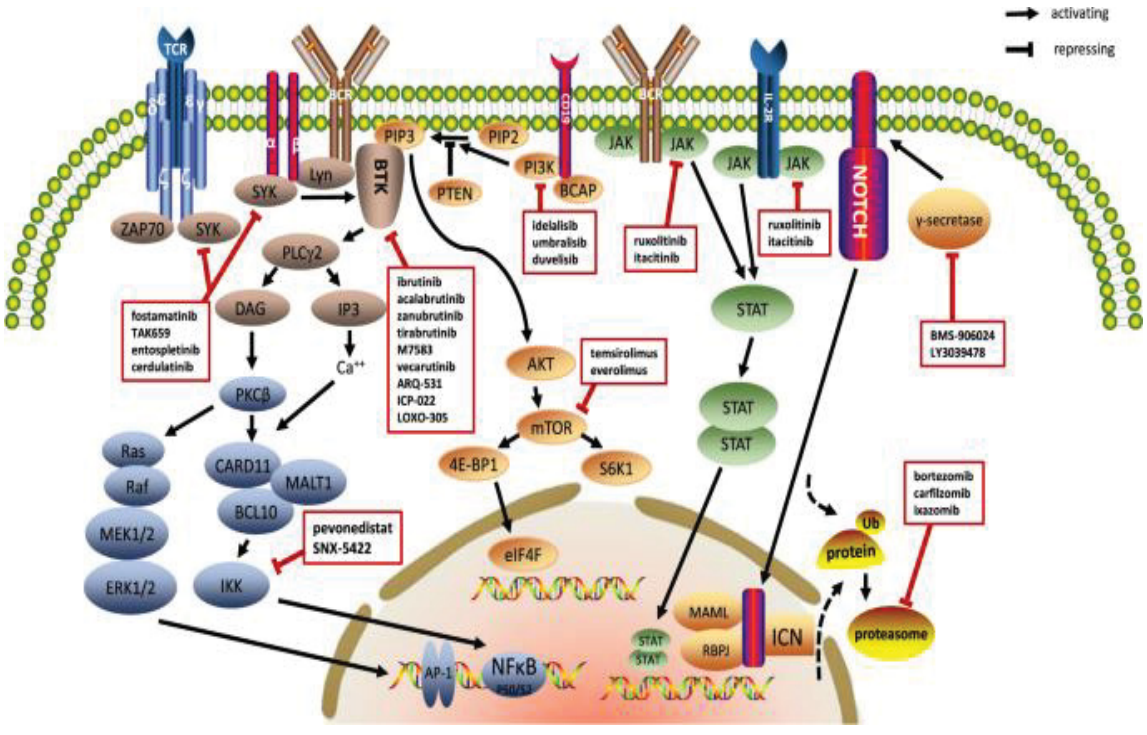

Figure 4. Signaling pathways responsible for lymphoma development
(adopted from Wang et al, 2020, Journal of Signal Transduction and Targeted Therapy)

Stem cell transplantation

Both autologous and allogenic stem cell transplantations (auto-SCT & allo-SCT) are effective consolidation therapies in relapsed or refractory lymphoma, and are capable of improving disease control as well as overall survival. Allo-SCT has the advantage of lacking any lymphoma cell contamination of the graft, and also offers the chance of harnessing a graft-versus-tumor effect (Ladetto et al. 2008; Sureda et al. 2018) .

CAR-T cell therapy

Among the evolving immunology-based treatment strategies of hematological malignancies, chimeric antigen receptor T-cell (CAR-T-cell) therapy is considered a real breakthrough and has attracted much attention in the field of lymphoma treatment. The CAR molecule has a hybrid structure consisting of two parts: 1) an extracellular domain based on a monoclonal antibody-like structure with the capability of surface antigen recognition on tumor cells in an MHC-independent manner, and 2) an intracellular part based on a T-cell receptor-like structure. After transfecting CAR-encoding viral vectors into T-cells isolated from patients, the genetically modified T-cells are expanded and finally injected into the patient (Roex et al. 2020), where they can attack lymphoma cells, for instance by binding to the CD20 antigen on the surface of B-cells.

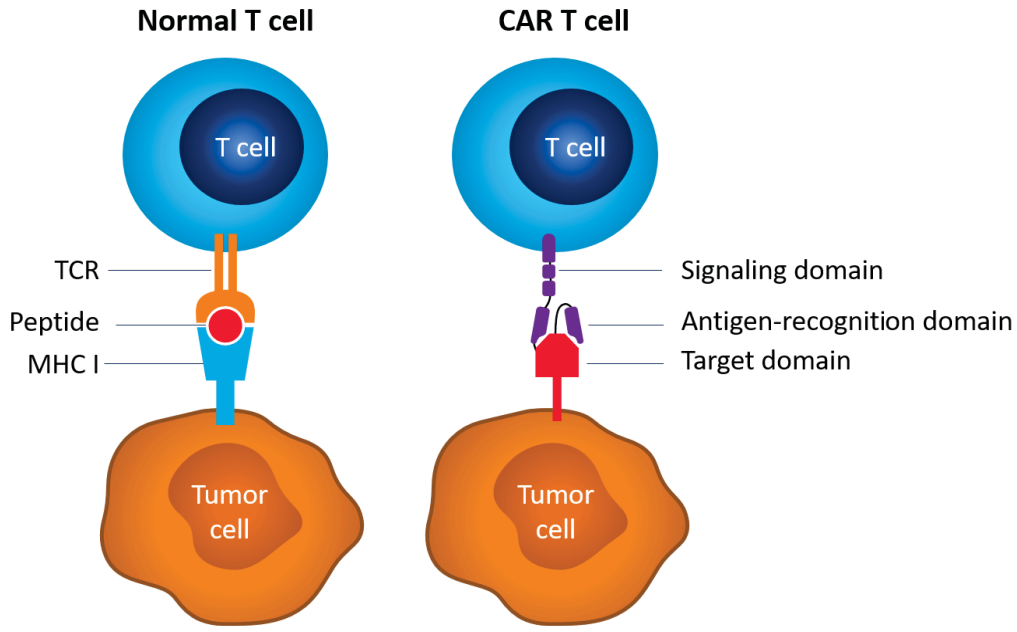

Figure 5. Normal vs CAR-T-cell
(adopted from: https://premier-research.com/a-brief-introduction-to-car-t-cell-therapy)

Aim of the study

Primary adrenal lymphoma is a rare lymphoid malignancy with poor prognosis. With fewer than 300 cases reported in the medical literature, data about clinical features and prognosis is limited, and there is no standardized diagnostic and treatment approach. Physicians confronted with a PAL patient usually have no previous experience with this disorder and may find it difficult to make the right decisions. Collecting and analyzing data from 14 centers worldwide, we aimed to refine the current understanding of the clinical features and prognosis of PAL and to identify current deficits in the management of this rare disease.

The retrospective analysis was approved by the Ethics Committee of the Medical Faculty of Heinrich Heine University (approval nr. 3027).

Publication

Clinical Study | F Majidi and others | Clinical spectrum of primary adrenal lymphoma | 183:4 | 453–462

Clinical spectrum of primary adrenal lymphoma: results of a multicenter cohort study

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European Journal of Endocrinology

Abstract

Purpose: We sought to refine the clinical picture of primary adrenal lymphoma (PAL), a rare lymphoid malignancy with predominant adrenal manifestation and risk of adrenal insufficiency.

Methods: Ninety-seven patients from 14 centers in Europe, Canada and the United States were included in this retrospective analysis between 1994 and 2017.

