National University of Athens, Greece

Systemic Autoimmune Diseases towards Stratified and Precision Medicine. The paradigm of Sjogren's Syndrome

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Disclosures

- Received Research Grants from Novartis, Pfizer, ABBVIE, Genesis, Eli-Lilly. NONE related to the current presentation
- Coordinator of HarmonicSS, an EU sponsored Research Grant
- Chairman of eSSential, the Study Group of EULAR, devoted to Sjögren's syndrome

Lecture Outline

- Sjogren's Disease. Clinical phenotyping based on harmonized integrated data
- NHL in Sjogren's Disease
- Predictors of Lymphoma development. Historical perspectives
- Predictors of Lymphoma development. Current status
- Conclusions-Work in progress

SJÖGREN SYNDROME

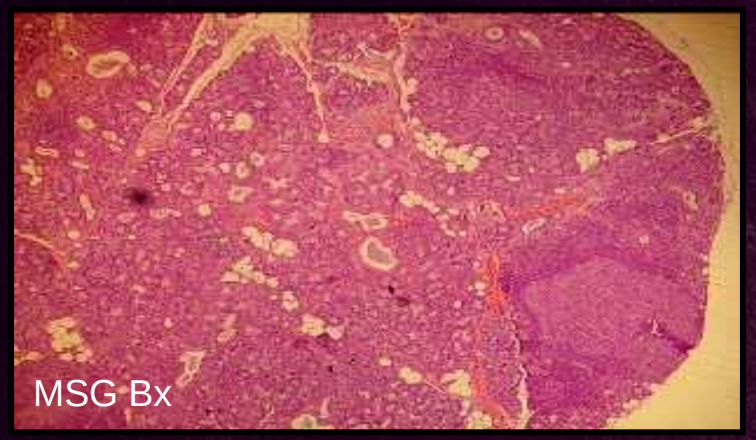
- Female disease
  ♀/♂ :20:1
- Common disease
  0,1-0,5 % prevalence
- 4th -5th decade of life
- Slowly progressive and difficult to treat
- Primary or associated with:
  - ❑ RA
  - ❑ SLE
  - ❑ Dermatopolymyositis
  - ❑ Scleroderma

2nd most common rheumatic disease

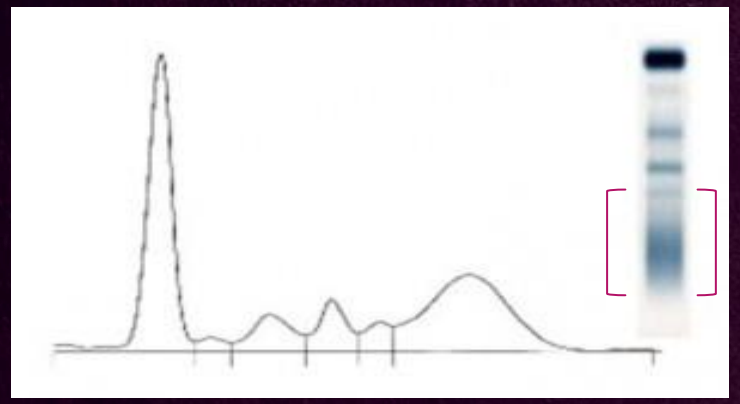
2nd most common autoimmune disease affecting women

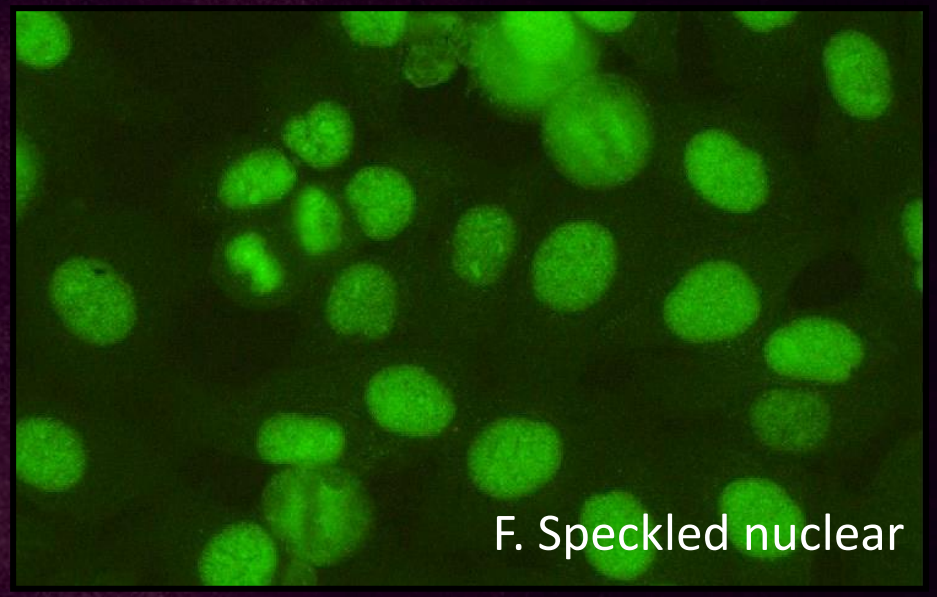
E. Pelechas et al., Illustrated Handbook of Rheumatic and Musculo-Skeletal Diseases

✓ Sjögren's Syndrome: Immunopathology

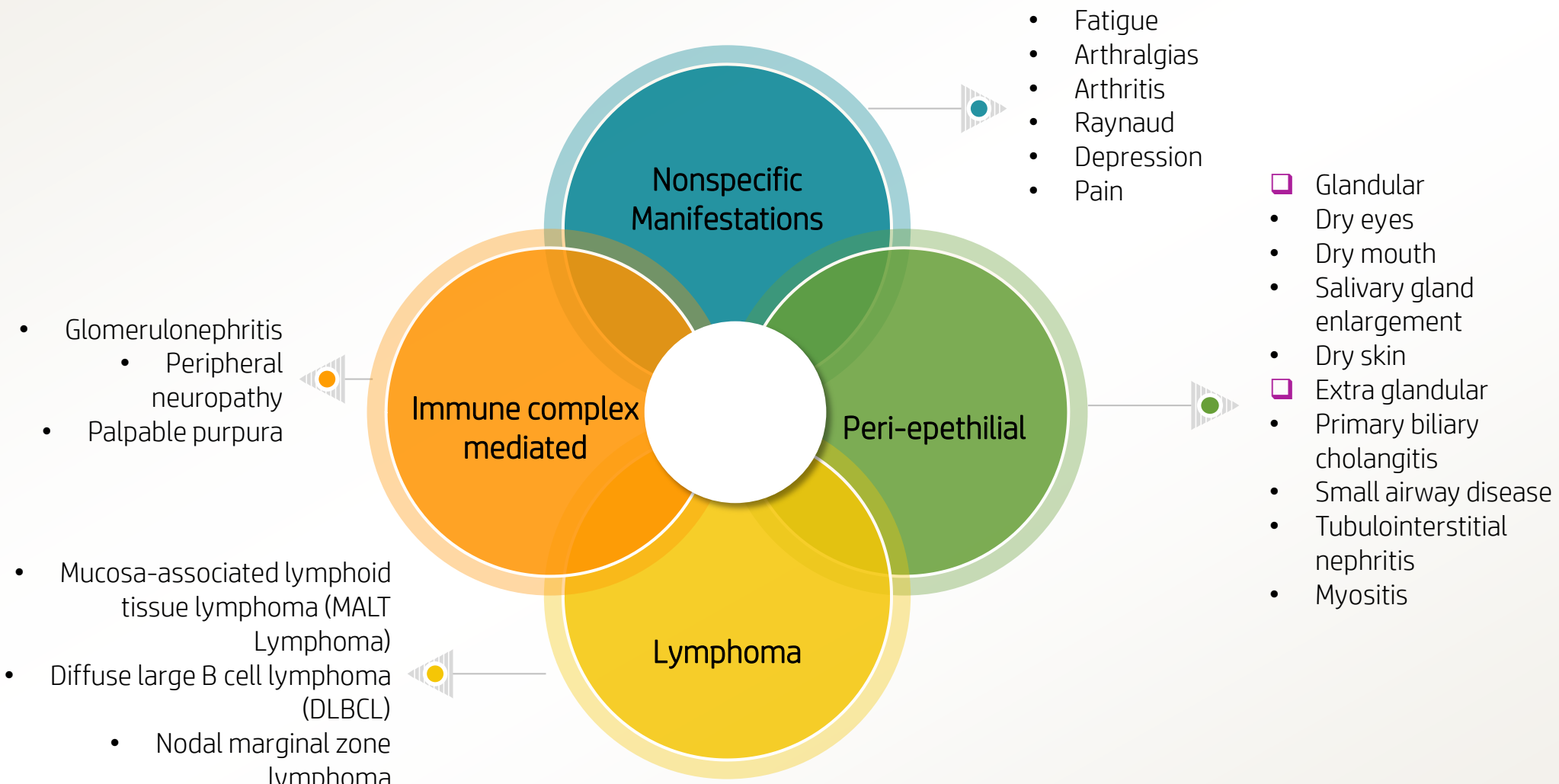


Infiltration by activated T- & B- cells





Sjögren's Disease
Clinical diversity



Mavragani et al. CMAJ 2014

Sjögren's Disease: *Classification Criteria*

	Item	Weight / Score
01	Labial salivary gland with focal lymphocytic sialadenitis and focus score ≥ 1	3
02	Anti-SSA (Ro) +	3
03	Ocular staining score ≥ 5 (or van Bijsterfeld score ≥ 4) on at least one eye	1
04	Schirmer ≤ 5 mm/5min on at least one eye	1
05	Unstimulated whole saliva flow rate ≤ 0.1 ml/min	1

A patient is classified as having SS when she has a score ≥ 4

Caroline H. Shiboski et al. 2016 ACR-EULAR Classification Criteria for primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts, Arthritis Rheumatol. , 2017

✓ The consortium

Cohorts

HarmonicSS Services and Tools

- Cloud infrastructure
- Semantic interlinking
- Harmonization
- Data Governance
- External information source retrieval
- Text mining
- Big data mining
- Genetics analytics
- Social media analytics
- Health policy impact assessment
- Visual analytics
- Clinical trial patient selection
- Segmentation of imaging tests
- Training/Education

Outcomes

- Network of partners
- Legal and privacy report on data sharing
- Integrative harmonized cohort
- Improved stratification for patient management
- Validation of existing biomarkers
- Identification of novel biomarkers
- Shared health policy
- Sustainability and expandability plan

How it was achieved

FEATURE	%, (1/n)
GLANDULAR MANIFESTATIONS	
Dry mouth	89,7 (6101/6800)
Dry eyes	89,0 (6046/6794)
Salivary gland enlargement	37,4 (1897/5083)
Parotid gland swelling	42,0 (2227/3843)
Submandibular swelling	5,0 (139/2787)
NON-SPECIFIC MANIFESTATIONS	
Raynauds phenomenon	26,5 (1577/5942)
Fatigue	54,0 (2840/5256)
Arthritis	16,6 (993/5999)
EXTRAGLANDULAR MANIFESTATIONS	
Renal disease	2,6 (162/6183)
Tubulointerstitial nephritis	1,4 (66/4729)
Glomerulopathy	0,9 (37/4330)
Pulmonary disease	7,1 (415/5466)
Small airway disease	3,0 (157/5169)
Interstitial lung disease	2,5 (112/4414)
Liver disease	2,4 (131/5431)
Autoimmune hepatitis	0,85 (40/4722)
Primary biliary cirrhosis	1,4 (81/5616)
Nervous System Disease	9,1 (560/6137)
Peripheral nervous disease	5,4 (267/4979)
Central nervous disease	2,7 (125/4612)
Palpable purpura	7,0 (396/5653)
Muscular System Disease	7,2 (357/4990)
Inflammatory myopathies	0,3 (10/3852)
Inclusion body myositis	3,3 (150/4579)

