

T-cell subpopulations quantified by flow cytometry in lymph node cell suspensions identify a group of patients with follicular lymphoma (FL) with favorable outcome

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BACKGROUND

- Tumor microenvironment plays an important role in the behavior of FL
- By gene expression and immunohistochemistry, an increase in macrophages has been associated with poor outcome, whilst an increase in T-cells is associated with good prognosis

OBJETIVE

- To explore the prognostic impact of subpopulations of T-cells using flow cytometry and to identify different groups of risk in FL patients

PATIENS AND METHODS

75 patients diagnosed of FL between 1994 and 2009 (median follow-up of 6.5 years) with samples at diagnosis

Main features	N (%)	Main features	N (%)
Age, median (range)	60 (29-84)	FLIPI	
Gender	36M/39F	Intermediate-high risk	56 (79)
Histology		Transformation to DLBCL	9 (12)
Grade 1-2	62 (87)	Treatment	
Grade 3a/3b	7(10)/2(3)	Watchful waiting	8(11)
Bulky disease	18 (25)	Adriamycin regimens	39 (52)
Stage IV disease	52 (69)	Fludarabine regimens	17 (22)
Bone marrow involvement	48 (64)	Others	11 (15)
High serum LDH	18 (25)	Rituximab Therapy	37 (49)
		10-year PFS, (95% CI)	41% (29-53%)
		10-year OS, (95% CI)	59% (47-71%)

Flow cytometer technique

Cell suspensions from lymph nodes obtained by squirting biopsies with culture medium.

