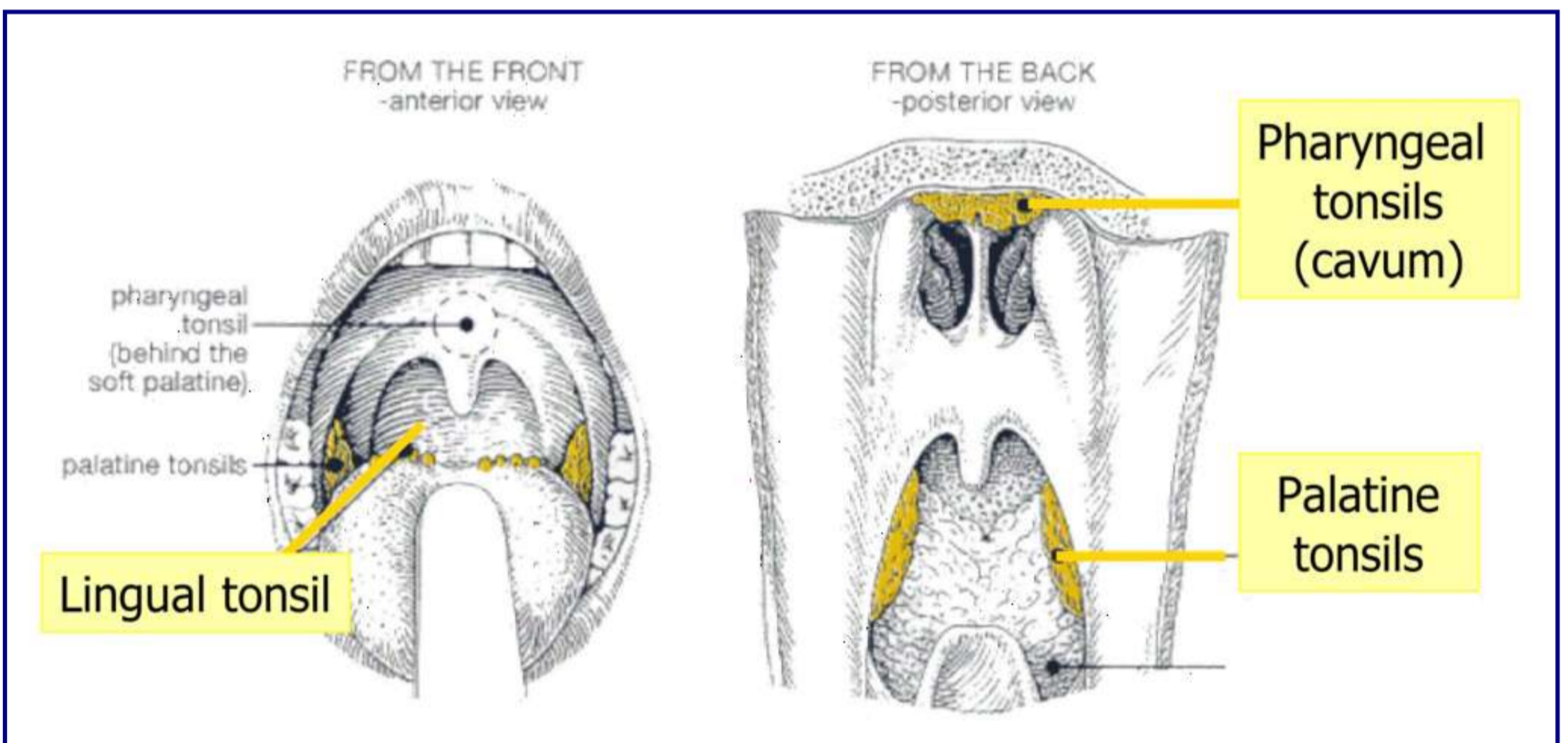


# DIFFUSE LARGE B-CELL LYMPHOMAS OF THE WALDEYER'S RING FREQUENTLY HAVE A GERMINAL CENTER-LIKE PHENOTYPE: A CLINICO-PATHOLOGICAL STUDY OF 209 PATIENTS FROM THE GROUPE D'ETUDE DES LYMPHOMES DE L'ADULTE (GELA)