SEROLOGY	
Anti Ro/SSA	72,8 (4564/6268)
Anti La/SSB	43,2 (2670//6169)
Rheumatoid Factor	49,1 (2282/4644)
Antinuclear Antibodies	79,9 (4354/5450)
Low C_4 levels	59,0 (2485/4210)
Cryoglobulinemia	5,7 (266/4675)
LYMPHOMA	
Any type of Lymphoma	5,7 (354/6007)
MALT lymphoma	4,4 (245/5569)
DLBCL lymphoma	0,8 (45/ 5371)

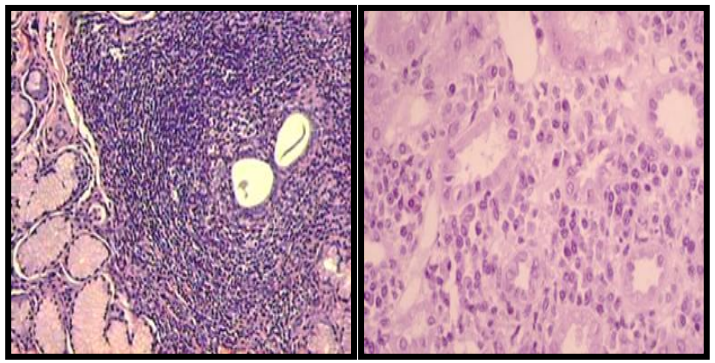
HarmonicSS

A Goules et al in preparation

Sjögren's Disease: *Clinical Manifestations*

Peri-epithelial

- Appear early in the course of the disease
- Remain stable for many years
- Low frequency of terminal organ damage



Extra-epithelial

- Late clinical sequel
- Severe organ damage if untreated
- Predictive factors for non-Hodgkin lymphoma development

Sjogren 18.8*

Lupus 7.4*

RA 3.9*

*Standardized Incidence Ratios

Zinzaras E, et al. Arch Inter Med 2005

Mortality in Sjogren's Disease with or without lymphoma

Outcome	SS patients with Lymphoma (53)	SS patients without Lymphoma (531)
Observed/Expected deaths	6/1.84	41/37.89
SMR (exact 95% CI)	3.25 (1.32 to 6.76)	1.08 (0.79 to 1.45)
Follow up, person years	556	1912
Excess Deaths due to Lymphoma	1.58 /1000 person-years	

Theander Ann Rheum Dis. 2006

✓ Precision medicine

VS Traditional Medicine

One-size-fits-all medicine

Stratified medicine

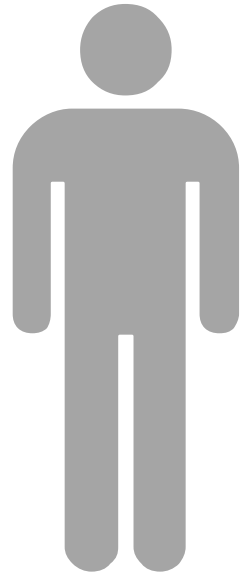
Precision medicine

Stratification

Patients are grouped by:
Disease Subtype
Demographics
Clinical features
Biomarkers

Personalisation

Patients individual:
Preferences,
Clinical features
Medication history
Environment
Behaviours & habits
Biomarker



Precision Medicine

Important prerequisites to achieve meaningful approaches towards Precision Medicine

- Correct clinical phenotyping
- Understanding of pathogenetic mechanisms underlying each clinical phenotype
- High-end –omics technologies
- Identification of clinically relevant biomarkers

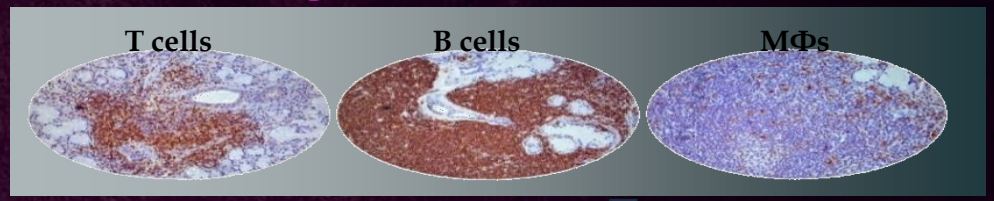
Sjögren's Disease

A conceptual step-wise categorization

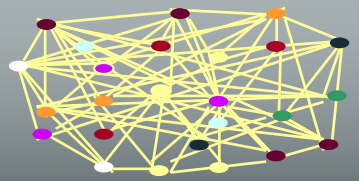
Clinical stratification

CLINICAL PHENOTYPING

Histologic stratification



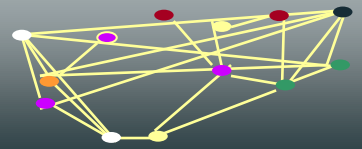
System biology approach



Candidate Biomarkers

- Preliminary
- Clinical utility
- Sensitivity

Molecular stratification



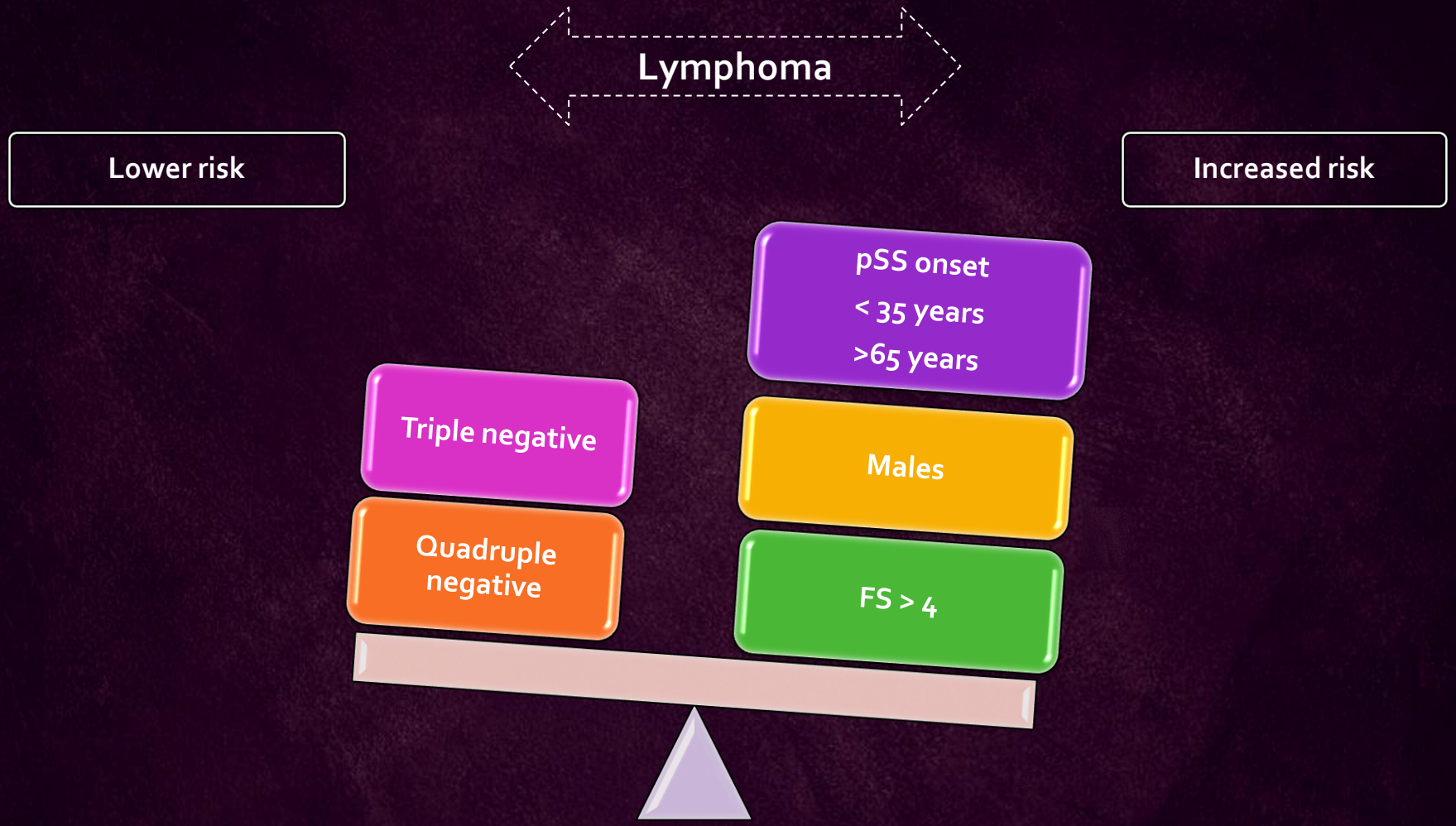
Homogenous patient groups for targeted therapy

Current perspectives in Clinical phenotyping

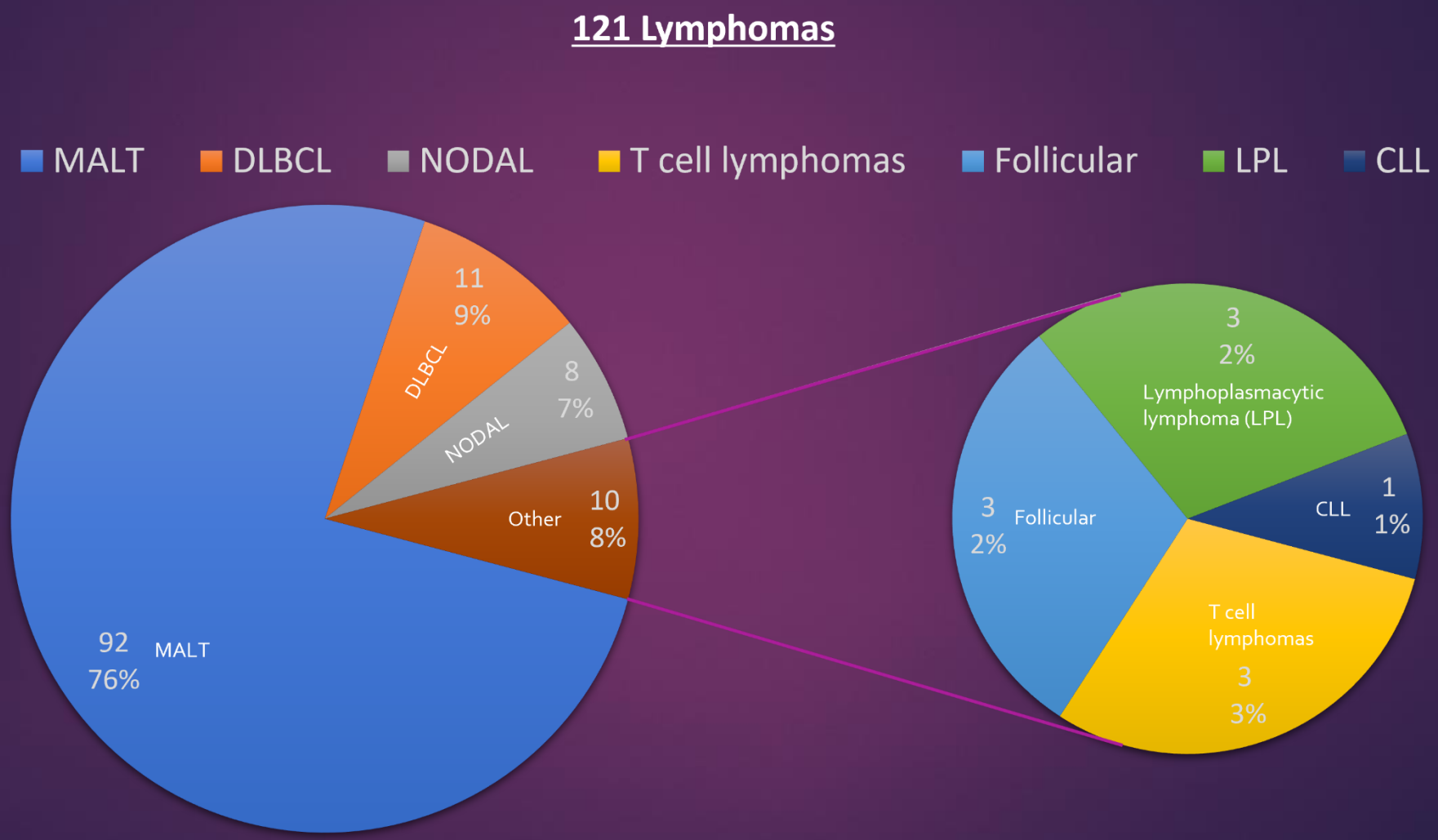
- SS in Male patients
- SS in patients with early and late disease onset
- The predictive role of minor salivary gland Bx
- Cryoglobulinemic vasculitis
- Sicca negative SS
- Autoantibody negative SS
- Non-Hodgkin's lymphoma

