Correlation of *HAVCR2/TIM3* and *TBET/EOMES* expression ratios with Granzyme B+ lymphocyte number and impact on the therapeutic response in pediatric patients with classical Hodgkin lymphoma

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INTRODUCTION

- Classical Hodgkin lymphoma (cHL) is characterized by 0.5-1% neoplastic Hodgkin and Reed-Sternberg cells, highly dependent on the tumor microenvironment (TME), which is formed by inflammatory and stromal cells.
- Cytotoxic (CD8+ and granzyme B+, GRZB+) cells are observed in the TME at variable numbers. In opposition to what is expected in an immunosurveillance model, high number of cytotoxic cells is associated to an unfavorable prognosis, suggesting that those cells could suffer from a hipofunctional state (i.e., exhaustion).
- The understanding of the central role of the PD1-PD1L axis in the tumor, despite fragmentary, has led to the recent approval of the use of anti-PD1 therapeutic antibodies (nivolumab and pembrolizumab) in relapsed patients.

AIM

We sought to quantify the expression levels of genes potentially associated to the immune checkpoint in cHL lymph nodes, aiming to disclose associations with the cellular composition of the TME and clinical response to prospect for potential immune biomarkers.

METHODS

In a group of 86 patients (children and adolescents) with cHL, the expression of mRNA form inhibitory receptors (*CTLA4, PDCD1, PD1L/CD274, HAVCR2/TIM3*, and *BTLA*) and transcription factors (*EOMES* and *TBX21/TBET*) associated with the immune checkpoint was quantified by RT-qPCR. The number of infiltrating lymphocytes expressing PD1+ was assessed by quantitative immunohistochemistry. The results were analyzed in relation to the clinical-epidemiological characteristics, the TME composition, and the therapeutic response. The sequence of experiments performed is shown in Fig. 1

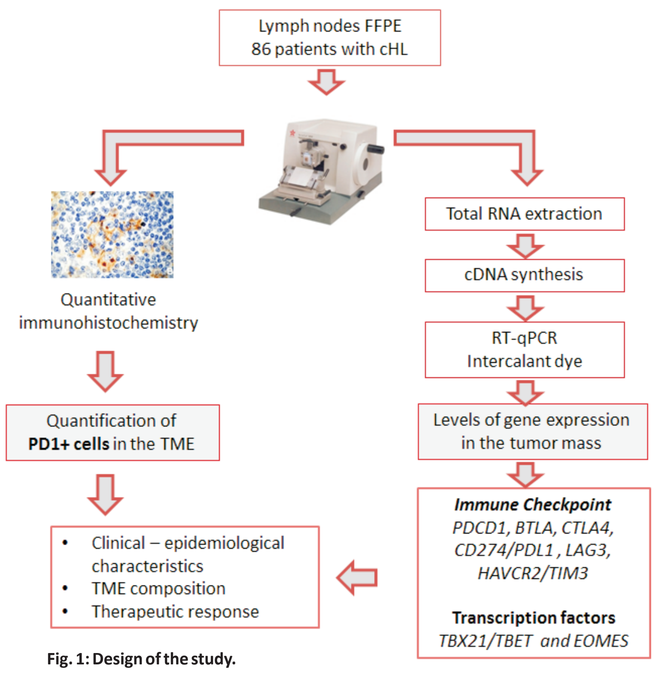

Fig. 1: Design of the study.

RESULTS

Expression levels of genes associated to the immune checkpoint

BTLA showed the highest expression level (median ± interquartil range: 2.219 ± 1.922), following by *CTLA4* (1.285 ± 1.790), *LAG3* (-0.347 ± 2.713), *PDCD1* (-0.954 ± 2.138), *CD274/PDL1* (-2.496 ± 3.088), and *HAVCR2/TIM3* (-5.281 ± 3.695). The *TBX21/TBET* transcription factor showed higher levels than *EOMES* (-0.565 ± 2.173 vs. -1.918 + 4.027) (Fig2)