L. de Leval<sup>1</sup>, C. Bonnet<sup>2</sup>, C. Copie-Bergman<sup>3</sup>, L. Seidel<sup>4</sup>, M. Baia<sup>3</sup>, J. Brière<sup>5</sup>, T. Molina<sup>5</sup>, B. Fabiani<sup>5</sup>, B. Falini, C. Gisselbrecht<sup>5</sup>, H. Thilly<sup>5</sup>, A. Albert<sup>4</sup>, G. Fillet<sup>2</sup>, P. Gaulard<sup>3</sup> - From the Departments of Pathology<sup>1</sup>, Hematology<sup>2</sup>, and Biostatistics<sup>4</sup> C.H.U. Sart-Tilman, Liège, Belgium, the Department of Pathology, Hôpital Henri Mondor<sup>3</sup>, Créteil, France, and for the GELA group<sup>5</sup>.

## Background and design

Diffuse large B-cell lymphomas (DLBCLs) are markedly heterogeneous, and there is increasing evidence that the biological features of these tumors vary according to the primary site of disease (lymph node or various extranodal organs). The Waldeyer's ring (WR), a circular band of mucosa-associated lymphoid tissue composed of the palatine, lingual and pharyngeal tonsils, is the second most common site of extranodal involvement by DLBCL..



## Patients and methods

The study comprised 209 adult patients with de novo DLBCL presenting in the WR consecutively included in the GELA protocols during a 10 year period (1993-2004). All patients (M/F ratio: 1,77/1, mean age 59 years) comprising 81% with stages I-II and 19% with stages III-IV disease, received intent-to-cure anthracyclin-based polychemotherapy.

The 5-years overall survival (OS) and 5-years event-free survival (EFS) were 74,2 +/- 3,4% and 66,9 +/- 3,6 %, respectively

Morphology and pattern of growth (further defined in a subset of cases by immunostaining with a follicular dendritic cell (FDC) marker) were evaluated on the original slides.

A tissue microarray (TMA) representative of 66 cases with available paraffin bloks was constructed. Immunohistochemistry was performed on archival unstained paraffin sections and/or on TMA sections, for the following markers: CD20, CD3, CD5, Bcl-2, Bcl-6, CD10, Mum-1. Individual markers and differentiation profile (by Hans' algorithm) were analyzed and correlated to the clinical features.

FISH was performed on TMA sections with break-apart probes sets to detect gene rearrangements of BCL2, BCL6 & c-MYC.

Survival and outcome were analyzed in comparison to a matched cohort of patients with primary nodal DLBCL.

## Morphology and pattern of growth

By morphology, 55% of WR DLBCLs were classified as centroblastic, 39% as centroblastic polymorphous, 3% as immunoblastic, and 3% unclassifiable.

Pattern	Large Resections n = 79	FDC staining n = 73
Diffuse (D)	37 (47%)	7/34 (20%) +
Nodular (N)	42 (53%)	28/39 (72%)
D>N	28 (35%)	15/25 (60%) +
N = or >D	14 (18%)	13/14 (93%) ++

DLBCL with follicular colonization  
DLBCL in association with FL

Among large biopsy specimens (n=79), 18% had a prominent nodular pattern consistent with transformed follicular lymphomas, 35% had a minor nodular component (<50%) interpreted as follicular colonization and 47% were diffuse. The nodular pattern correlated with centroblastic morphology (p=0,0007).