Chatzis et al J Clin Med 2019
Chatzis et al Frontiers Immunol 2020
Argyropoulou et al Sem Arthritis Rheum 2020
Goules et al J Autoimmunity 2020
Chatzis et al Clin Exp Rheum 2021
Chatzis et al Clin Exp Rheum 2022

✓ *Sjögren's Disease: different Sjogren's Disease groups display different risk for lymphoma development*

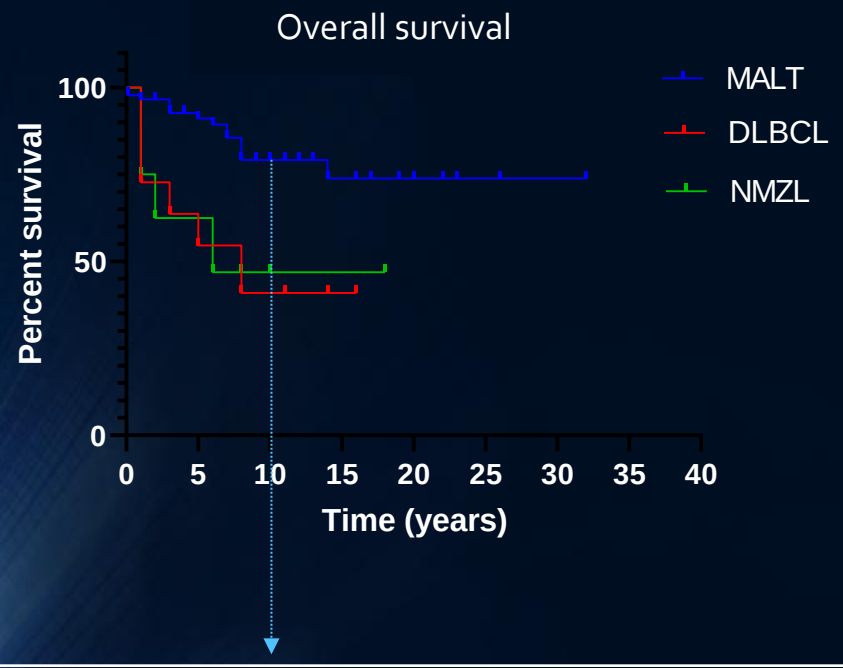


Sjögren's Disease Associated Lymphomas (NKUA cohort)

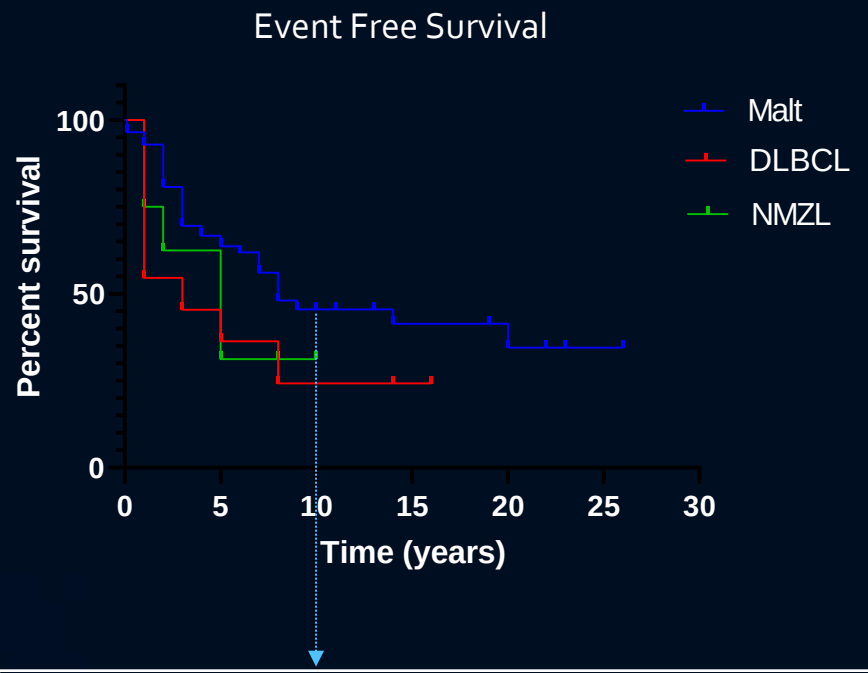


OR and EFR Survival curves

Event
- Disease progression
- Lymphoma relapse
- Histologic transformation
- Starting treatment after a watch and wait approach
- Death from any cause

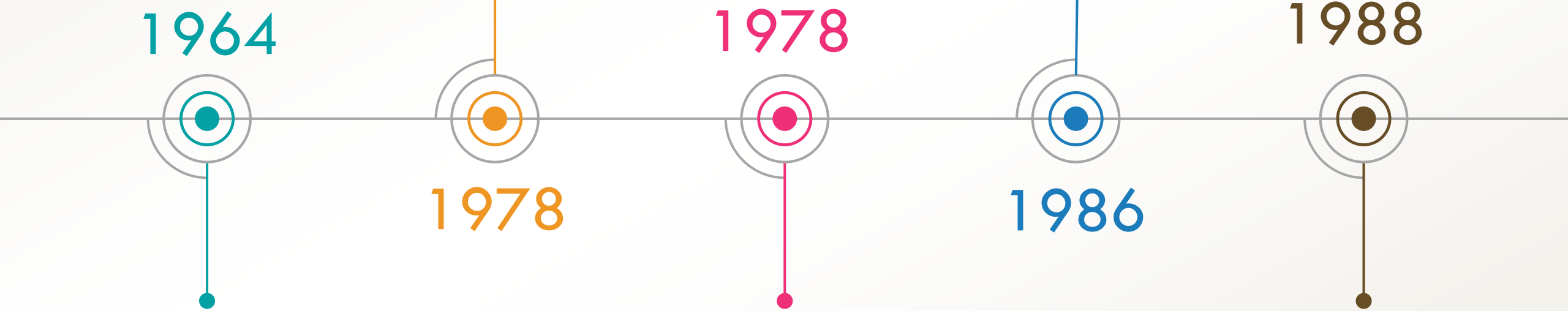


10-year OS	Malt	DLBCL	NMZL
	79.140	40.909	46.875



10-year EFS	Malt	DLBCL	NMZL
	45.552	24.242	31.250

Historical perspectives



Historical perspectives

1996

Tzioufas et al proposed that mixed monoclonal cryoglobulinemia may serve as a predictive factor for lymphoma development

2000

Skopouli et al highlighted the importance of the initial clinical presentation of SjD for the subsequent outcome. Low C4 complement, cryoglobulinemia and purpura were associated with the development of lymphoproliferative disorders

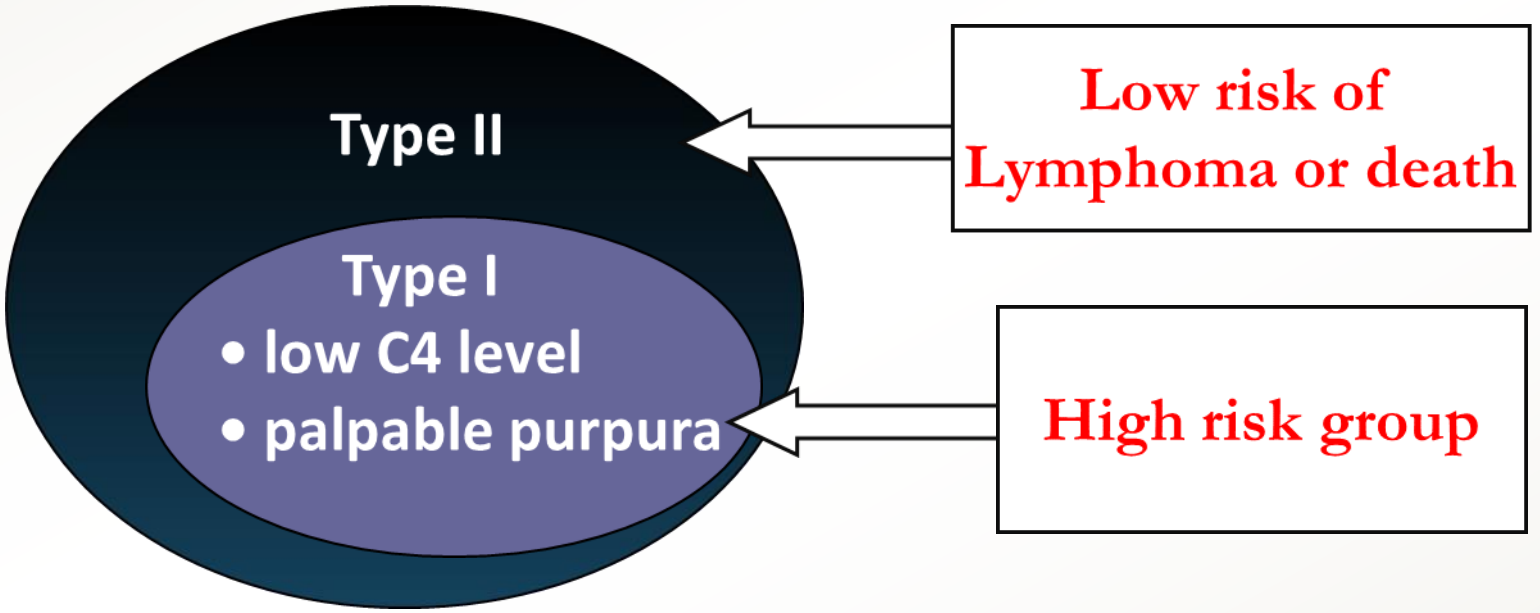
2002

Ioannidis et al. found that the 10-year risk of lymphoma was 4%, while the lifetime risk is estimated to be 5–10%. Palpable purpura and low C4 levels distinguishes high-risk patients from patients with an uncomplicated disease course