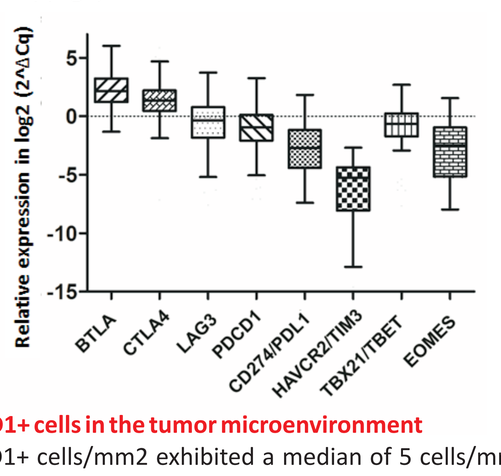

Fig. 2: Levels of gene expression of molecules associated with immune checkpoint and transcription factors *TBX21/TBET* and *EOMES* in the total group of pediatric patients diagnosed with cHL. Each box plot represents the expression levels of the different genes evaluated in the patient group.

PD1+ cells in the tumor microenvironment

PD1+ cells/mm2 exhibited a median of 5 cells/mm2 (range 0-363). PD1+ lymphocytes were homogeneously distributed in lymph node tissue in most cases, while sometimes showed a rosette-like pattern around H-RS cells (Fig. 3).

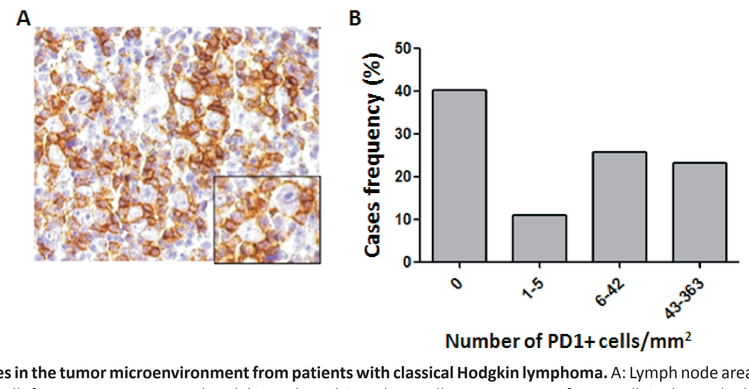

Fig. 3: PD1+ lymphocytes in the tumor microenvironment from patients with classical Hodgkin lymphoma. A: Lymph node area with PD1+ cells evenly distributed. In the square, are PD1+ cells forming rosettes around Hodgkin and Reed-Sternberg cells; B: Frequency of PD1+ cells in the studied pediatric group.

Correlations between gene expression and number of cells in the tumor microenvironment

A correlation between *PDCD1* expression and PD1+ cells/mm² was observed (Rho=0.408), as well as *TBX21* expression and TBET+ cells/mm2 (Rho=0.319), and *HAVCR2/TIM3* and GRZB+ cells/mm² (Rho=0.395) (P<0.01, Spearman's correlation) (Fig. 4).

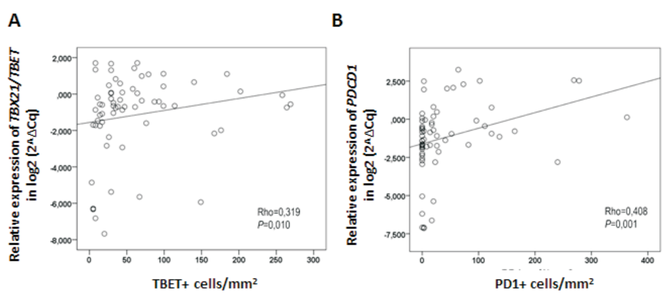

Fig. 4: Correlations between gene expression and cells numbers in the tumor microenvironment. A: *TBX21/TBET* expression vs. *TBET*+ cells/mm²; B: *PDCD1* expression vs. PD1+ cells/mm².

Partial-Least Square Regression (PLSR)

This method was performed to evaluate the weight (relationship) of gene expression values on the TME cells, specifically GRZB+ cells.