## Immunohistochemistry

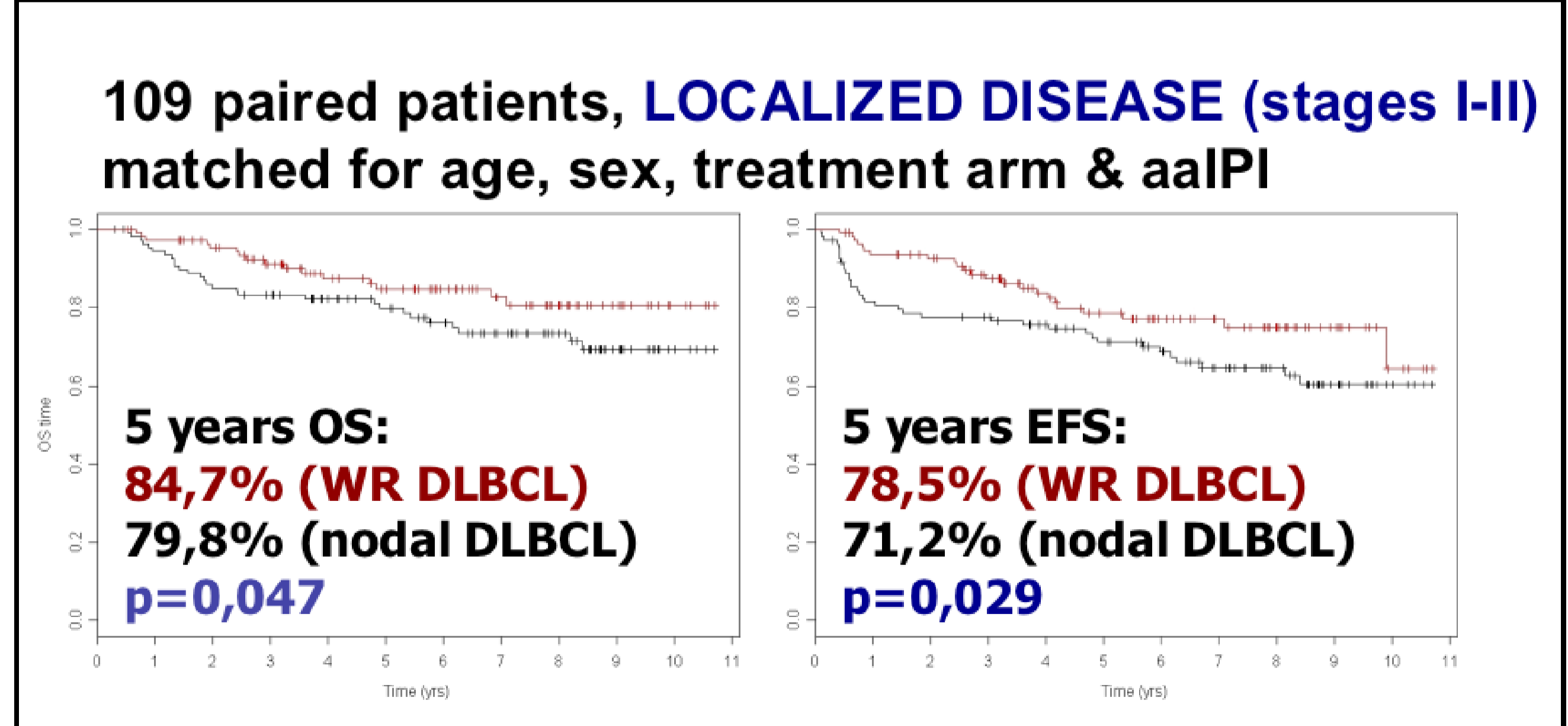
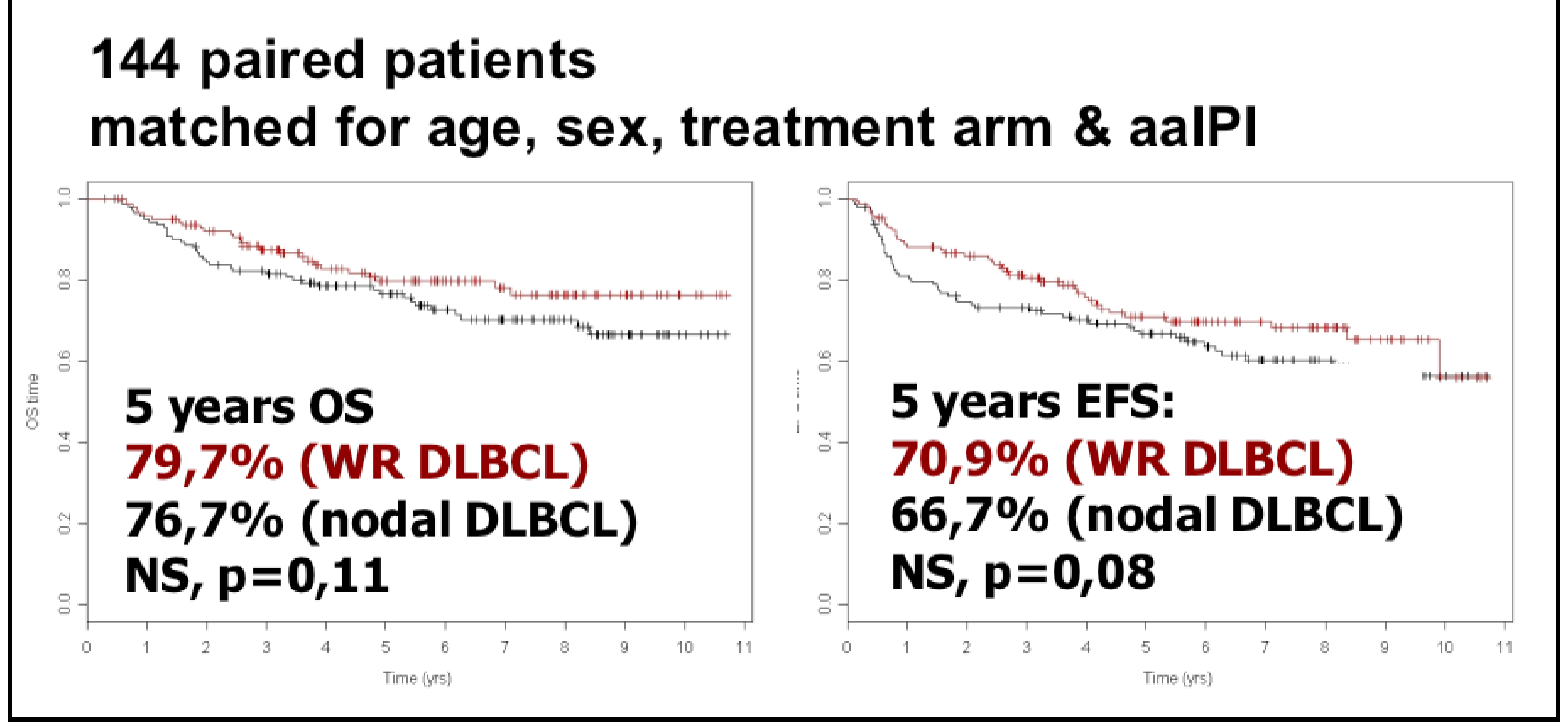
	Bcl2	CD10	Bcl6	MUM1	GC/non-GC
Full slide	n = 180	n = 160	n = 60	n = 75	
Non interpretable	32	23	47	30	
Contributive	148 (82%)	141 (88%)	13 (23%)	45 (60%)	84
	82 pos	56 pos	11 pos	33 pos	56 GC
	66 neg	85 neg	2 neg	12 neg	28 non-GC
	55% pos	40% pos	85% pos	73% pos	67% GC
TMA	n = 66	n = 66	n = 66	n = 66	n = 66
	39 pos	33 pos	31 pos	8 pos	39 GC
	27 neg	33 neg	35 neg	58 neg	27 non GC
	59% pos	50% pos	46% pos	12% pos	59% GC
Full slide + TMA	n = 189	n = 178	n = 76	n = 109	n = 136
	105 pos	75 pos	40 pos	40 pos	82 GC
	84 neg	103 neg	36 neg	69 neg	54 non-GC
	56% pos	42% pos	53% pos	37% pos	60% GC

The GC-like profile correlated with absence of bcl-2 expression (p<0,0001) and with centroblastic morphology (p=0,04). In multivariate analysis, the GC-like phenotype correlated with better OS (p=0.014), and CD10 expression correlated with better EFS (p=0.0061).

## FISH analysis

FISH results	18q21 BCL2	3q27 BCL6	8q24 C-MYC
n	52	52	52
interpretable	42	35	41
colocalized signals	39	26	38
split signals (translocation)	3	9	3
% rearrangement	7,10%	25,70%	7,30%

## WR versus nodal DLBCL



In conclusion, WR DLBCLs frequently have a partially follicular pattern of growth, and a GC-like phenotype. In DLBCL patients with no factor of the age-adjusted IPI, the tonsillar localization appears to confer a better outcome than primary nodal involvement.