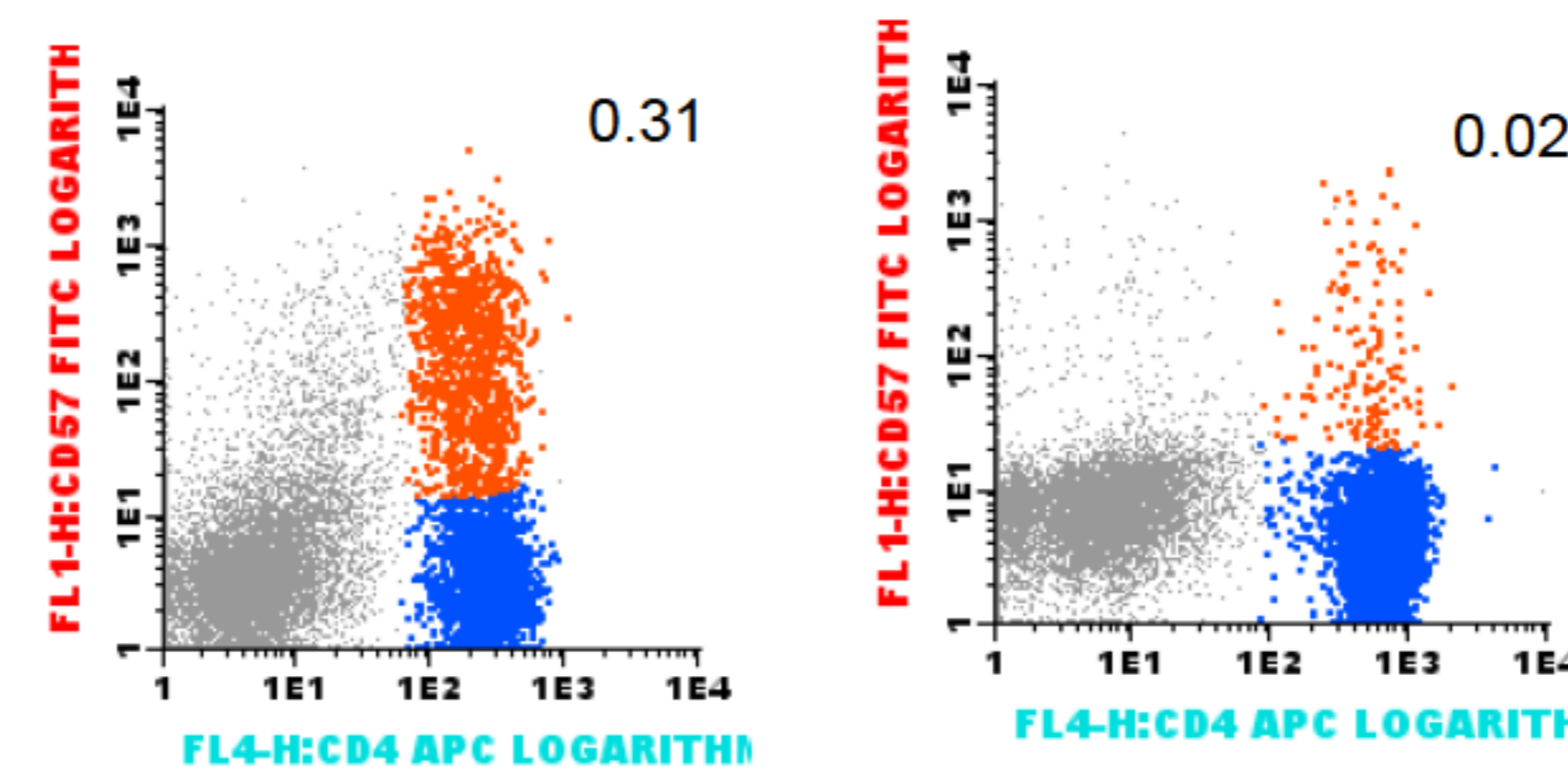


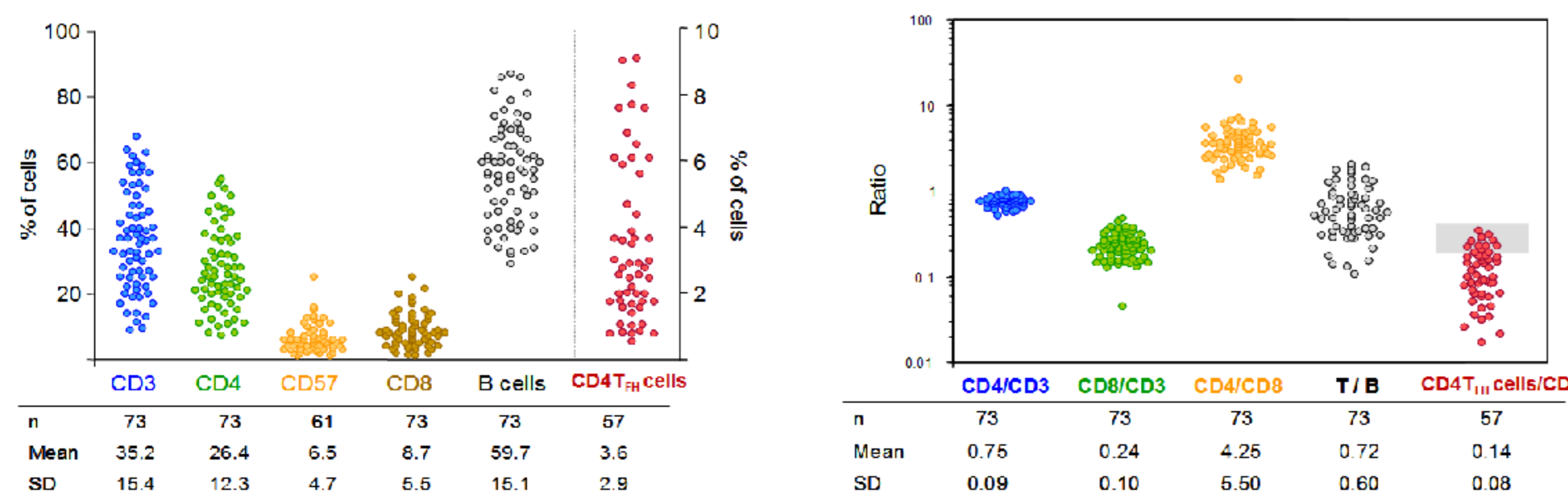
Figure. Sample stained with CD57 and CD4. CD4_{T_{FH}} cells (CD4⁺CD57⁺) cells are depicted in orange and the rest of CD4 cells in blue. Inside each box is shown the value of the CD4_{T_{FH}} cells /CD4 ratio.

Flow cytometry: All samples were obtained at diagnosis. Directly labelled antibodies against B and T cells were used to identify subpopulations in a FACScalibur flow cytometer. The percentage of CD3, CD4, CD8, CD57, and T-follicular helper (CD4_{T_{FH}} cells, CD4+CD57+) as well as their ratios were calculated for each sample (figure).