Results: Of the 81 patients with imaging data, 19 (23%) had isolated adrenal involvement (iPAL), while 62 (77%) had additional extra-adrenal involvement (PAL+). Among patients who had both CT and PET scans, 18FDG-PET revealed extra-adrenal involvement not detected by CT scan in 9/18 cases (50%). The most common clinical manifestations were B symptoms (55%), fatigue (45%), and abdominal pain (35%). Endocrinological assessment was often inadequate. With a median follow-up of 41.6 months, 3-year progression-free (PFS) and overall (OS) survival rates in the entire cohort were 35.5% and 39.4%, respectively. The hazard ratios of iPAL for PFS and OS were 40.1 (95% CI: 2.63–613.7, $P = 0.008$) and 2.69 (95% CI: 0.61–11.89, $P = 0.191$), respectively. PFS was much shorter in iPAL vs PAL+ (median 4 months vs not reached, $P = 0.006$), and OS also appeared to be shorter (median 16 months vs not reached), but the difference did not reach statistical significance ($P = 0.16$). Isolated PAL was more frequent in females (OR = 3.81; $P = 0.01$) and less frequently associated with B symptoms (OR = 0.159; $P = 0.004$).

Conclusion: We found unexpected heterogeneity in the clinical spectrum of PAL. Further studies are needed to clarify whether clinical distinction between iPAL and PAL+ is corroborated by differences in molecular biology.

European Journal of Endocrinology (2020) **183**, 453–462

https://eje.bioscientifica.com
https://doi.org/10.1530/EJE-19-0506

Published by Bioscientifica Ltd.

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Introduction

Primary adrenal lymphoma (PAL) is a rare entity that seems to have a poor prognosis. Patients may not only suffer from lymphoma-related symptoms such as fever, night sweats and weight loss (B symptoms), and abdominal or lumbar pain, but have also been reported to develop life-threatening adrenal insufficiency in case of bilateral involvement (1, 2). Therefore, a multidisciplinary approach is needed, involving oncologists and endocrinologists. However, due to the rarity of PAL, most oncologists and endocrinologists do not have much experience in treating this disease. Knowledge of PAL is based on less than 250 cases reported in the retrievable scientific literature; the largest series includes 31 patients (2, 3).

The diagnostic work-up for PAL includes imaging studies like ultrasonography, CT, MRI and PET, but histopathology is required to confirm the diagnosis (2, 4). This does not conflict with the recommendation that adrenal biopsy should only be performed if the expected findings are likely to alter the management of the individual patient and after biochemical exclusion of catecholamine-producing tumors to prevent potentially life-threatening complications (5, 6). A comprehensive endocrine work-up is also strongly recommended according to recent guidelines (7). As the exact cause of an adrenal mass is difficult to ascertain on the basis of imaging characteristics, some PALs are diagnosed only after surgical removal. This should be avoided, because surgery exposes the patient to additional risks, including a considerable delay in starting chemotherapy.

In general, treatment of PAL follows the principles of therapy for B cell non-Hodgkin lymphomas. Reliable prognostic factors have not been identified. Controversial results have been reported as to the prognostic impact of tumor stage, bilateral vs unilateral involvement, involvement of other organs, presence of adrenal insufficiency, serum lactate dehydrogenase (LDH) level, and achieving complete remission after immunochemotherapy (1, 3). We present a multicenter case series of 97 patients with PAL, focusing on clinical presentation, radiographic features, immunohistochemistry, treatment results, and prognosis.

Patients and methods

Data collection

Fourteen academic medical centers in Europe, Canada and the USA contributed to this study. Ninety-seven patients with PAL were identified between 1994 and 2017. As explained by Rashidi and Fisher (2) in their systematic review of PAL, 'primary adrenal lymphoma is histologically proven lymphoma of one or both adrenal glands in patients with no prior history of lymphoma. If other organs or lymph nodes besides the adrenal glands are involved, the adrenal lesion must be unequivocally dominant'. This definition of PAL is generally used and was therefore adopted in our retrospective multicenter study.

With approval from each participating centers' institutional review boards, retrospective clinical data, laboratory results, imaging and histopathology reports were extracted from patients' medical records and pathology databases. Most of the participating centers are part of the ENS@T registry (European Network for the Study of Adrenal Tumors), which has been approved by the local ethics committees. The study was approved by the following ethics committees: Heinrich Heine Universität Düsseldorf (Ethikkommission der Medizinischen Fakultät); University of Birmingham, Universität Würzburg (Ethik-Kommission bei der Medizinischen Fakultät); Comite de protection des personnes Est1 for university hospitals Dijon, Besancon, Lyon, Strasbourg and Nancy in France; Institutional Review Board at MD Anderson Cancer Center; Johns Hopkins University (Office of Human Subjects Research, Institutional Review Board); Ethikkommission bei der Medizinischen Fakultät der Ludwig-Maximilians-Universität München; British Columbia Cancer Agency Research Ethics Board; Karolinska Institute Ethics Committee, Stockholm; and Universitetssjukhuset Linköping (Regionala ethic-prövningsnämnden BESLUT I Linköping).

Due to the retrospective design of the study, informed consent could not be obtained from most patients. Regarding imaging studies, data collection was restricted to the retrieval of radiology reports. Images for repeat measurements were rarely available. Data were entered into a common study-specific data capture sheet by local investigators. The database included patient demographics, clinical presentation, laboratory findings, for example, serum adrenocorticotrophin (ACTH) level, baseline cortisol level, ACTH stimulating test, lactate dehydrogenase (LDH) and Epstein–Barr virus DNA, imaging results (CT and PET scans), histopathological analysis including immunohistochemistry (proliferation marker Ki-67 and lymphoma markers including CD3, CD5, CD10, CD20, CD79a, BCL2, BCL6 and MUM1), treatment data, and clinical follow-up. PAL was defined as histologically proven extra-nodal lymphoma that primarily affects the adrenal gland(s) (3). If there was involvement

of other organs and/or lymph nodes, the diagnosis of PAL was accepted only in the case of unequivocal dominant involvement of the adrenal gland(s) (3). We divided the disease into two subtypes: cases with synchronous extra-adrenal involvement at diagnosis (PAL+) and cases with isolated involvement of the adrenal(s) (iPAL). While there is no unmistakeable evidence of adrenal origin in cases with additional extra-adrenal involvement (PAL+), these cases were deemed primary adrenal by treating physicians in the centers, based on clinical and radiological findings.

In the entire study cohort of 97 patients with histopathologically proven PAL, imaging data was available for 81, detailed patient history for 71, information about treatment modality for 69, data on initial treatment response for 62, and follow-up information for 59 patients.

Statistical analysis

Survival data were collected until 31 April 2018. Patient characterstics were reported with median and range for continuous variables and as frequencies (%) for categorical variables. The univariable association of categorical variables was evaluated using Fisher's exact test. Overall survival (OS) was calculated with the non-parametric method of Kaplan–Meier, considering the date of first diagnosis and either the time of death (complete data), irrespective of cause, or the time of last follow-up (censored data). Progression-free survival (PFS) was defined as the time from the date of diagnosis until the date of progression or death from any cause and calculated in the same manner as OS. Maximum observation time was 10 years. In the Kaplan–Meier analysis, comparison between survival curves was made according to the log-rank test. Univariable analysis using Cox proportional model was carried out to identify factors associated with OS and PFS. Because the number of complete datasets appeared too small, we refrained from multivariable analysis. All tests were two sided and *P*-values less than 0.05 were considered statistically significant. All data were analyzed with IBM SPSS statistics software version 24.

Results

Clinical and laboratory findings

The study cohort included 68 males (70%) and 29 females (30%). Bilateral adrenal involvement was reported in 49% of the patients (44/90, missing data in 7). As the clinical information was captured mostly from tertiary referral centers, the exact date of first diagnosis and the duration of survival were not available for all patients. Detailed records of clinical manifestations were available in 71 patients. B symptoms, fatigue, abdominal or back pain, and anorexia were the most common symptoms. Shortness of breath/exertional dyspnea, hypotension, and pruritus were less common (Fig. 1). Adrenal insufficiency may have contributed to the patients' symptoms, particularly fatigue and hypotension. It is difficult for the clinician, though, to attribute these symptoms to adrenal insufficiency, as fatigue is commonly found in patients with malignant lymphoma and hypotension may be part of an infectious complication.