The number of GRZB+ cells/mm² (log10) was used as indicator variable, all genes studied were positively correlated; *TBX21/TBET and PD1L/CD274* showing the highest weights (Fig. 5).

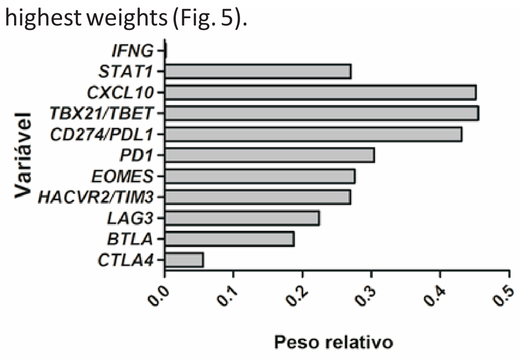

Fig. 5: Relative weight of gene expression levels in respect of the number of granzyme B positive cells in the tumor microenvironment of Hodgkin lymphomas (log10 of GRZB + cells).

HAVCR2/TIM3 and *TBET/EOMES* expression ratios associated to therapeutic response

High expression of *TIM3* showed a trend with short time of progression free survival (PFS) and *EOMES/TBX21* expression ratio >2.0 were associated to short PFS, as shown in Table 1 and Fig. 6.

Table 1: Progression-free survival (PFS) according to the expression levels of genes associated to immune checkpoint in pediatric patients diagnosed with classical Hodgkin lymphoma

		Confidence Interval (95%)		
Variables	HR (ß)	Lower	Upper	*P*-value
BTLA	0.95	0.68	1.32	0.772
CTLA4	0.97	0.85	1.03	0.476
HAVCR2/TIM3	1.18	0.99	1.50	0.056
LAG3	1.04	0.88	1.26	0.630
PDCD1	0.95	0.78	1.19	0.628
CD274/PD1L	0.99	0.80	1.24	0.975
TBX21/TBET	1.03	0.85	1.31	0.760
EOMES	0.98	0.82	1.20	0.871
***EOMES/TBET* >2.0**	4.86	1.82	14.66	0.001

P-values calculated with a Firth's correction strategy.

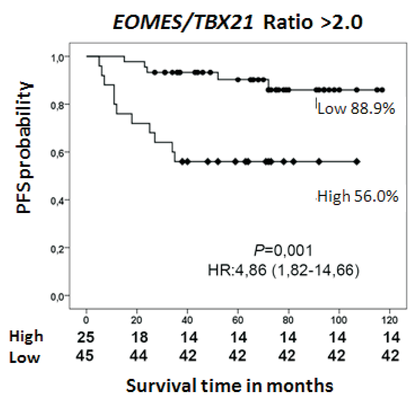

Fig. 6: Kaplan-Meier curve, progression free survival (PFS) by EOMES/TBX21 ratio >2.0 in pediatric patients with classical Hodgkin lymphoma. On axis "X" is shown the number of patients at risk for each category over time.

These variables maintained the prognostic impact in multivariated analysis, as shown in Table 2.

Table 2: Multivariate survival analysis (PFS) using Cox (proportional hazards) regression model, considering clinical and levels of gene expression as variables in pediatric patients with classical Hodgkin lymphoma.

		Confidence Interval (95%)		
Variables	HR (ß)	Lower	Upper	*P*-value
Mixed cellularity subtype	3.962	1.146	13.428	0.030
Extranodal sites	7.903	2.042	29.561	0.004
GRZB+ cells/ *HAVCR2* expression	6.464	1.182	15.004	0.028
EOMES/TBX21 ratio>2.0	4.143	1.362	76.455	0.012

P-values calculated with a Firth's correction strategy. GRZB+ cells/*HAVCR2* expression: patients with both high (>25 percentile) *HAVCR2/TIM3* expression and GRZB+ cells.

CONCLUSIONS

- The ratio EOMES/TBX21 has potential as biomarker to be used in the context of conventional and targeted therapies, if validated in independent, larger series.
- A potential explanation to the unfavorable association of cytotoxic cells with clinical response in cHL.
- Functional studies are warranted to characterize the level of functional impairment of effector cells in cHL.

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