RESULTS

Flow Cytometry

Lymphocyte distribution and ratios



Factors associated to T-cell subsets in FL nodes

	CD3 ⁺ P≥50	CD4 ⁺ P≥50	CD8 ⁺ P≥50	CD3/B-cell P≥50	CD4/CD8 P≥50	CD4 _{T_{FH}} /CD4 ⁺ P≥75
Age						
<60 y (%)	39	34	29*	21*	55	21
≥60 y (%)	59	56	57	43	43	39
Histological grade						
1-2 (%)	45*	43	35*	27	58**	29
3 (%)	89	67	89	67	0	11
Bulky disease						
No (%)	57*	53*	46	60*	46	23
Yes (%)	22	17	33	11	56	29
Extranodal sites						
≤1 (%)	58**	52*	51*	58*	47	23
>1 (%)	21	21	21	26	53	29
Leukemic phase						
No (%)	56 (34/61)*	50*	48*	32*	60	22
Yes (%)	10 (1/10)	10	10	0	46	25

* P<0.05; **P<0.01

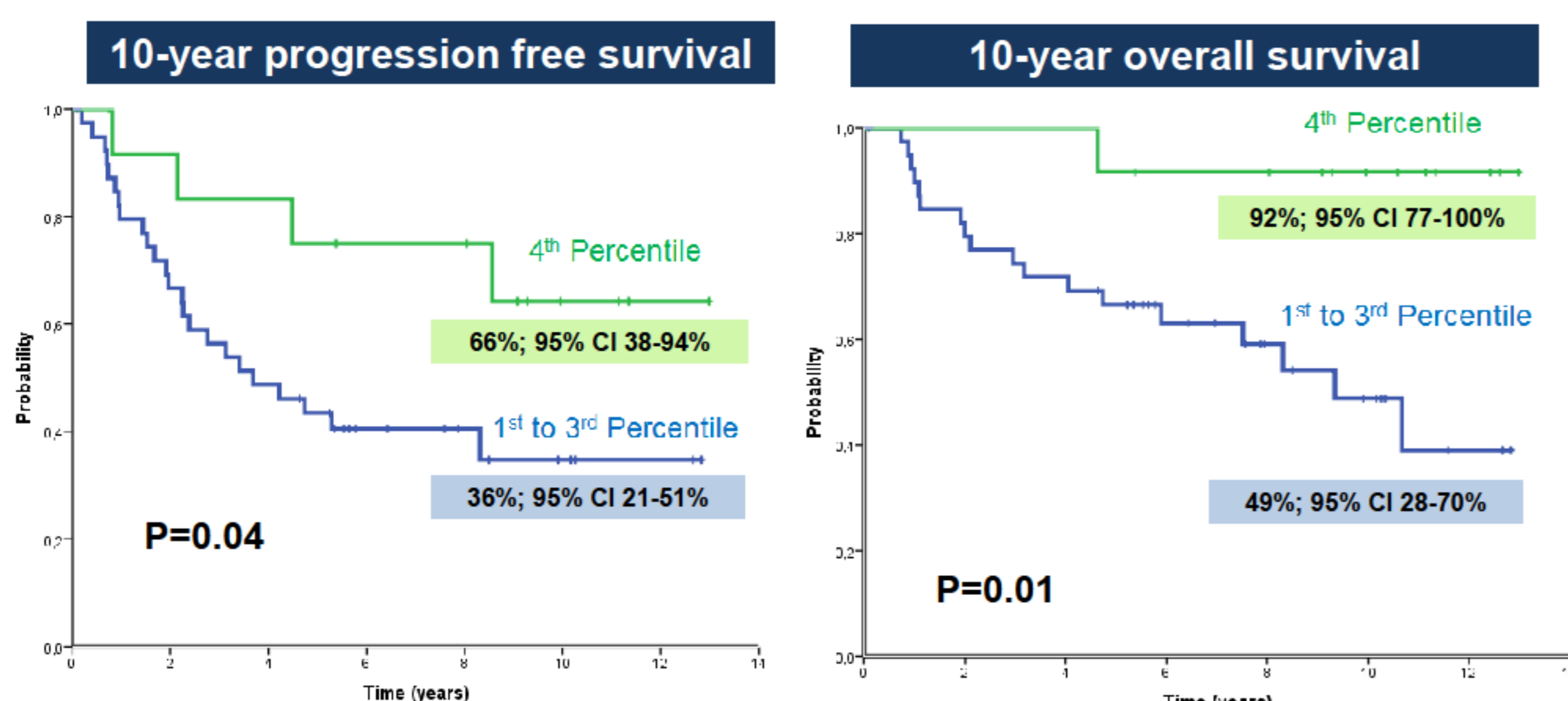
Immunohistochemistry

- CD3⁺, CD4⁺ and CD57⁺ cells were mainly observed in the intrafollicular compartment
- CD8⁺ cells were found mainly in interfollicular or perfollicular areas
- FL histological grade 1-2 more frequently showed CD57 intrafollicular pattern (p=0.017) and CD8 interfollicular or perfollicular pattern (p=0.024) than cases with histological grade 3
- Cases with purely follicular pattern were associated with a CD57 intrafollicular pattern (p=0.02)