PAL was an incidental finding on CT scan in 10 of 71 cases (14%) detected during clinical workup for other disorders. Five patients had a history of immune dysfunction, related to human immunodeficiency virus (HIV) infection (2/71) or autoimmune disease (rheumatoid arthritis (2/71), Evans syndrome (1/71)). A concurrent or past diagnosis of cancer was noted in 13 of 71 cases (18%), in particular prostate cancer (7/51 male patients), breast cancer (1/24 female patients), non-melanoma skin cancer (3/71), and chronic lymphocytic leukemia (2/71). Serum LDH was increased in 53 of 65 (82%) patients tested. A test for Epstein–Barr virus DNA in the serum was done in 30 patients and was positive in 22 cases (73%). Thirty-seven patients underwent measurement of baseline cortisol level and/or an ACTH stimulation test ($n=13$). In 20 of these patients, bilateral adrenal involvement was present. Adrenal insufficiency was detected in 14 (70%) of 20 patients with a bilateral mass, but none of 17 patients with unilateral PAL.

Of the 81 patients with available imaging data, 19 (23%) had iPAL, while 62 (77%) had PAL+. The sex distribution (f:m) was 53%:47% for iPAL and 22%:78% for PAL+. Using the Fisher's exact test, we found a significant association of female sex with the iPAL phenotype (odds ratio: 3.81, 95% CI: 1.294–11.213, $P=0.01$). Median age at diagnosis was 66 years (range: 25–89), with no significant difference between PAL+ and iPAL. Interestingly, isolated PAL was significantly less frequently associated with B symptoms at diagnosis (OR=0.159; $P=0.004$).

Histopathological diagnosis

B cell non-Hodgkin lymphoma was the most common histopathological finding, reported in 91% (88/97) of patients. The most frequent subtype was diffuse large B cell lymphoma (DLBCL) (74/97) including one T cell/histiocyte-rich B cell lymphoma, an uncommon morphological variant of DLBCL. As shown in Table 1,

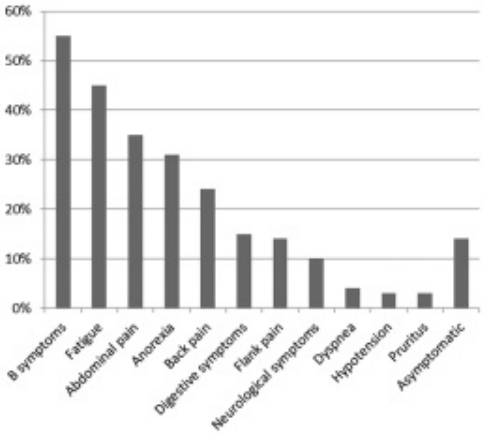

Figure 1
Symptoms of primary adrenal lymphoma (PAL) at diagnosis.

six patients were diagnosed as T cell lymphoma, two had a NK/T cell lymphoma, and one patient had Hodgkin's lymphoma.

On immunohistochemistry, the B-cell marker CD20 was expressed in 36 of 37 evaluated B cell lymphomas. CD10, BCL2, BCL6 and MUM1 were expressed in 18% (6/34), 93% (13/14), 72% (18/25), and 100% (17/17) evaluated cases with DLBCL, respectively. All cases classified as DLBCL showed strong expression of the proliferation marker Ki-67 (median 85%, range 50–100%), confirming the highly proliferative nature of this lymphoma.

Radiological findings

CT and PET imaging reports were available for 81 and 18 patients, respectively. Information regarding the size of the adrenal mass was available for 68 patients. The median diameter of the lesion was 80 mm (range 27–180 mm). Extra-adrenal involvement was detected through radiological examination in 77% of the patients (62/81). The most common extra-adrenal organ manifestations were brain and spleen, followed by involvement of lung, kidney and inferior vena cava. Thirty patients (37%) had associated lymphadenopathy, nine of them without further extra-adrenal organ involvement. Of the 81 patients, 19 (23%) had isolated adrenal lymphoma (iPAL). In all patients with available 18-fluorodeoxyglucose (18FDG)-PET (n=18), standard uptake values (SUV) were elevated in the adrenal glands, with a median SUVmax of

Table 1 Patient characteristics.

Characteristics	Values
Total, *n*	97
Women	29 (30%)
Men	68 (70%)
Age	
Median	66
Range	25–79
Lymphoma localization	
Isolated adrenal involvement	19/81 (23%)
Extra-adrenal involvement	62/81 (77%)
Data not available	16
Laterality	
Bilateral	44/90 (49%)
Unilateral	46/90 (51%)
Data not available	7
LDH	
Elevation above normal limit	53/65 (81%)
Median	850 U/L
Range	174–6515 U/L
Adrenal insufficiency	14/37 (38%)
With bilateral involvement*	14/20 (70%)
With unilateral involvement*	0/17 (0%)
Unknown/not evaluated	60
Histopathology	
B cell non-Hodgkin lymphomas	88/97 (91%)
Diffuse large B cell lymphoma	74/97
Mantle cell lymphoma	3/97
Marginal zone lymphoma	2/97
Follicular lymphoma	2/97
B cell lymphoma not otherwise specified	7/97
Non-B cell lymphoma	9/97 (9%)
T cell lymphoma	6/97
NK/T cell lymphoma	2/97
Hodgkin lymphoma	1/97

* In evaluated patients.

17 (range, 3.5–48). Among patients who had both CT and PET scans, 18FDG-PET revealed extra-adrenal involvement not detected by CT scan in 9/18 cases (50%). PET-CT images are displayed for four patients who showed a marked response to systemic treatment (Figs 2, 3, 4 and 5).

Treatment

Data regarding lymphoma treatment was available for 69 patients. Of five patients who died without receiving chemotherapy, two were treated with corticosteroids (dexamethasone and prednisolone, respectively). The other 64 patients received chemotherapy, in four cases (6%) combined with radiotherapy and in six cases (9%) as adjuvant therapy after adrenalectomy. The most common first-line chemotherapy protocol was CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone), given to 53 patients (77%). One patient with B-cell lymphoma received an intensive

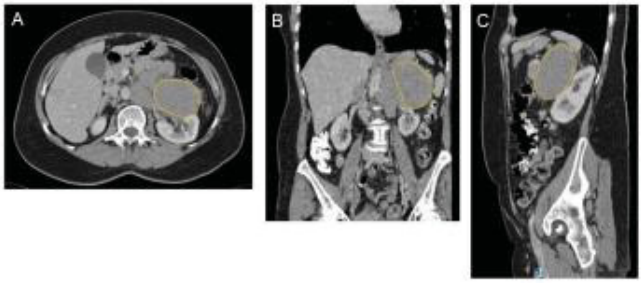

Figure 2
CT images illustrating unilateral iPAL. CT scans (A, axial plane; B, coronal plane; C, sagittal plane) showing a unilateral large (10.7 cm) left adrenal mass in a 53-year-old female patient who presented with abdominal pain and pruritus. On biopsy, a primary adrenal B-cell lymphoma was diagnosed.

Hyper-CVAD protocol (course A: cyclophosphamide, vincristine, adriamycin and dexamethasone; course B: methotrexate and cytarabine). Another patient, who showed histopathological findings of Hodgkin lymphoma, was treated according to the ABVD protocol (adriamycin, bleomycin, vinblastine, and dacarbazine). In 46 of 61 patients with B-cell lymphomas (75%), rituximab (R) was part of the treatment regimen. One patient was treated with rituximab and bendamustine during first-line therapy and with R-CHOP after disease progression. Five patients received CNS prophylaxis, of whom two patients received intrathecal methotrexate (MTX); one patient underwent whole brain irradiation; one patient was given high doses of i.v. MTX (two cycles); and another patient received intrathecal triple therapy (MTX, dexamethasone, cytarabine).