2016

Fragkioudaki et al presented a diagnostic scoring tool for NHL development in the context of SS based on the presence of 7 risk factors

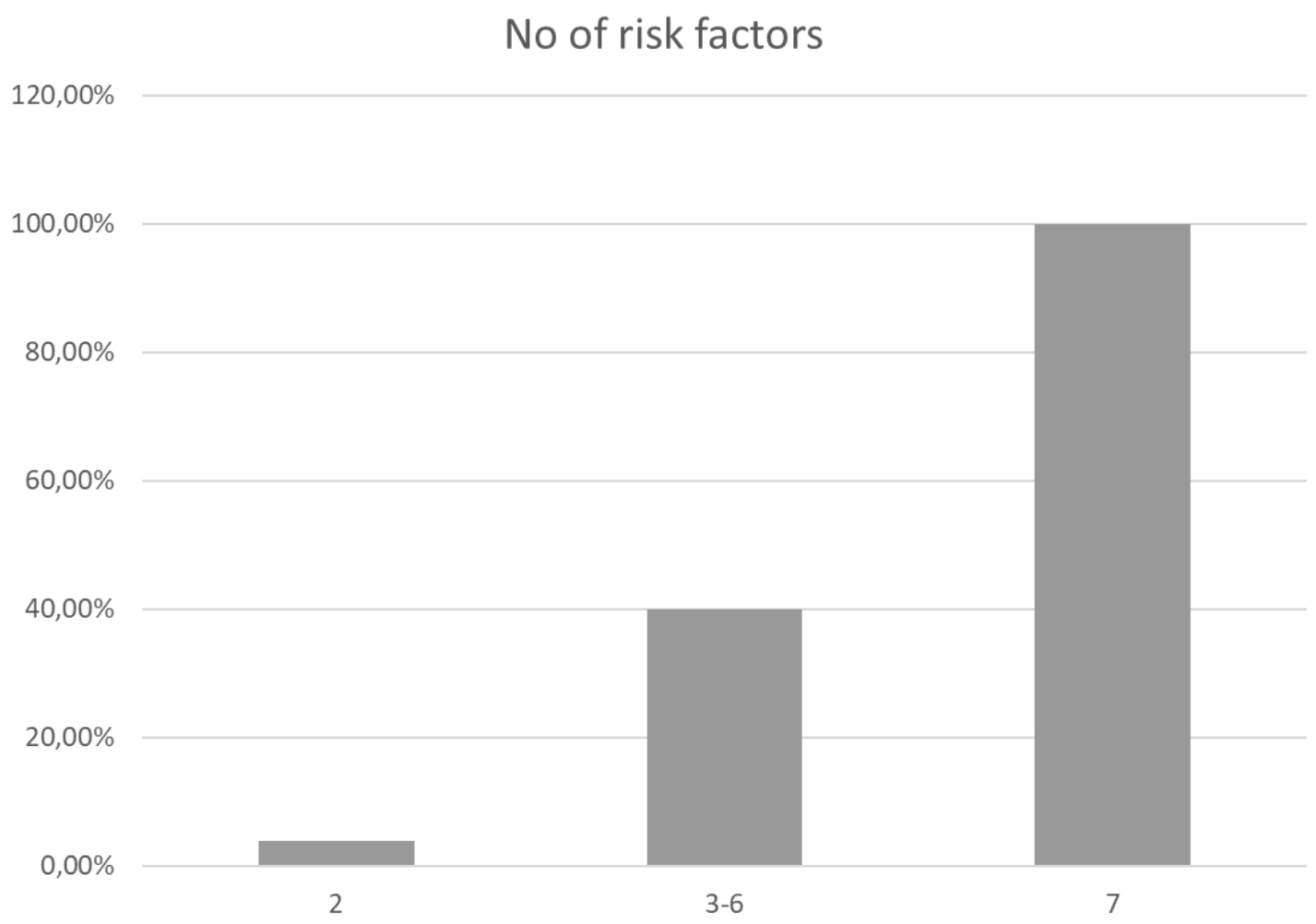
Sjögren's disease
Stratification risk for lymphoma development



Ioannidis JP, et al. Arthritis Rheum 2002

Independent risk factors for lymphoma

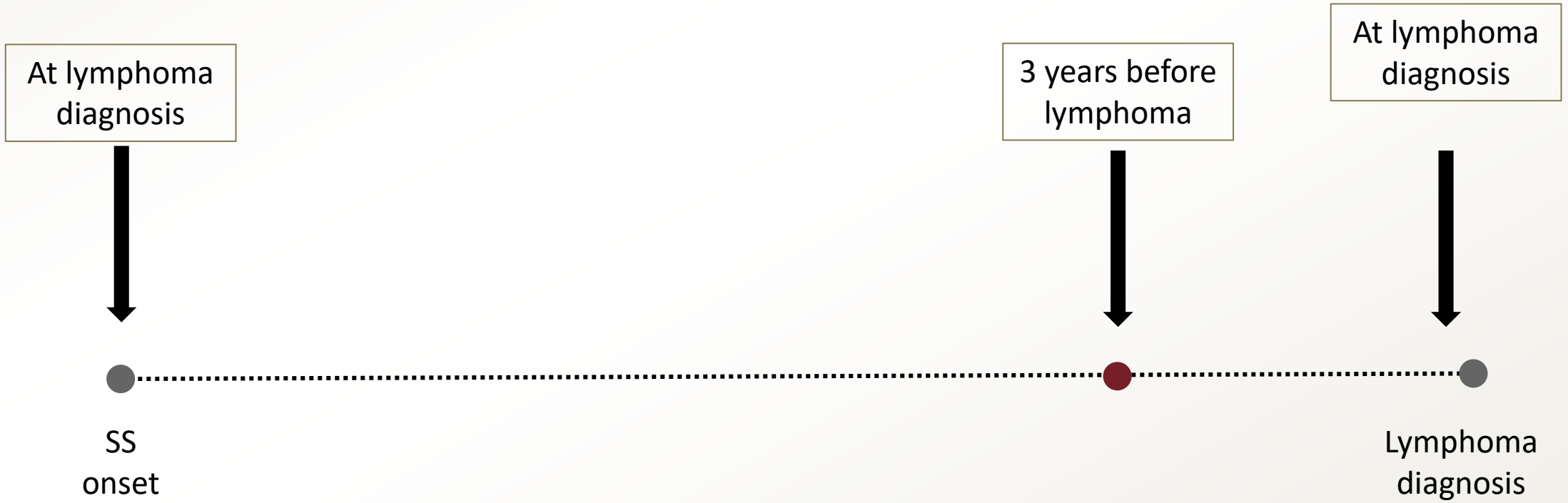
- Salivary gland enlargement
- Lymphadenopathy
- Raynaud's phenomenon
- Anti-Ro/SSA and/or anti-La/SSB positivity
- Rheumatoid factor positivity
- Monoclonal gammopathy
- C4 hypocloplementemia



Fragkioudaki et al. Medicine 2016

Unmet needs

- Previous studies recruited patients with Sjögren's disease and lymphoma of mixed histological types
- Absence of a temporal distance between lymphoma diagnosis and Sjögren's disease diagnosis leads to confusion regarding the real predictors or merely manifestations of lymphoma



Identification and evolution of predictors of Sjögren's disease-associated mucosa-associated lymphoid tissue lymphoma development over time: a case-control study

Andreas V Goules, Loukas Chatzis, Vasilis C Pezoulas, Markos Patsouras, Clio Mavragani, Luca Quartuccio, Chiara Baldini, Salvatore De Vita, Dimitrios I Fotiadis, Athanasios G Tzioufas

THE LANCET Rheumatology

Sjögren's Disease
Lymphomagenesis-Predictors

Study Design

- Three SjD centers: NKUA, Pisa, Udine

- SjD-MALTLs patients with the following criteria were identified: i) MALT lymphoma diagnosis was according to the WHO classification criteria, ii) MALT lymphoma diagnosis was ***> 3 years from SjD diagnosis*** and iii) no other systemic autoimmune disease was present

- Matched in 1:1 ratio, according to age, sex, disease duration from SjD diagnosis to last follow up and treatment modalities

- 3 time points: V1/at the time of SjD diagnosis, V2/3-4 years before lymphoma diagnosis and V3/0.5-1.5 years before lymphoma diagnosis

Goules AV. et al. Lancet Rheum 2024

Domain weights of original ESSDAI

DOMAIN	ESSDAI
Constitutional (0-2)	3
Lymphadenopathy (0-3)	4
Glandular (0-2)	2
Articular (0-3)	2
Cutaneous (0-3)	3
Pulmonary (0-3)	5
Renal (0-3)	5
Muscular (0-3)	6
Peripheral nervous system (0-3)	5
Central nervous system (0-3)	5
Hematological (0-3)	2
Biological (0-2)	1
Range of total score	**0-123**

Sjögren's Disease
Lymphomagenesis-Predictors

Study Design

- Lymphoma patients whose SjD diagnosis was 3-4 years from lymphoma diagnosis were utilized once and were included in the V1 dataset
- 33-34 features/variables for each dataset
- Features with missing values more than 10% during the quality control were excluded from the analysis of each dataset
- Data driven analysis: FCBF/LR and 10-fold cross validation

Goules AV. et al. Lancet Rheum 2024

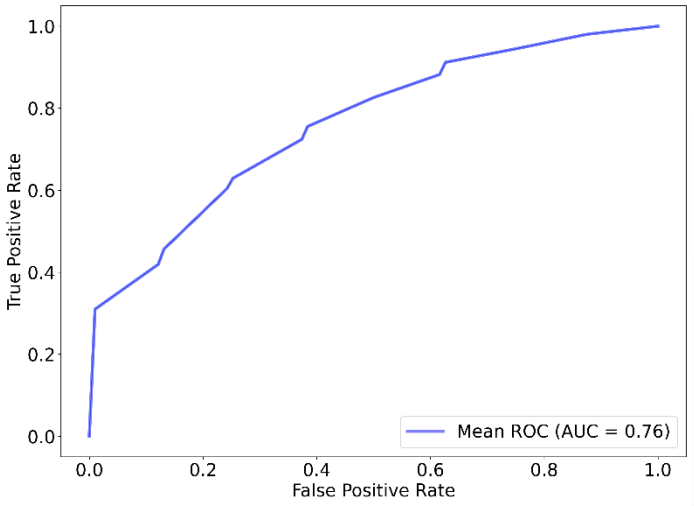
Sjögren's Disease Lymphomagenesis-Predictors

FCBF-based multivariable logistic regression analysis for MALT lymphoma predictors at SjD diagnosis* (V1) (n=80, MALT lymphoma patients with > 3 years from SjD diagnosis)

Prominent feature*	Regression coefficient	Odds ratio	p-value	CI upper	CI low
Rheumatoid Factors**	**1.2**	**3.332**	**<0.001**	**5.644**	**1.967**
Cryoglobulinemia	1.112	3.071	0.193	16.374	0.581
ACA pattern	1.441	4.237	0.084	21.565	0.842
ESSDAI≥5	0.693	2.022	0.072	4.048	1.01
Lacrimal Gland enlargement	0.885	2.438	0.524	37.321	0.163