T-cells subsets and outcome

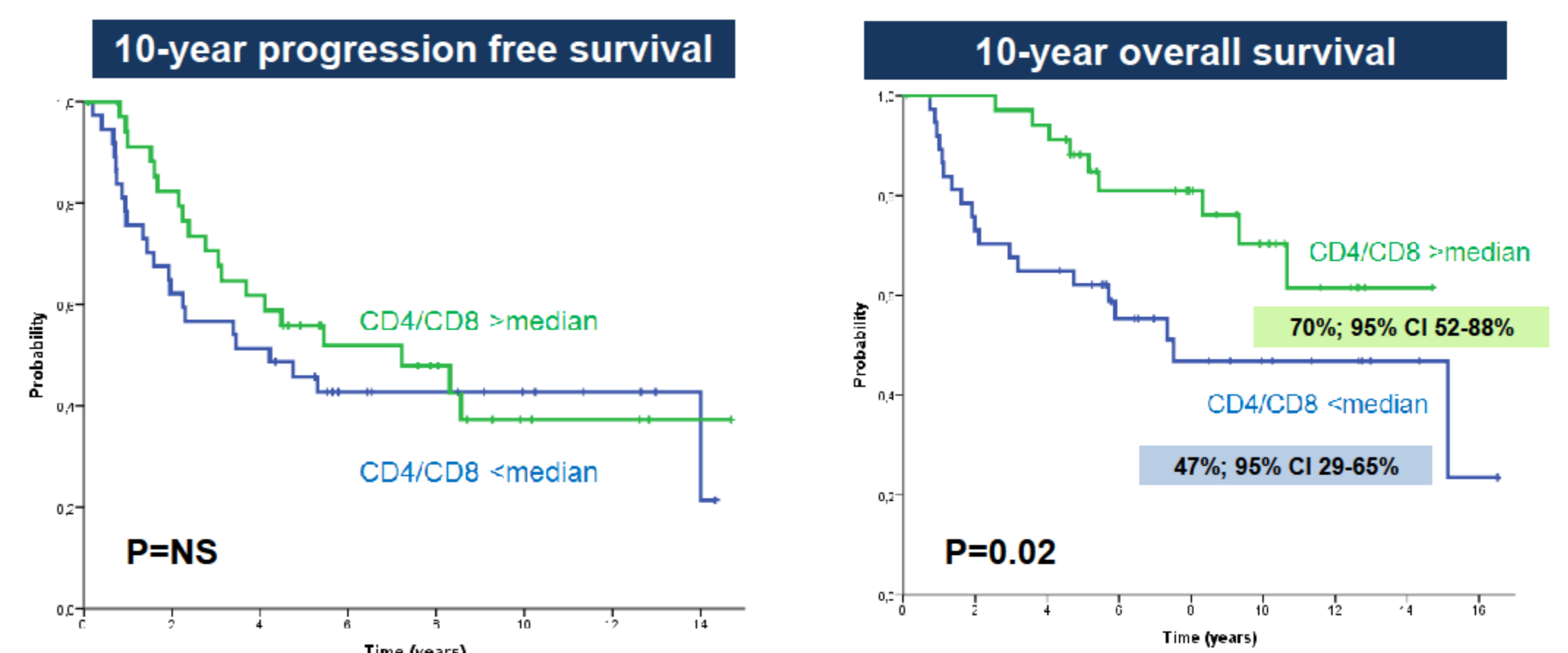
Outcome according to CD4_{T_{FH}} cells

4th percentile >0,19



Outcome according to CD4/CD8 ratio

Median CD4/CD8 ratio: 2.6



A multivariate Cox analysis was performed including FLIPI score (low vs. intermediate vs. high) and CD4_{T_{FH}}/CD4 ratio (1-3 percentile vs. 4 percentile) with CD4_{T_{FH}}/CD4 ratio being the most important variable to predict OS in the final model with 52 patients (relative risk: 32; p=0.01)

CONCLUSION

Flow cytometry allows the identification of T-Cell subpopulation in FL, showing that a high percentage of CD4_{T_{FH}} cells is associated with more favorable prognosis