Since endocrinological testing of adrenal status, in particular by using the short synacthen test, was only performed in a small fraction of patients ($n=13$) at the time of diagnosis and follow-up SSTs were not available at all, it was not possible to assess the impact of lymphoma treatment on adrenal status.

Outcomes and prognostic factors

For 62 patients, response to first-line therapy was evaluable: 34% (21/62) achieved partial remission (PR) and 44% (27/62) achieved complete remission

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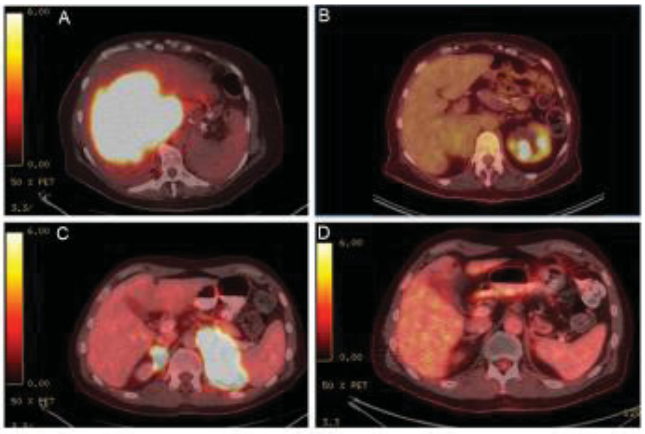

Figure 3
PET-CT images illustrating unilateral iPAL and bilateral iPAL, respectively, at diagnosis and during follow-up after treatment. (A) FDG PET-CT scan showing a large right adrenal mass at diagnosis in a 65-year-old female patient who presented with backpain and B-symptoms. On biopsy, a primary adrenal B-cell lymphoma was diagnosed. (B) The patient responded to six cycles of R-CHOP immuno-chemotherapy. (C) FDG PET-CT scan showing a bilateral adrenal mass at diagnosis in a 62-year-old male patient with primary adrenal B-cell lymphoma who presented with fatigue, weekness, abdominal pain and confirmed adrenal insufficiency at diagnosis. (D) After treatment failure with R-CHOP, the patient achieved a remarkable remission with the Bruton's tyrosine kinase inhibitor Ibrutinib.

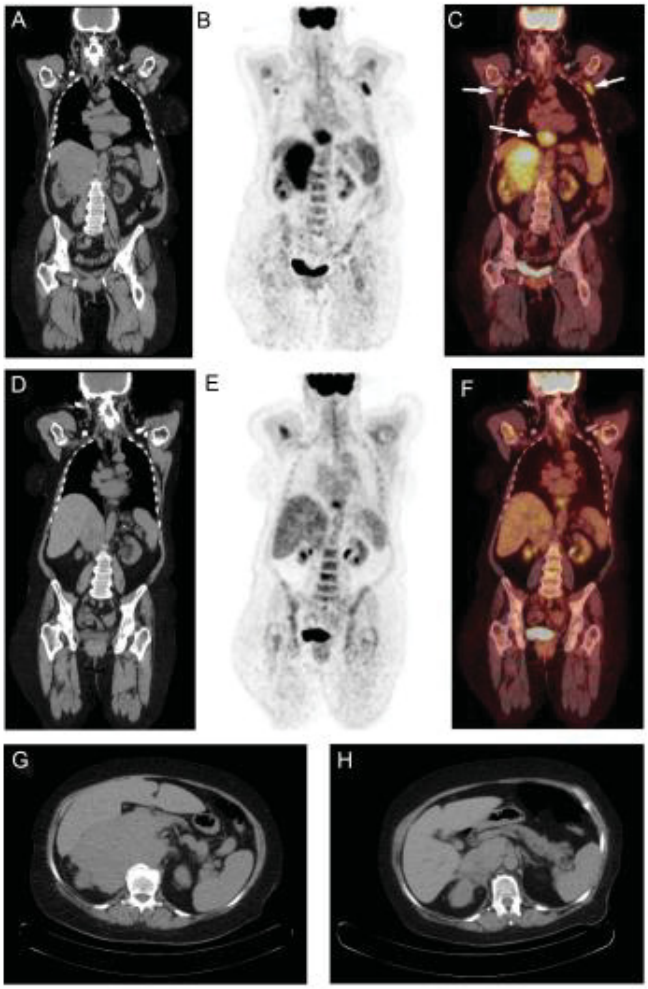

Figure 4
CT (coronal reconstruction), PET and fusion PET/CT images illustrating unilaterial PAL+ before and after treatment with immuno-chemotherapy. (A, B and C) Prior to treatment, CT, PET and fusion PET/CT showed a unilateral FDG positive large left adrenal mass (14.1 cm, SUV max 9.2) and, in addition, axillary and mesenteric lymphadenopathy (see arrows, SUV max 7.4–8.6) in a 66-year-old female patient who presented with weakness, anorexia and abdominal pain. On biopsy, a primary adrenal B-cell lymphoma was diagnosed. (D, E and F) The patient showed a good partial response to four cycles of R-CHOP immuno-chemotherapy. (G and H) CT images (axial sections) before and after treatment

(CR). Fourteen of 62 patients (23%) showed disease progression (DP) during or shortly after first-line therapy. Eighteen of 62 patients (29%) relapsed after an initial response to treatment. Six patients (three in CR and three with DP after first-line therapy) had secondary involvement of the brain, one of them despite prior CNS prophylaxis using whole brain irradiation.

Median follow-up of patients was 42 months. The 3-year PFS and OS were 36% and 39%, respectively. Median PFS and OS were 10.5 (range 0–120) and 16 (range 0–120) months, respectively (Fig. 6).

We evaluated several potential prognostic factors by univariable analysis (Table 2). B cell lymphomas were associated with better PFS and OS than other lymphoma types. There was a statistically significant association between iPAL and shorter progression-free survival (HR for progression 2.721, 95% CI 1.280–5.787, $P=0.009$) (Fig. 7A). Patients with iPAL also appeared to have considerably shorter overall survival (Fig. 7B), but the difference did not reach statistical significance on univariable analysis (HR 1.93, $P=0.11$).

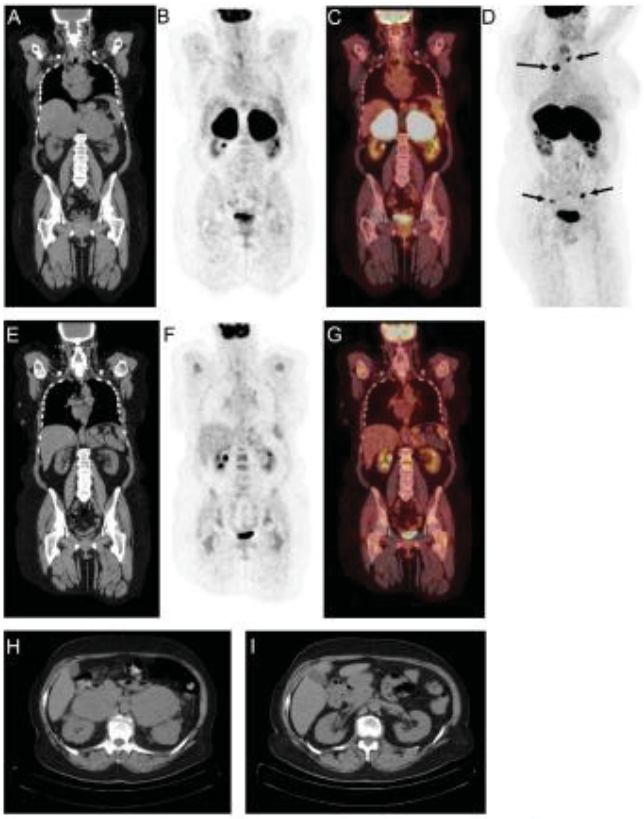

Figure 5
CT (coronal reconstruction), PET and fusion PET/CT images illustrating bilaterial PAL+ before and after treatment with immuno-chemotherapy. (A, B, C and D) Prior to treatment, CT, PET and fusion PET/CT showed bilateral adrenal masses (SUV max 23.5) plus further extranodal lesions including thyroid, lung, ovary and bones (see arrows in D (PET-MIP), SUV max (right ovary) 5.7–14.8 (Th3)) in a 59-year-old female patient who presented with B-symptoms, anorexia, and abdominal and back pain. (E, F and G) The patient achieved complete remission after two cycles of cytarabine (pre-phase chemotherapy), four cycles of chemoimmunotherapy with obinutuzumab/ifosfamide/etoposide, followed by two cycles of high-dose methotrexate (for CNS prophylaxis). (H and I) CT images (axial sections) before and after treatment.