* 33 Features/Variables analysed by the FCBC algorithm: sex, age at SjD diagnosis, dry mouth, dry eyes, salivary gland swelling, lacrimal gland enlargement, Raynaud's phenomenon, arthritis, arthralgias, palpable purpura, lymphadenopathy, renal disease-glomerulopathy, renal disease-tubulointerstitial nephritis, pulmonary disease-small airways disease, interstitial lung disease, liver disease-autoimmune hepatitis, primary biliary cirrhosis, peripheral nervous system disease, central nervous system disease, autoimmune thyroiditis, ANA, ACA pattern, anti-La antibodies, anti-Ro antibodies, rheumatoid factors, cryoglobulinemia, low C4, anemia, leukopenia, neutropenia, lymphopenia, thrombocytopenia, ESSDAI≥5

** < 0.05 (95% confidence interval)



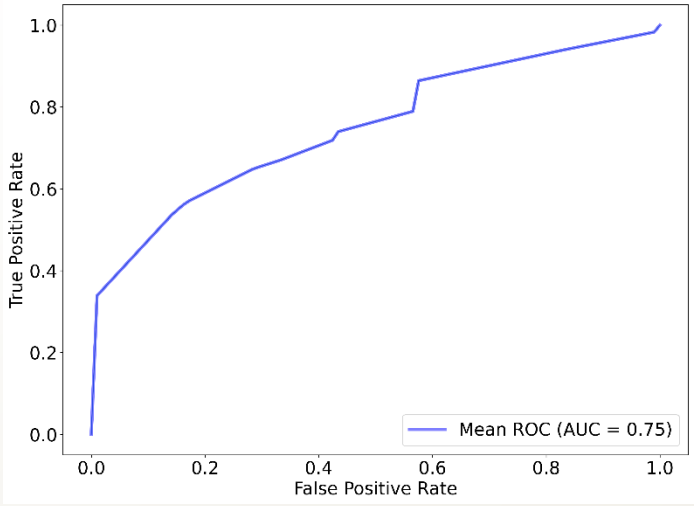
Sensitivity:63%, Specificity 62%

FCBF-based multivariable logistic regression analysis for lymphoma predictors at 3-4 years (V2) before lymphoma diagnosis* (n=68, MALT lymphoma patients with > 3 years from SjD diagnosis)

Prominent feature*	Regression coefficient	Odds ratio	p-value	CI upper	CI low
ESSDAI≥5**	**1.342**	**3.871,**	**0.002**	**8.852,**	**1.694,**
Rheumatoid Factors**	**1.294**	**3.683,**	**<0.001**	**6.467,**	**2.097**
ACA pattern	1.382	3.995	0.107	21.246	0.763

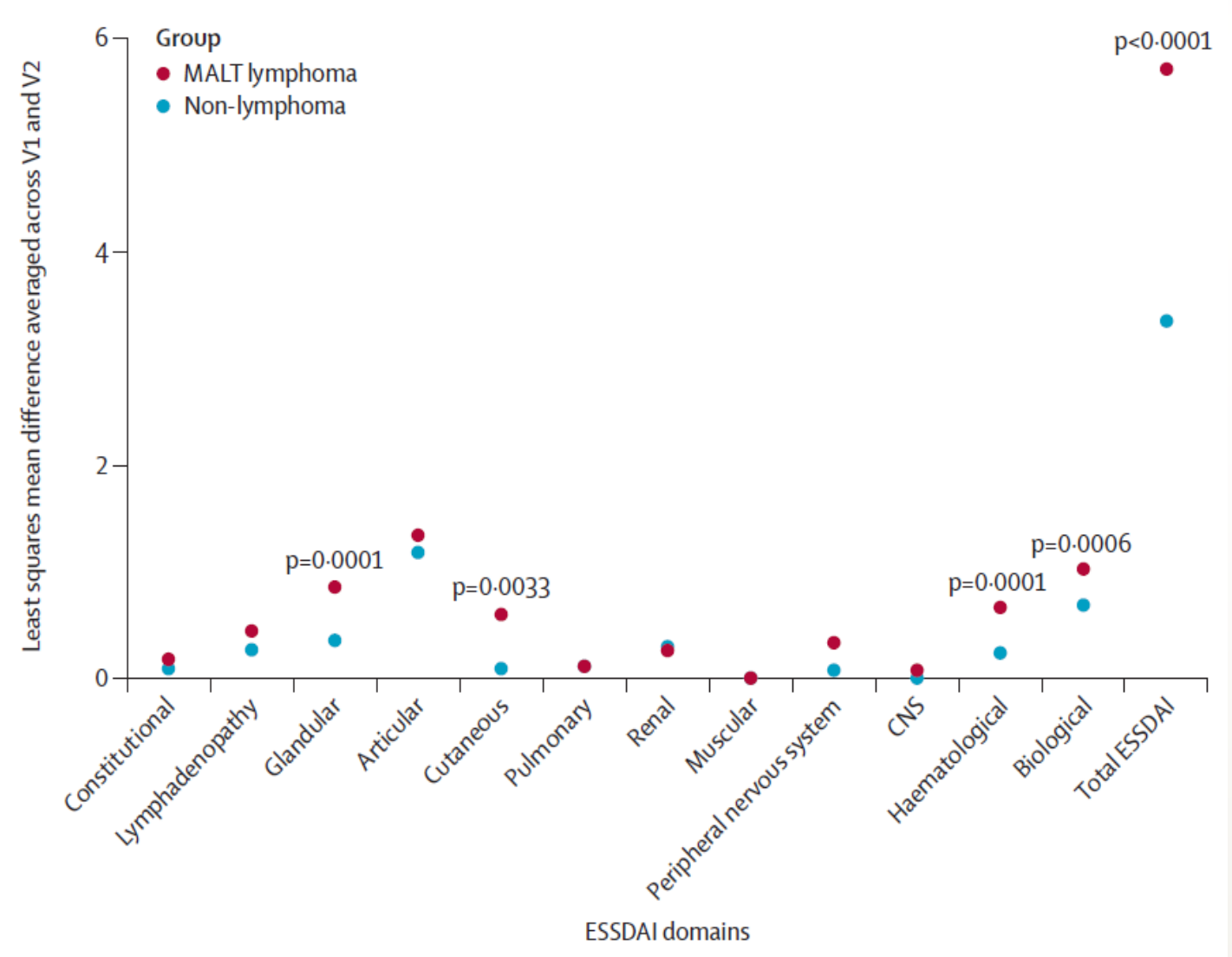
* 34 Features/Variables analysed by the FCBC algorithm: sex, age at SjD diagnosis, disease duration until V2, dry mouth, dry eyes, salivary gland swelling, lacrimal gland enlargement, Raynaud's phenomenon, arthritis, arthralgias, palpable purpura, lymphadenopathy, renal disease-glomerulopathy, renal disease-tubulointerstitial nephritis, pulmonary disease-small airways disease, interstitial lung disease, liver disease-autoimmune hepatitis, primary biliary cirrhosis, peripheral nervous system disease, central nervous system disease, autoimmune thyroiditis, ANA, ACA pattern, anti-La antibodies, anti-Ro antibodies, rheumatoid factors, cryoglobulinemia, low C4, anemia, leukopenia, neutropenia, lymphopenia, thrombocytopenia, ESSDAI≥5

** < 0.05 (95% confidence interval)



Sensitivity:65%, Specificity 71%

The evolution of systemic disease activity as a MALT lymphoma predictor across V1 and V2



Sjögren's Disease and Predictors of Lymphoma-Concluding remarks

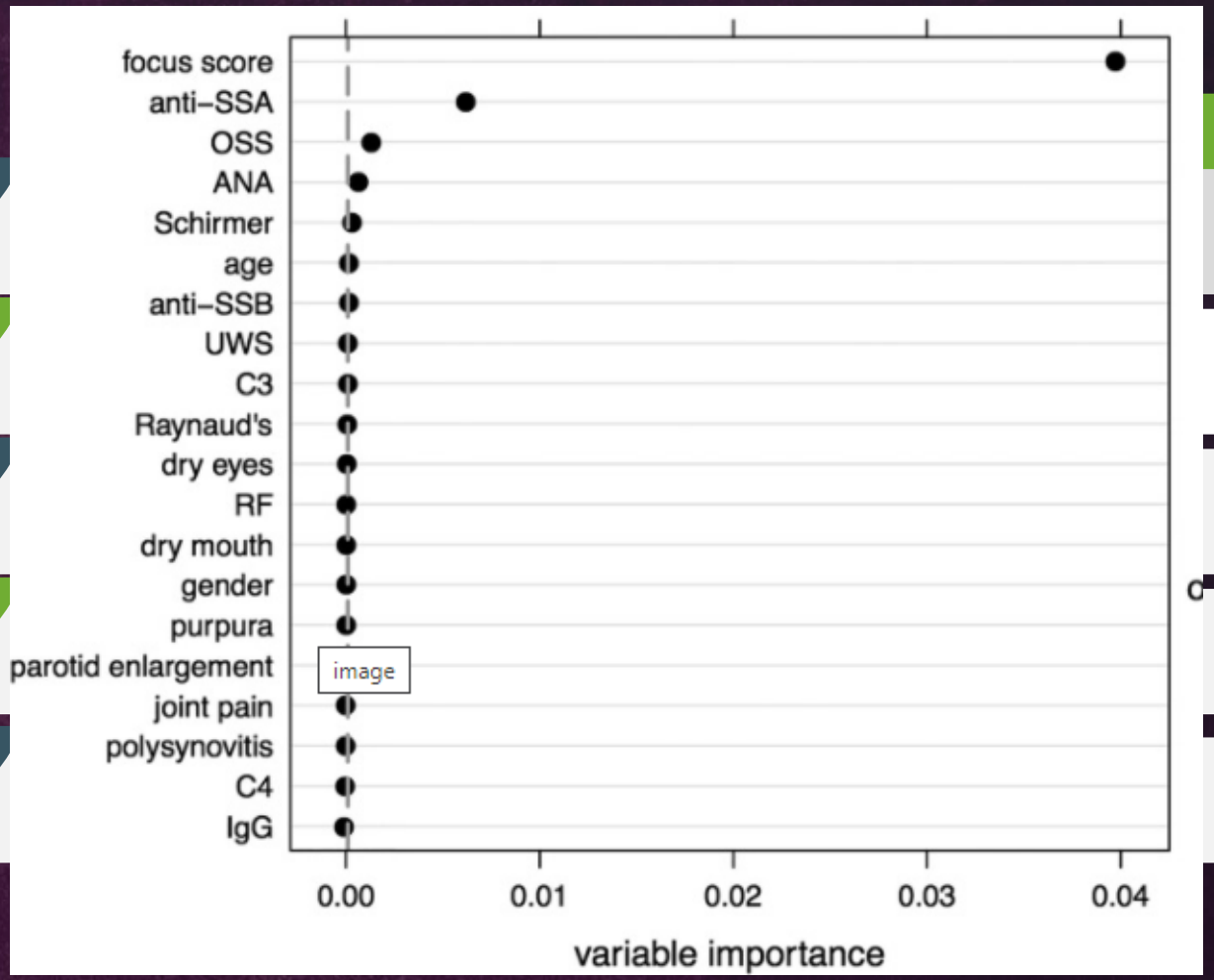
- RFs are the most persistent and chronically distant MALTL predictor
- The addition of cutaneous, glandular, hematologic and biologic manifestations define a definite time point of lymphomagenesis
- Cryoglobulinemia and salivary gland enlargement are findings of underlying or future lymphoproliferative disorder