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Too many possible confounders precluded formal assessment of treatment effects. However, it was our impression that treatment regimens other than R-CHOP did not substantially alter the outcome. Neither did we observe any substantial differences in treatment regimens between iPAL and PAL+ that may explain the worse outcome in iPAL. Seven responding patients (five in CR and two in PR) received autologous stem cell transplantation. Follow-up information was available for four of these patients, who all showed relatively long overall survival (median 93, range 72–120 months). However, the small number of cases precluded meaningful statistical analysis.

We suspect that the failure to perform adequate endocrinological testing (and subsequent failure to institute cortisol replacement therapy) may have contributed to the bad prognosis in some of the patients. For instance, one of the patients who underwent autologous stem cell transplantation without prior endocrinological testing died soon after the procedure, with no clear cause of death ascertained. This patient may have suffered from adrenal insufficiency, which may have made it impossible for him to tolerate the stresses and strains of the procedure.

Discussion

Primary adrenal lymphoma (PAL) was first described in 1961 (8). With fewer than 250 published cases in the medical literature, there are limited data regarding the epidemiology, clinical features, and pathophysiology of this rare disease. Our current retrospective analysis of 97 patients from 14 centers is among the largest surveys so far. Expectedly, the heterogeneity of the reported investigations and treatments, as well as the problem of

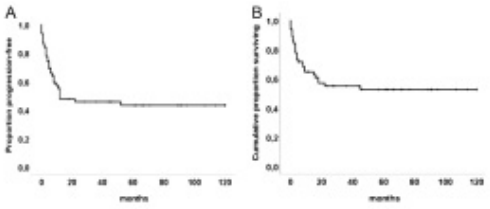

Figure 6
Kaplan–Meier estimates of progression-free survival (A) and overall survival (B) in patients with primary adrenal lymphoma.

missing data, places limitations on our retrospective study. Therefore, we tried to avoid unsubstantiated conclusions, for instance, regarding the efficacy of various treatment regimens. The lack of adequate endocrinological assessment in a large proportion of patients must also be considered a limiting factor. This inadequacy, however, reflects the real-life situation of patient care for PAL, which is in need of improvement.

Regarding our distinction between iPAL and PAL+, the respective diagnoses obviously depend on diagnostic imaging. It would have been desirable to have FDG-PET scans available in the majority of patients. However, due to the retrospective nature of our study, this was not possible. Nevertheless, absence of 18F-FDG PET in the majority of our retrospective cases is a limitation of the study, since we cannot exclude with certainty that PET might have detected a few additional extra-adrenal manifestations.

Based on 18 evaluable patients, our study suggests that PET is superior to CT scan in the detection of extra-adrenal manifestations. We found that, among patients who had both CT and PET scans, FDG-PET revealed extra-adrenal involvement not detected by CT scan in 9/18 cases (50%). All of these extra-adrenal manifestations presented as enlarged lymph nodes. Although the quality of tomographic imaging studies has improved over the years, older CT scans were of sufficient quality to detect large adrenal tumors as well as substantial lymphadenopathy. Our conclusion that PET-CT appears to be superior to CT regarding the detection of extra-adrenal involvement is based on patients who had both CT and PET scans within a relatively short period of time. This intra-individual comparison avoids comparing recent PET-CT technology with older versions of CT scanning.

Novel, clinically relevant prognostic factors are needed for PAL because current lymphoma staging systems and prognostic scores may not be reliable. This is illustrated by our finding that, in contrast to recent publication on primary adrenal lymphoma by Li *et al.* (9), increased tumor burden in the form of bilateral adrenal involvement or large tumor diameter had no impact on prognosis.

We presume that, in the future, knowledge of the mutational landscape of PAL may aid in the prognostic assessment of patients. Recently, Chapuy *et al.* showed that primary CNS lymphoma (PCNSL) and primary testicular lymphoma (PTL) have a mutational landscape and immunohistochemical appearance that differ from nodal diffuse large B-cell lymphoma. For instance, the extranodal lymphomas harboured more numerous somatic mutations and showed more frequent overexpression of the PD-1 ligand (10). Nothing is known about the corresponding features of PAL. Therefore, it is also difficult to predict whether modern lymphoma therapies, like those targeting the B cell receptor pathway, will be more successful than conventional immunochemotherapy.

In order to improve the treatment of PAL, prompt endocrinological management is as important as effective cancer therapy. In a systematic review by Rashidi *et al.*, 70/115 (61%) of evaluated patients had either relative or absolute adrenal insufficiency (2). Of concern, endocrinological assessment was performed in less than 40% of our cases and just 44.4% of patients with bilateral

Table 2 Factors influencing PFS and OS according to univariable analysis.

Variables	n/total	PFS			OS		
		HR	95% CI	P	HR	95% CI	P
Age <65	33/97	0.370	0.159–0.864	0.022	0.476	0.198–1.143	0.097
Diameter >8 cm	36/68	1.136	0.423–1.832	0.733	0.994	0.469–2.106	0.987
Bilateral adrenal involvement	44/90	0.755	0.664–2.643	0.426	0.684	0.320–1.462	0.327
iPAL	19/81	2.721	1.280–5.787	0.009	1.920	0.863–4.273	0.110
LDH >250 U/L	53/65	1.244	0.465–3.327	0.664	1.272	0.477–3.390	0.631
Adrenal insufficiency	14/37	0.809	0.243–2.690	0.730	0.927	0.352–2.439	0.878
B cell lymphoma	88/97	0.276	0.118–0.646	0.003	0.335	0.135–0.832	0.018

* iPAL, isolated adrenal involvement.

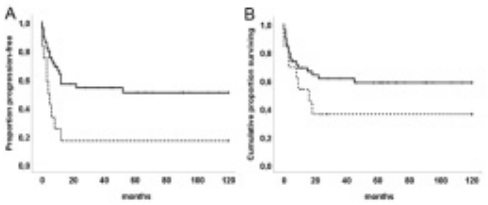

Figure 7
Kaplan–Meier estimates of progression-free survival (A) and overall survival (B) in patients with iPAL (isolated adrenal lymphoma involvement; dotted lines) and PAL+ (featuring additional extraadrenal manifestation; solid lines).

involvement. In most of the patients, adrenal insufficiency was only assessed by baseline cortisol measurement, which helps ruling out adrenal insufficiency only if a level is above a value that different studies placed between 285 nmol/L (10.3 µg/dL) and >480 nmol/L (17 µg/dL) (11). Although destruction of 90% of the adrenal glands is required for the development of clinical signs and symptoms of adrenal insufficiency, subclinical adrenal insufficiency may be present earlier, due to cytokine-driven, paracrine effects of lymphoma cells on the adrenal gland microenvironment (2). This degree of adrenal insufficiency would not be detected by simply measuring baseline cortisol, even if performed in the morning, since the result may fall within the normal range. Inadequate adrenal reserve may become a clinical problem in case of severe illness, surgical intervention or in the context of corticosteroid withdrawal after immunochemotherapy (12). It is thus necessary not only to be vigilant regarding signs and symptoms of adrenal insufficiency, including skin hyperpigmentation, fatigue, hypotension, and electrolyte abnormalities, but also to conduct a formal assessment of adrenal reserve. Failure to do so may have contributed to the worse prognosis in our patients with iPAL. It is possible that adrenal insufficiency was at least partially responsible for the early demise of six of nine patients with iPAL whose death was not attributable to progressive lymphoma but to adrenal crisis, sepsis, poor general condition, or adrenalectomy.

In conclusion, our retrospective analysis suggests that PAL is a heterogeneous disease and comprises cases with isolated involvement of adrenal tissue (iPAL) and cases with additional extra-adrenal organ manifestations (PAL+). It is possible that iPAL, which has an unusual male/female ratio, less B symptoms, and a particularly poor prognosis, may have biological characteristics that are associated with a pronounced tendency for early destruction of adrenal endocrine tissue and subsequent adrenal insufficiency. Accordingly, we emphasize the need for careful endocrine assessment of patients with PAL and for close cooperation between oncologists and endocrinologists when treating patients with this rare disease.

Declaration of interest
M F is an associate editor and W A is Editor in Chief for the *European Journal of Endocrinology*. However, they were not involved in any way in the review or editorial process for this paper, on which they are listed as authors. The other authors have nothing to disclose.

Funding
W A receives support from the National Institute for Health Research (NIHR) Birmingham Biomedical Research Centre at the University Hospitals Birmingham NHS Foundation Trust and the University of Birmingham (Grant Reference Number BRC-1215-20009). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care. V C receives support from the Academy of Medical Sciences UK (Starter Grant for Clinical Lecturers SGL020/1018).

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Received 2 July 2019
Revised version received 31 May 2020
Accepted 16 June 2020

Discussion

Extra-nodal lymphomas (ENLs) are characterized by infiltration of malignant lymphocytes into organs other than lymph nodes. They are designated as primary ENL if they originate from a non-lymphoid organ, whereas secondary ENLs result from hematogenous spread of malignant lymphoid cells from a nodal origin into a non-lymphoid organ (Das et al. 2014). ENLs involving the gastrointestinal tract, central nervous system (CNS), lung, liver, spleen and bone are well described (Das et al. 2014). Primary adrenal lymphoma (PAL) is a rarely documented lymphoma type with a poor prognosis. Considering the absence of commonly agreed diagnostic criteria or a useful staging system, the lack of treatment studies, and the inadequate awareness regarding endocrinological complications, there is no doubt that PAL qualifies as an “orphan disease”.

In order to gain more insight into the clinical spectrum of PAL, we embarked on an international multicenter analysis and collected 97 cases from Europe, Canada and the USA (Majidi et al. 2020). This is the second largest cohort of PAL patients ever reported. Only Li et al in China assembled more cases, compiling clinical data on 136 PAL patients. (Li et al. 2019). Compared with the investigation by Li et al, we tried to consider both oncological and endocrinological features of PAL, enabling us to recognize more comprehensively the current deficits in the management of this rare disease. Another advantage of our study is that all patients were collected from either a tertiary center of endocrine oncology or a center affiliated with the European network for the study of adrenal tumors (ENSAT), suggesting that we are dealing with accurate diagnoses. Nevertheless, as expected in a multicenter study of an orphan disease, we encountered challenges due to the heterogeneity of diagnostic and therapeutic approaches.

Deficits in the management of primary adrenal lymphoma

Patients with diagnosis of PAL may not only suffer from lymphoma-related symptoms, but in case of bilateral involvement can also present with life-threatening adrenal insufficiency (Majidi et al. 2020). Therefore, proper clinical management of PAL requires a multidisciplinary approach involving pathologists, oncologists, endocrinologists, and experts in the field of radiology and nuclear medicine. Based on our findings, we categorize the diagnostic and therapeutic pitfalls of PAL management as follows:

1. Inadequate endocrinological work-up:

Since cortisone was discovered seventy years ago, adrenal insufficiency (AI) has become a treatable condition. However, AI can still be fatal, due to diagnostic errors and delayed treatment (Puar et al. 2016). Therefore, the neglect and/or underestimation of AI we observed in our study raises concerns as to the proper clinical management of PAL. The following endocrinological aspects should be taken into consideration during PAL treatment.

AI is characterised by inadequate production of glucocorticoids, mineralocorticoids and adrenal androgens. Based on the cause of adrenal hypofunction, three types of AI have been defined: 1) Primary adrenal insufficiency (PAI), due to destroyed or damaged adrenal tissue. Causes of primary AI are summarized in table 4. 2) Secondary adrenal insufficiency (SAI), due to pituitary damage. 3) Tertiary adrenal insufficiency (TAI), caused by damage to the hypothalamic region or suppression of hypothalamic activity.

In PAL with bilateral involvement, massive infiltration of both adrenal glands with malignant lymphatic cells can cause PAI. However, TAI can also occur, due to lymphoma treatment with corticosteroids (Hahner et al. 2021). It has been shown that neither duration nor dose nor type of administration of corticosteroid therapy correlate with the probability of developing TAI (Broersen et al. 2015).

Aetiology	Pathogenesis	Diagnostic tools
Autoimmune	T and B cell autoimmunity against adrenocortical cells	21-Hydroxylase autoantibodies
Infection	Mycobacteria, bacteria (e.g. *Neisseria meningitidis*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*), viruses (e.g. human immunodeficiency virus, herpes simplex, cytomegalovirus) or fungi (e.g. *Pneumocystis jirovecii*)	Culture, QuantiFERON test, PCR, adrenal CT
Tumour	Primary tumour (bilateral), metastasis (bilateral), adrenal lymphoma (bilateral)	Adrenal CT
Bleeding	Anti-phospholipid syndrome, anticoagulant therapy, disseminated intravascular coagulation	Adrenal CT, phospholipid autoantibodies
Surgery	Bilateral adrenalectomy	Patient history
Infiltrative	Amyloidosis Haemochromatosis Histiocytosis	Adrenal CT, subcutaneous fat biopsy Ferritin, *HFE* sequencing Adrenal imaging
Genetic[a]	Congenital adrenal hyperplasia, congenital lipoid adrenal hyperplasia, adrenoleukodystrophy (X-linked), adrenal hypoplasia congenita, autoimmune polyglandular syndrome type 1	Sequence of relevant gene
Medication[b]	Enzyme inhibition (ketoconazole, fluconazole, itraconazole, etomidate, aminoglutethimide, metyrapone, trilostane, osilodrostat); adrenolytic effect and increased cortisol metabolism (mitotane); inflammation (checkpoint inhibitors)	Medication and patient history

Table 4: Causes of primary adrenal insufficiency
(adopted from Hahner et al, Nature Reviews 2021)

Once AI is suspected, both morning serum cortisol and plasma ACTH should be measured. Combined morning cortisol <140 nmol/l (5 µg/dl) and ACTH higher than twice the upper limit of normal confirms the diagnosis of PAI. However, cortisol levels ≥140 nmol/l (5µg/dl) cannot exclude PAI. An ACTH stimulation test, using 250 µg synthetic ACTH, is considered the gold standard in the diagnosis of AI. A serum cortisol <450 nmol/l 30 min. after ACTH stimulation, or less than 500 nmol/l 60 min. after ACTH stimulation, confirms the diagnosis of PAI (Hahner et al. 2021). In our study, we repeatedly noted that AI was erroneously ruled out on the basis of a cortisol level ≥140nmol/l.