Prognostic

SS

Diagnostic

Guide patients follow up



01

02

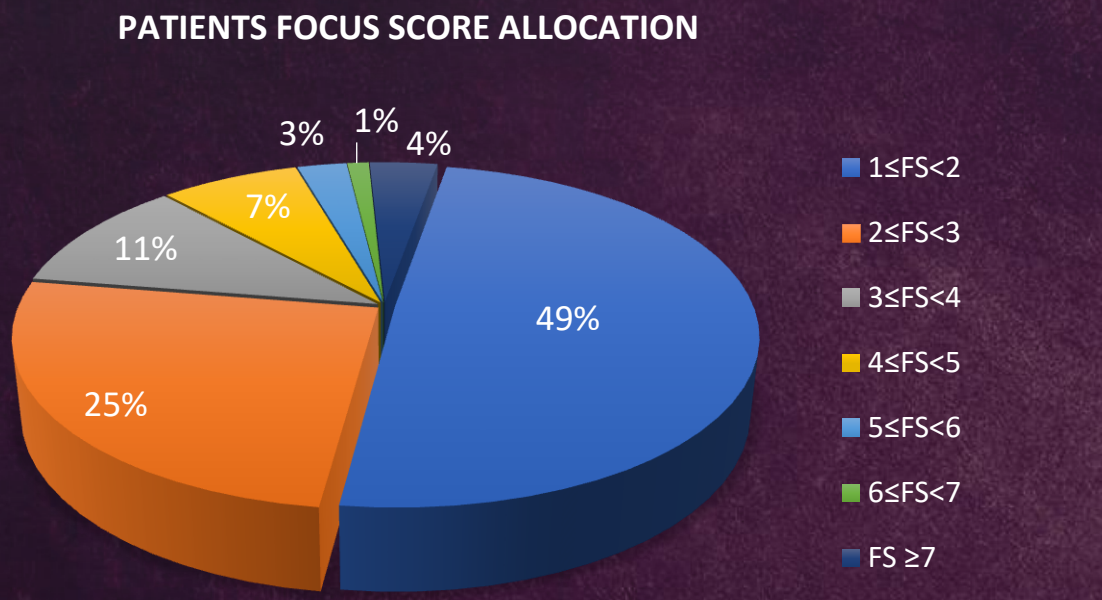
03

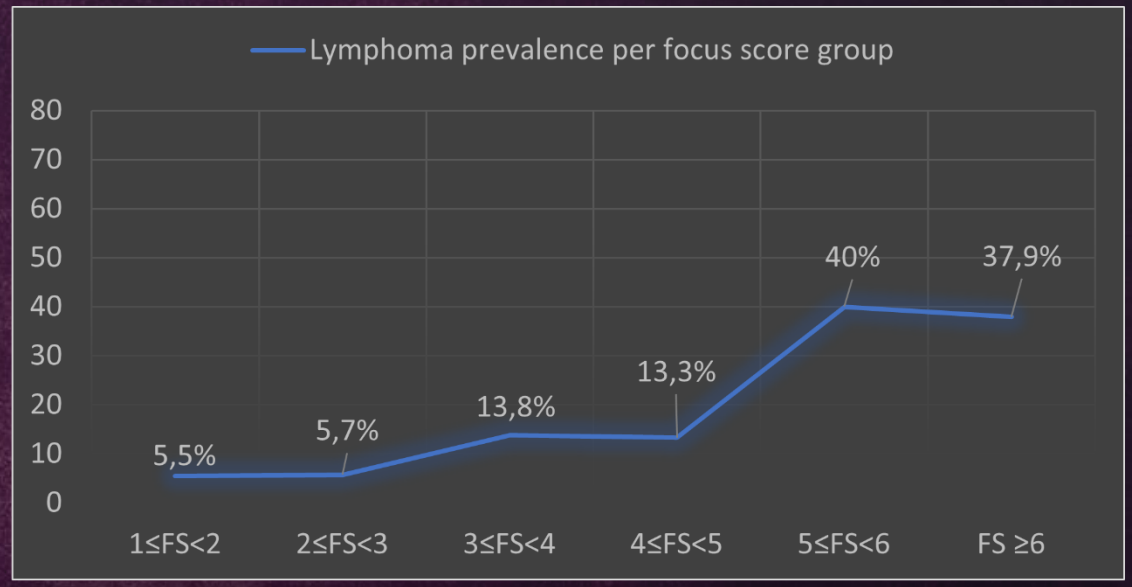
04

05

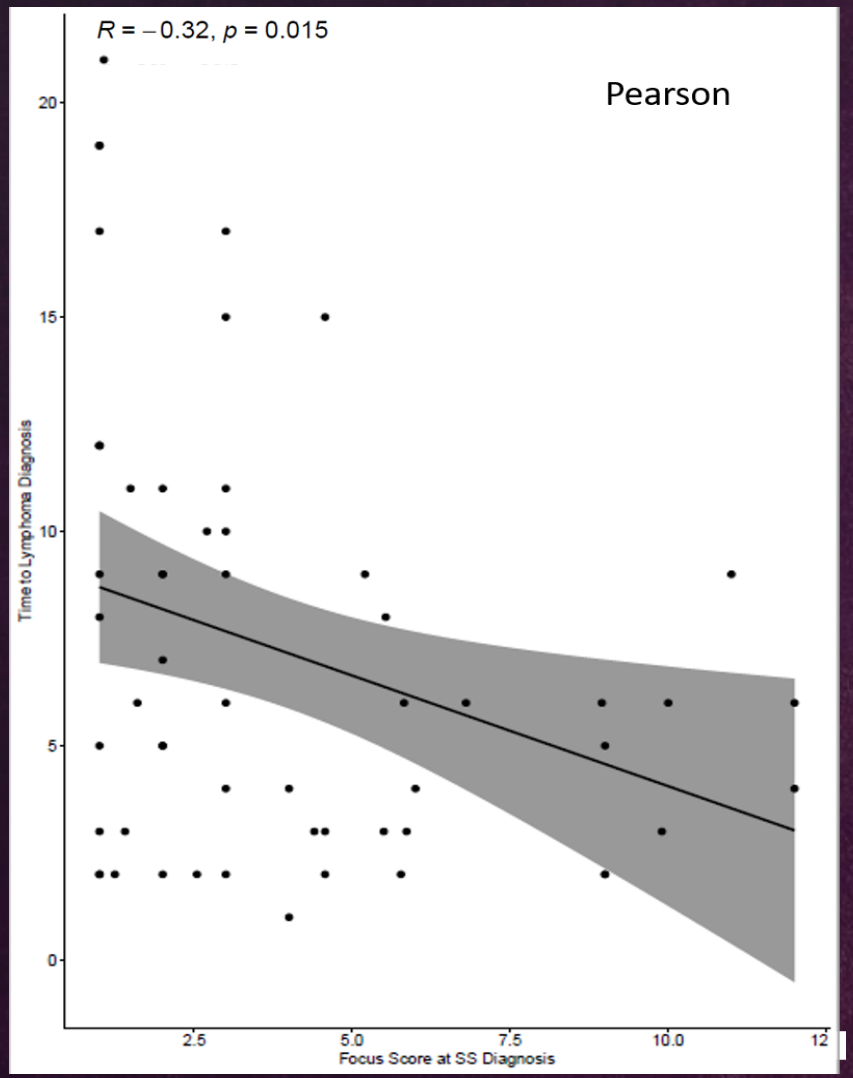
Caroline H. Shiboski et al. 2016 ACR-EULAR Classification Criteria for primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts, Arthritis Rheumatol. , 2017

Focus score





Chatzis LG et al A biomarker for lymphoma development in Sjogren's syndrome: Salivary gland focus score. J Autoimmun. 2021

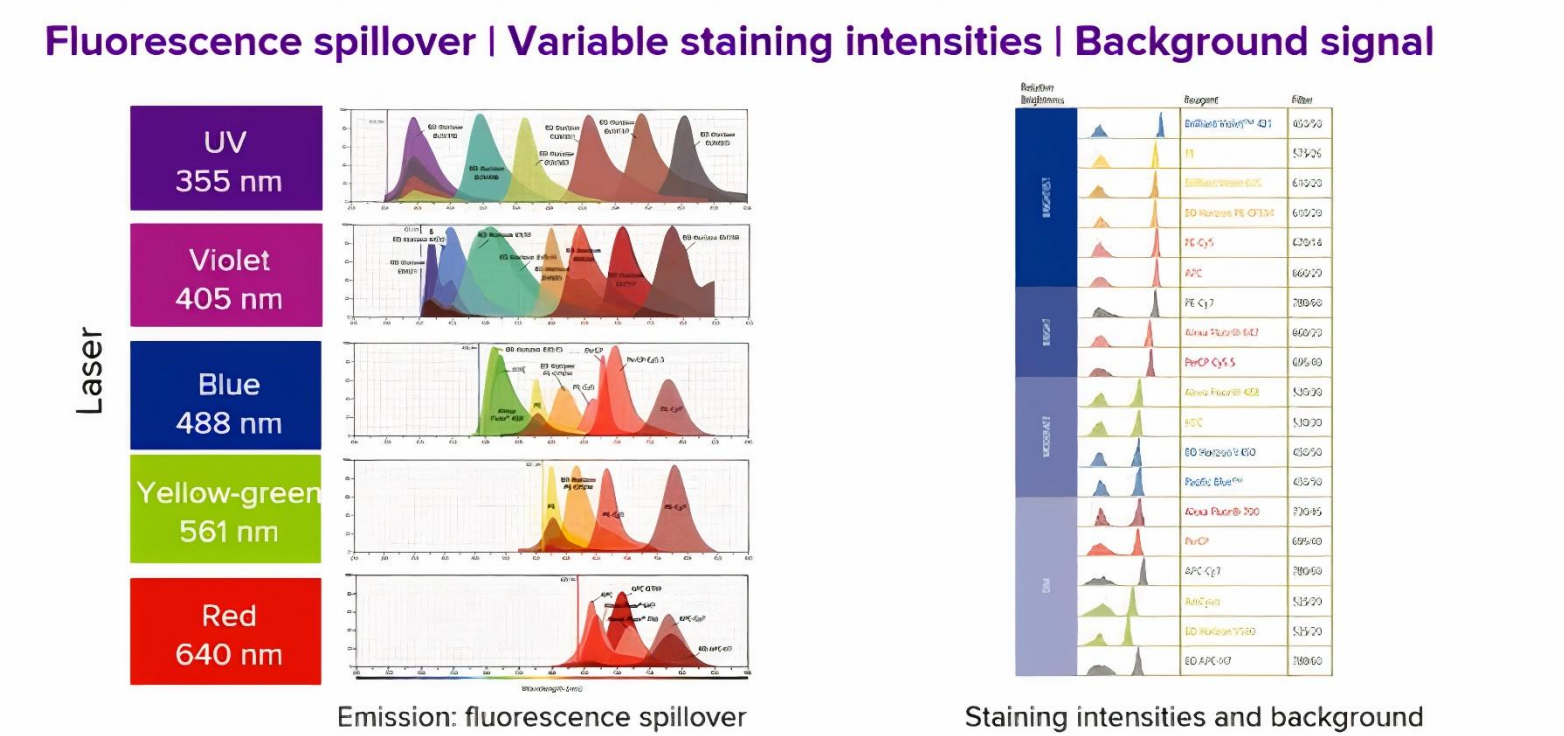


Sequential algorithm for time interval from SS until lymphoma diagnosis for each FS threshold	
FS range	**P Value***
FS≥2 vs FS<2	0,0795
FS≥3 vs FS<3	0,0956
FS≥4 vs FS<4	**0,0080**
FS≥5 vs FS<5	0,0857
FS≥6 vs FS<6	0,1820
FS≥7 vs FS<7	0,2004
FS≥8 vs FS<8	0,2004
FS≥9 vs FS<9	0,1716
FS≥10 vs FS<10	0,9005
FS≥11 vs FS<11	0,9146