Clinical practice guidelines recommend replacement therapy with 15-25 mg hydrocortisone daily and, in case of aldosterone deficiency, 50-100 µg fludrocortisone daily for treating AI (Bornstein et al. 2016). It seems that the corticosteroid requirement of a patient with PAL is fully covered by the steroid component of most lymphoma protocols during the first days of treatment. For instance, a patient with PAL receiving immuno-chemotherapy according to the R-CHOP protocol will receive 100 mg prednisolone on days 1-5, which is more than adequate to treat AI (see table 5.) However, in case of undiagnosed AI, this patient may run into problems after day 5. This is particularly worrisome if PAI due to lymphomatous destruction of adrenal tissue is accompanied by TAI due to hypothalamus-pituitary-adrenal (HPA) axis suppression as a consequence of preceding high-dose corticosteroid therapy.

APPROXIMATE RELATIVE POTENCY

Compound (tablet strength, mg)	Anti-inflammatory (glucocorticoid) effect	Sodium-retaining (mineralocorticoid) effect	Equivalent[a] dosage (for anti-inflammatory effect, mg)[b]
Cortisone (25)	0.8	1.0	25
Hydrocortisone (20)	1.0	1.0	20
Prednisolone (5)	4	0.8	5
Methylprednisolone (4)	5	Minimal	4
Triamcinolone (4)	5	None	4
Dexamethasone (0.5)	30	Minimal	0.75
Betamethasone (0.5)	30	Negligible	0.75
Fludrocortisone (0.1)	15	150	Irrelevant
Aldosterone (none)	None	500[c]	Irrelevant

[a]Note that these equivalents are in approximate inverse accord with the tablet strengths.
[b]The doses in the final column are in the lower range of those that may cause suppression of the hypothalamic–pituitary–adrenocortical axis when given daily continuously. Much higher doses, e.g. prednisolone 40 mg, can be given on alternate days or daily for up to 5 days without causing clinically significant suppression.
[c]Injected.

Table 5: Relative potencies of steroids
adopted from https://basicmedicalkey.com/adrenal-corticosteroids-antagonists-corticotropin/

2. Lack of standardized Imaging and staging criteria

Except for two PAL patients with a marginal zone lymphoma subtype, 95/97 (97%) of the cases in our study are regarded as FDG-avid lymphomas. Current guidelines recommend the use of FDG-PET scanning for the staging and assessment of treatment response in such lymphomas. However, in our retrospective study, PET-CT was only applied in 18/81 patients with available radiological findings. We acknowledge that our results regarding treatment response and survival may have been slightly different if PET-CT had been used as standard imaging technique, and if the modern Lugano instead of conventional RECIST response criteria had been applied (Table 6).

For example, a patient with FDG-avid PAL whose CT scan after chemotherapy shows a >50% reduction in the perpendicular diameter of an adrenal mass will be regarded as having a partial response according to conventional criteria. However, according to the PET-CT-based Lugano criteria, the same condition can be classified as a complete response if the FDG uptake, assessed by a 5-point-score, is in the range of 1-3.

Modality	Complete Response	Partial Response	Stable Disease	Progressive Disease
CT	Lymph nodes ≤1,5 cm in Ldi Complete disappearance of radiologic evidence of disease	Single lesion: ↓ ≥ 50% in PPD Multiple lesions: ↓ ≥ 50% in SPD of up to six lymph nodes or extranodal sites	↓ ≤ 50% in SPD of up to six lymph nodes or extranodal sites (no criteria for progressive disease are met)	1) New lymphadenopathy or ↑; single node must be abnormal with: a) Ldi > 1.5 cm and b) PPD ≥ 50% and c) Ldi or Sdi ↑ 0.5 cm if ≤ 2.0 cm and ↑ 1.0 cm if > 2.0 cm 2) ↑ splenic volume: a) with prior splenomegaly: ↑ > 50% of its prior ↑ beyond baseline b) without prior splenomegaly: ↑ > 2.0 cm c) New or recurrent splenomegaly 3) New or larger nonmeasured lesions 4) Recurrent previously resolved lesions 5) New extranodal lesion > 1.0 cm in any axis (new lesions < 1.0 cm in any axis are included if attributable to lymphoma) 6) A new node > 1.5 cm in any axis
FDG PET-CT	Scores 1, 2 ,3 in nodal or extranodal sites with or without a residual mass	Scores 4 or 5 with ↓ uptake compared with baseline and residual mass(es)	Scores 4 or 5 with no obvious change in FDG uptake	Scores 4 or 5 in any lesion with ↑ uptake from baseline and/or New FDG-avid foci

Ldi = longest transverse diameter; Sdi = shortest transverse diameter; PPD = product of perpendicular diameters; SPD = sum of the product of the perpendicular diameters of multiple lesions; ↑ = increase; ↓ = decrease

Table 6. Adopted from Vitor Vita Ricci, Radiopaedia.org, rID: 45845

3. Lack of systematic molecular pathology study

In accordance with other ENLs such as primary central nervous lymphoma (PCNL) and primary testicular lymphoma (PTL), DLBCL was the most common histopathological subtype in our PAL cohort. As expected in a multicenter retrospective study, there was considerable inter-institutional heterogeneity regarding molecular pathological diagnostics. However, a general finding was that certain molecular tests like those ruling out *double hit* lymphoma were missing in most of the patients, despite their therapeutic relevance.

Double hit / double expressing lymphomas (DHL/DEL) are an aggressive variant of B-cell lymphoma with poor prognosis, characterized by genetic aberrations in MYC, BCL2 and/or BCL6 genes or an alteration of the respective protein expression. These lymphomas have a tendency for extranodal manifestation. DHLs are predominantly of the germinal center B-cell (GCB) subtype, whereas DELs can belong to the GCB or the activated B-cell (ABC) subtype (Figure 6). DHL/DEL comprise about 15% of DLBCLs and 75% of Burkitt lymphomas (BL). According to the WHO 2016 classification of lymphomas, DHL/DEL cases are called *"high grade B-cell lymphoma (HGBL) with rearrangements of MYC and BCL2 and/or BCL6"*. It has been shown that patients with DHL/DEL who fail to respond to first-line therapy rarely achieve disease control with subsequent standard therapies and are thus candidates for participation in clinical trials. Therefore, testing for DHL/DEL at diagnosis is recommended for all DLBCLs and BLs because patients with a DHL/DEL should be treated according to an intensified immuno-chemotherapy protocol (dose-adjusted R-EPOCH) instead of receiving the standard R-CHOP regimen as first line therapy. Furthermore, patients with DHL/DEL are at increased risk of developing CNS involvement, and should thus have a high-dose methotrexate component in their treatment regimen (Landsburg and Schuster 2016; Friedberg 2017; Rosenthal and Younes 2017). Our retrospective study showed that, due to lack of molecular analyses, such oncological reasoning was not applicable to the PAL patients under consideration.

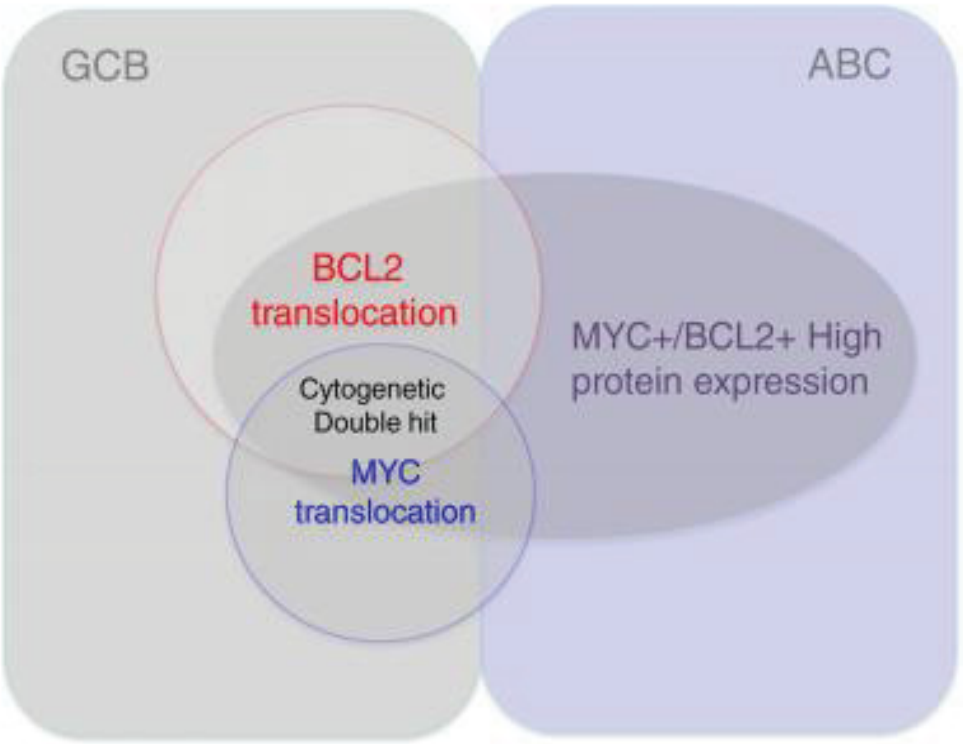

Figure 6: Cell of origin in double hit and double expressing lymphomas
(adopted from Rosenthal & Younes , Blood Rev, 2017)

Concluding remarks and future perspective

We showed for the first time that PAL is a heterogeneous disease with two subtypes including isolated primary adrenal lymphoma (iPAL) and primary adrenal lymphoma with additional extra-adrenal manifestations (PAL+). Unexpectedly, iPAL showed an unusual preponderance for females, less B-symptoms and significantly worse clinical outcome (Majidi et al. 2020). We acknowledge that due to the retrospective nature of our study and the limitations regarding standardized diagnosis and treatment, our distinction between iPAL and PAL+ should be confirmed by analyzing independent patient cohorts in the future. The current management of PAL leaves room for improvement. We should like to emphasize the importance of careful endocrine assessment and multidisciplinary care when treating patients with this rare disease. Our study confirmed that the histopathological subtype in the majority of PAL cases (76%) is DLBCL. The result of three whole exome sequencing projects revealed that DLBCL in turn consists of several prognostically relevant clusters, which are summarized in Table 7 (Chapuy et al. 2018; Schmitz et al. 2018; Lacy et al. 2020). Chapuy et al. also evaluated the genetic landscape of three primary extranodal lymphomas, including primary testicular lymphoma (PTL), primary central nervous system lymphoma (PCNSL), and primary mediastinal B cell lymphoma (PMBL). They compared these entities with nodal DLBCL and identified unique combinations of genetic features in discrete DLBCL subtypes. PTLs and PCNLs showed more pronounced genomic instability in comparison to nodal DLBCL and PMBL. In addition, there was frequent biallelic loss of CDKN2A, frequent MYD88 mutations, frequent NFKBIZ amplification indicating oncogenic Toll-like receptor signaling, and more frequent deregulation of BCL6. Furthermore, PCNSLs, PTLs, and PMBLs exhibited genetic alterations and overexpression of PD-L1 and PD-L2, whereas nodal DLBCLs rarely showed these features (Chapuy et al. 2016). According to all these studies, mutations of MYD88 and CD79B play a substantial role in lymphomagenesis with extra-nodal manifestation and lead to poor prognosis. The studies performed on more common types of lymphoma have provided great reference datasets, which can help to better characterize the mutational landscape of PAL. Chen et al evaluated the frequency of MYD88 and CD79B in 29 PAL patients with a DLBCL subtype, of which 24% showed a MYD88 mutation and 52% harbored a CD79B mutation (Chen et al. 2020). These findings need to be confirmed in a larger cohort with an expanded panel including the PIM, ETV and CDKN2A genes, which are of clinical relevance in ENLs.

Lacy et al. 2020	Chapuy et al. 2018	Schmitz et al. 2018	
MYD88 MYD88, PIM1, CD79B, ETV6, CDKN2A	**C5** CD79B, MYD88, ETV6, PIM1, TBL1XR1	**MCD** MYD88, CD79B	Strongly associated with ABC-type DLBCL. The most robust group, occurring in all reports. Contains the majority of cases with PCNSL and primary testicular lymphoma. Associated with a poor prognosis
BCL2 EZH2, BCL2, CREBBP, TNFRSF14, KMT2D	**C3** BCL2, CREBBP2, EZH2, KMT2D, TNFRSF14	**EZB** BCL2 translocation, EZH2	Strongly associated with GCB-type DLBCL. Mutational profile is shared with follicular lymphoma. Contains the majority of cases of transformed follicular lymphoma and cases with a concurrent diagnosis of follicular lymphoma. Generally favorable prognosis, although enriched for cases of double-hit lymphoma and MHG
SOCS1/SGK1 SOCS1, CD83, SGK1, NFKBIA, HIST1H1E	**C4** SGK1, HIST1H1E, NFKBIE, BRAF, CD83		Predominantly GCB-type DLBCL. Shares genetic and gene expression features of PMBCL. Associated with the most favorable prognosis
TET2/SGK1 TET2, BRAF, SGK1, KLHL6, ID3			A less strongly identifiable subtype emerging from SGK1 when applying the Akaike information criterion (supplemental Methods). Has very strong similarity to SOCS1/SGK1 but differentiated by the addition of TET2 and BRAF and the lack of SOCS1 and CD83. Associated with a favorable prognosis
NOTCH2 NOTCH2, BCL10, TNFAIP3, CCND3, SPEN	**C1** BCL6 translocation, BCL10, TNFAIP3, UBE2A, CD70	**BN2** BCL6 translocation, NOTCH2	Not associated with any cell of origin. Shares mutational similarity to MZL but not enriched for cases of transformed MZL. Less strongly defined than other subgroups (supplemental Methods)
NEC NOTCH1, REL amplification, TP53		**Other**	A default category, containing cases that could not be classified elsewhere. Contains cases with no detected mutation. Likely to also contain cases belonging to both NOTCH1 and TP53/CNA subgroups. Even though 3 abnormalities are significantly enriched in this group, their q-values are far less extreme than those of characteristic mutations from the other subtypes
	C2 TP53, frequent deletions		Characterized by TP53 mutation and widespread copy number changes. Due to limited CNA in our study, these cases were predominantly allocated to the NEC group
	C0 No detected abnormalities		Cases with no detectable mutation were allocated to the NEC group
		N1 **NOTCH1**	Characterized by NOTCH1 mutation, this was significantly elevated in our NEC group but only mutated in 2.5% of samples. Associated with poor outcome

Table 7. Comparison of main genetic clusters in DLBCL
(adopted and modified from Lacy et al, Blood 2020)

Sophisticated molecular characterization of PAL biopsies, careful lymphoma staging using PET-CT, identification of prognostic biomarkers, and development of a suitable staging system for PAL will contribute to improved oncological management of patients with PAL and will also help to determine whether our distinction between iPAL and PAL+ is of sustained clinical relevance. In order to optimize the clinical care of patients with PAL, it is also necessary to remedy the current defects in endocrinological assessment and provide adequate corticosteroid replacement therapy if needed. Good clinical management of PAL requires a truly multidisciplinary effort.

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Acknowledgment

First and foremost, I am extremely grateful to my supervisor, Prof. Dr. Norbert Gattermann, whose immense knowledge and neverending support encouraged me to continue my difficult journey and keep pursuing my goals.

I also appreciate the kind support that I received from my co-supervisor Prof. Dr. Matthias Schott.

Finally, I wish to thank my colleagues all over the world who contributed data to this study. Their active collaboration was pivotal for the completion of the project.