Chatzis LG et al A biomarker for lymphoma development in Sjogren's syndrome: Salivary gland focus score. J Autoimmun. 2021

Antibody-mediated multiparameter protein detection

- Fluorochrome-conjugated antibodies are widely used but have limited utility for high-parameter studies. Theses limitations contribute complexities into experimental design and interpretation



Analysis

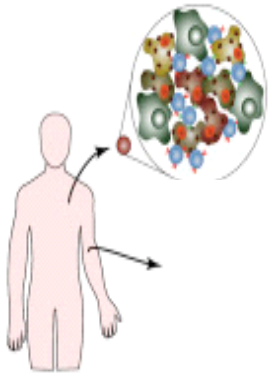
Plateforme de cytométrie en flux et de masse

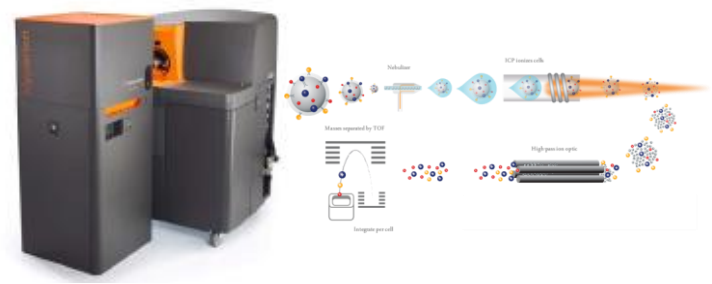
HYPERION

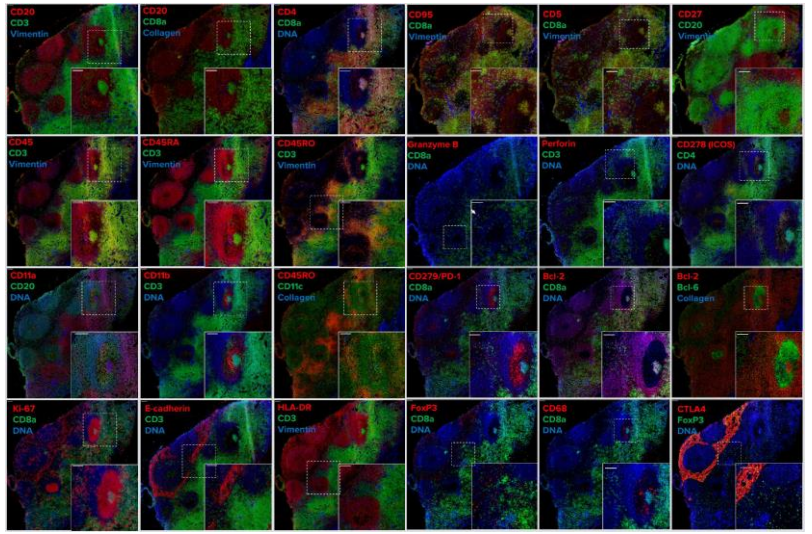
HYPE Research in Immunology and ONcology

Imaging mass cytometry (IMC) is a new multiparametric and quantitative technique for phenotypic and functional analysis of cells and tissue sections. It can measure up to 50 parameters simultaneously in tissues at a spatial resolution of 1 µm.

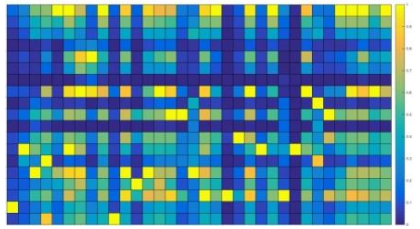
Tissue

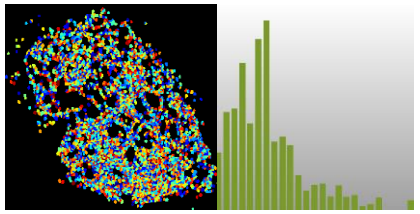






Phenograph



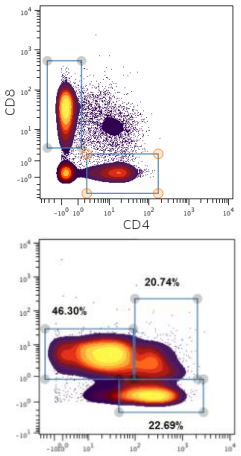


Neighborhood analysis

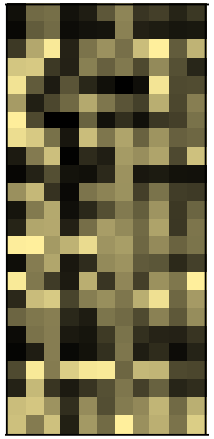
Panel

CD45
CD196 CCR6
CD123
CD19
CD4
CD8a
CD11c
CD16
CD45RO
CD45RA
CD161
CD194 CCR4
CD25
CD27
CD57
CD183 CXCR3
CD185 CXCR5
CD28
CD38
CD56 NCAM
TCRγδ
CD294
CD197 CCR7
CD14
CD3
CD20
CD66b
HLA-DR
IgD
CD127
Cell-ID
CD24
CD11b
CD163
IgM
CD21
CD5

Dot Plot

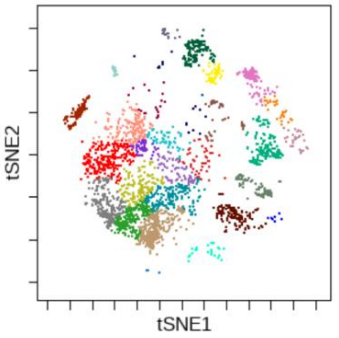


Heatmap

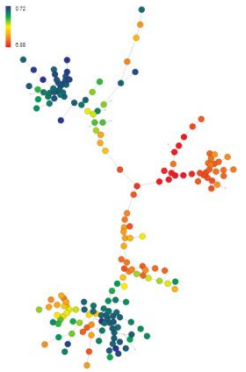


Supervised analysis

tSNE



SPADE



Unsupervised analysis

Background

1. 5 sicca controls
2. 5 Mild infiltrates
3. 5 Intermediate infiltrates
4. 5 Severe infiltrates
5. 4 patients with non-SS associated NHL

MILD

FS:0-1, Anti – Ro/SSA (-), Anti-La/SSB (-)

FS:1-1.79, TS:1

Interm ediate

Based on B and T cell infiltrates

FS:1.8-3.5, TS:2

Severe

FS: 3.6-11, TS: 3-4

MALT Lymphoma diagnosis

Chatzis LG et al Unpublished data

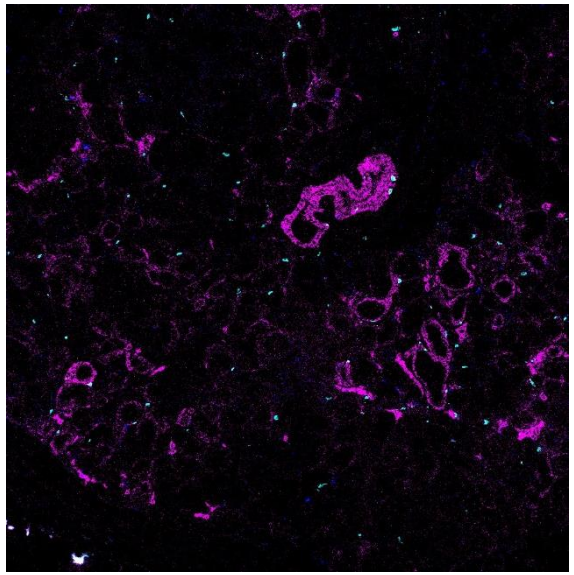
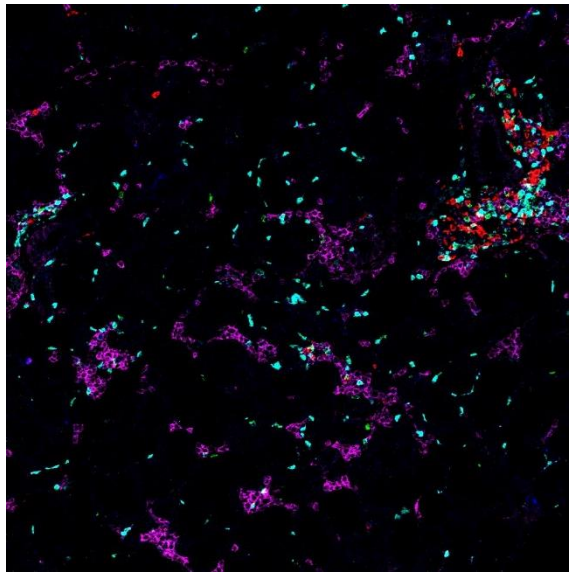
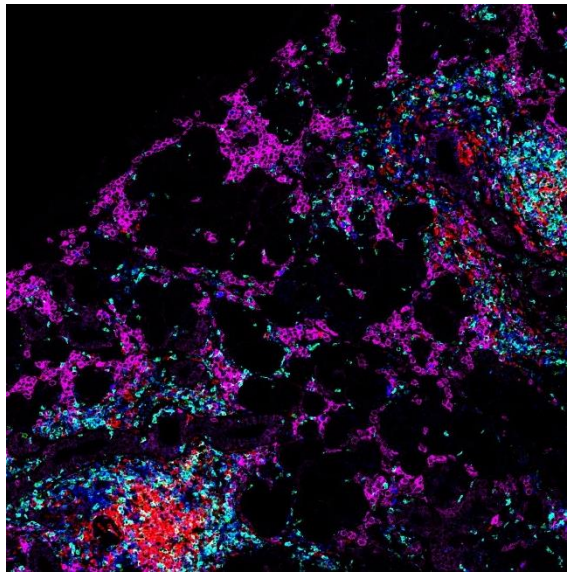
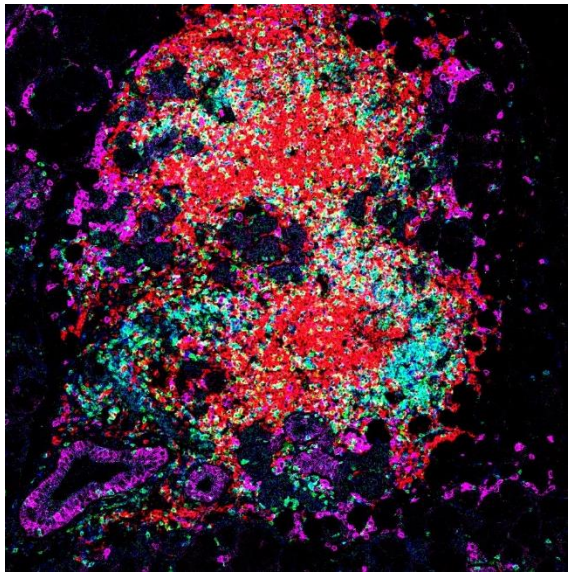
2 Antibody Panels

General Panel	Properties
CD38	Plasma cells, Immature/ transitional b cells, effector T cells
CD204	M2 macrophage
Vimentin	Mesenchymal cells
CD14	**Macrophages**, dendritic cells, neutrophils
Tbet	Th1 T cells
CD34	Hematopoietic progenitor cells, stem cells
CD163	M2 macrophages
Pan-Keratin	Epithelial cells
CD11b	Monocytes, B1 B cells, natural killer (NK) cells, and dendritic cells, cDC2
TSLP	Lymphoma, Epithelial cells
CD31	Endothelial cells in blood vessels, platelels
Ki-67	Proliferation
IgD	Mantle zone B cells, naive B cells
IgM	Mantle zone B cells, naive B cells
FoxP3	T regulatory cells
Cd4	T helper cells
cKit	Cancer, Stem cells
CD68	M1 Macrophages
IgA	Plasma cells and germinal center immunoblasts
AID	Somatic hypermutation and immunoglobulin class switch recombination

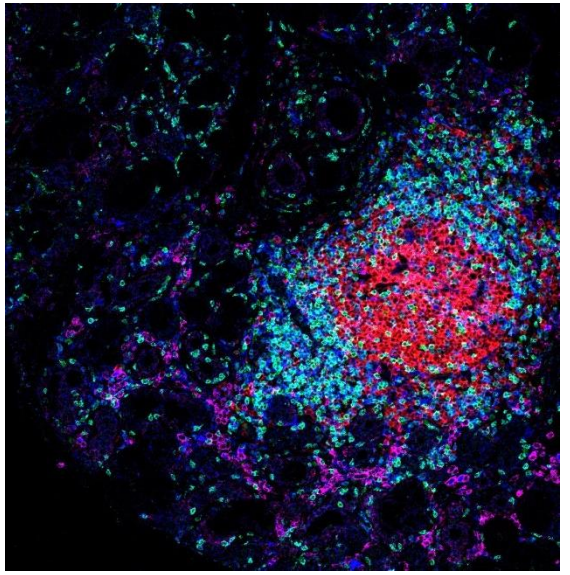
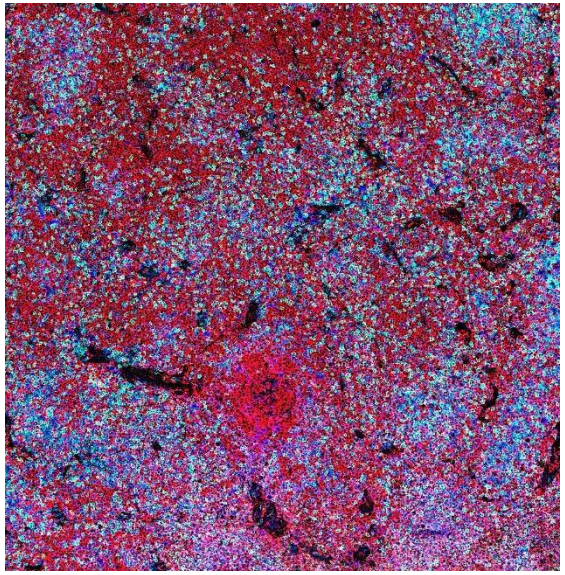
General Panel	Properties
Bcl6	GC B cells and T follicular helper cells
CD20	B lymphocytes
CD8a	Cytotoxic T cells
CD138	Plasma cells
MPO	Neutrophils
Flt3Ligand	T cells
CD56	NK cells
CD127	Memory and Effector T cells not on Treg, Precursos B cells
Collagen	Collagen type I
CD3	T cells
CD27	Memory T cells (except an effector memory population), B cells (not effector), Plasmablasts (High) NK cells
Caspase-3	Cell apoptosis
Podoplanin	Follicular DCs, Lymphatic endothelial cells
HLA-DR	Dendritic cells, macrophages, B cells, stimulated epithelial cells
pS6	Akt/PI3K/mTOR pathway related proliferation
Flt-3	Plasmacytoid DCs, B progenitor cells
CXCL13	Follicular dendritic cells
CXC3	Activated Th1 cells, CD8 effector cells, Nk cells, proinflmmatory B cells

>>>>>>

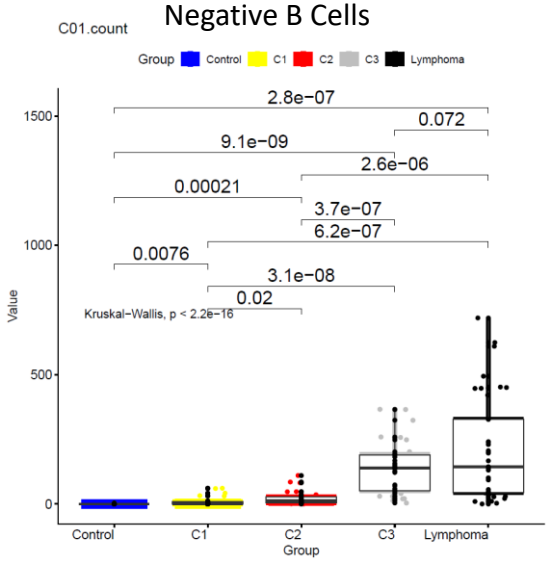
B cell panel	Properties
CD45RB	Memory B cells, Cd38 positive B cells outside of GM
Vimentin	Mesenchymal cells
Tbet	Th1 T cells
Pan-Keratin	Epithelial cells
Ki-67	Proliferation
IgD	Mantle zone B cells
FoxP3	T regulatory cells
CD4	T helper cells
CD68	M1 Macrophages
Bcl6	GC B cells and T follicular helper cells
CD20	B lymphocytes
CD8a	Cytotoxic T cells
CD138	Plasma cells
MPO	Neutrophils
PD-1	T follicular helper cells
Collagen	Collagen type I
CCR6	Bmem
CD3	T cells
CD27	Memory B cells (except an effector memory population), T cells (not effector), NK cells
Podoplanin	Follicular DCs, Lymphatic endothelial cells
SMA	Smooth muscle antibodies
CD38	Plasma cells, Immature/transitional B cells, effector T cells
AID	Somatic hypermutation and immunoglobulin class switch recombination

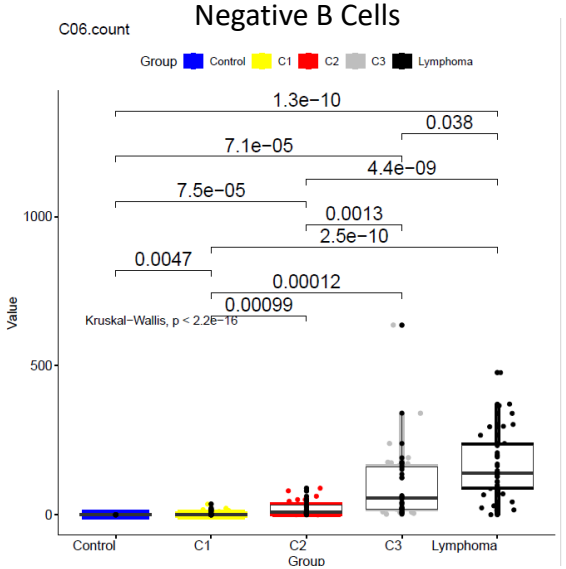
CD20
CD3
CD4
CD8
CD138

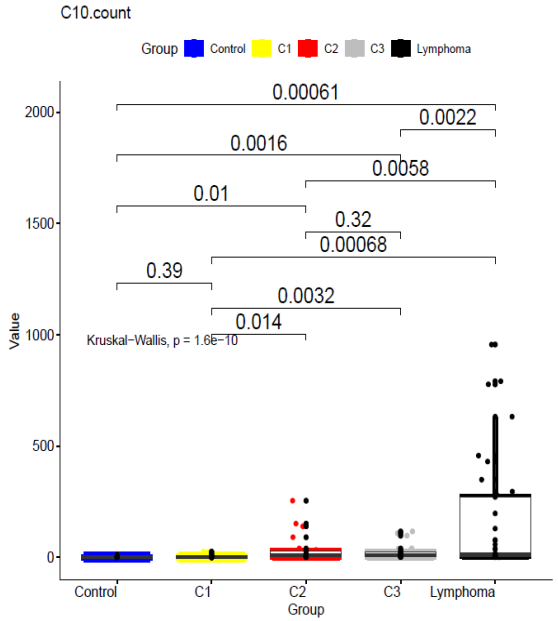
CD45RBhigh Double Negative B Cells



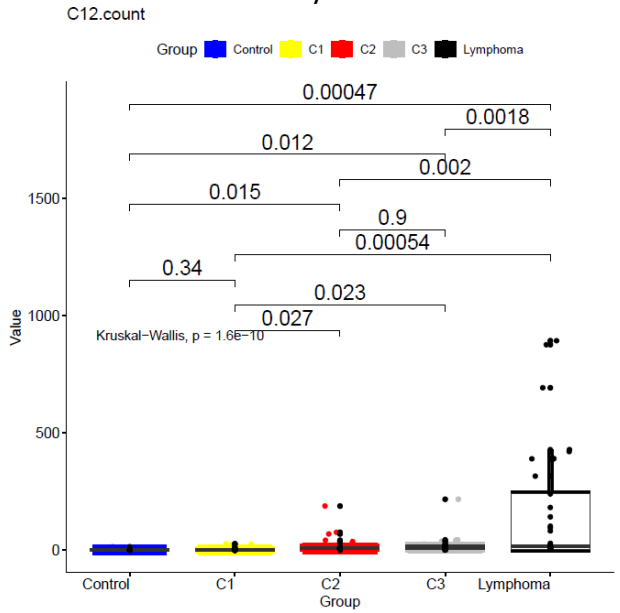
Podoplanin⁺ Double Negative B Cells



Double Negative B cells



CD45RB⁺ Switched Memory B cells



Next steps

- Definition of pathogenetically relevant biomarkers in tissue injury and peripheral blood, per disease phenotype, using:
- Integration of multi –omics technologies
- Validation
- "Sensitivity to change" after treatment to define clinically relevant cells/molecules

- **Definition of next generation biomarkers per disease phenotype (new targets, new stratification tools, advanced treatment selection strategies)**

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LBAI U1227

Pathophysiology Dept. School of Medicine

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Autoimmune Diseases

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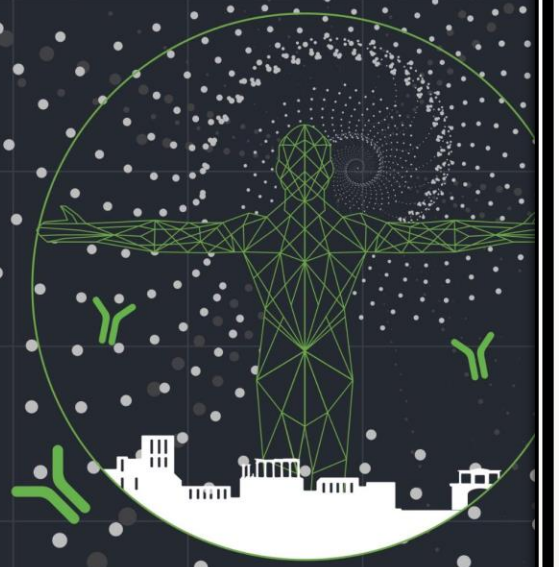

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