Exploring of the prognostic value of eight immunohistochemical markers expressed in the tumor microenvironment and on Hodgkin Reed-Sternberg cells in pediatric patients with classical Hodgkin lymphoma

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Abstract

Immunohistochemical markers are associated with treatment outcome in adults with classical Hodgkin Lymphoma (cHL). Studies in children are scarce and inconsistent. We investigated in 67 children with cHL, whether the expression of CD15, CD30, PAX5, PD-1, PD-L1, CD68, CD163 and TARC is associated with the event-free survival (EFS) and the interim remission status. Low expression of PD-L1 was significantly associated with complete remission at interim PET-scan. There was no association between marker expression and EFS. Our data suggest a difference between pediatric and adult cHL. This underlies the importance of future research into specific molecular drivers in pediatric cHL.

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Abbreviations key

AUC	Area Under the Curve
cHL	Classical Hodgkin Lymphoma
CR	Complete remission
CT	Computed Tomography
EBV	Epstein Barr Virus
EFS	Event-Free Survival
HRS	Hodgkin and Reed-Sternberg
OR	Odds Ratio
PAX-5	Paired Box 5
PD-1	Programmed Death 1
PD-L1	Programmed Death Ligand 1
PET	Positron Emission Tomography
PFS	Progression Free Survival
PHL	Pediatric Hodgkin Lymphoma
ROC	Receiver Operating Curve
TARC	Thymus and Activation-Regulated Chemokine
TME	Tumor Microenvironment

AbstractImmunohistochemical markers are associated with treatment outcome in adults with classical Hodgkin Lymphoma (cHL). Studies in children are scarce and inconsistent. We investigated in 67 children with cHL, whether the expression of CD15, CD30, PAX5, PD-1, PD-L1, CD68, CD163 and TARC is associated with the event-free survival (EFS) and the interim remission status. Low expression of PD-L1 was significantly associated with complete remission at interim PET-scan. There was no association between marker expression and EFS. Our data suggest a difference between pediatric and adult cHL. This underlies the importance of future research into specific molecular drivers in pediatric cHL.

IntroductionClassical Hodgkin lymphoma (cHL) contains a notably small amount of 0.1-10% malignant Hodgkin and Reed-Sternberg (HRS) cells, surrounded by inflammatory cells. These cells produce different cytokines and chemokines, maintaining a specific tumor microenvironment (TME) in which the HRS cells can thrive [1,2]. There is a strong variation among individual patients in the frequency and distribution of HRS cells and the TME [3]. These variations may be used to identify prognostic markers in pediatric patients to develop risk-adapted treatment strategies, leading to better outcome and less treatment-related toxicities [1,2]. Furthermore, these markers could be new treatment targets. Multiple studies have demonstrated that the presence of certain immune cell types and immunohistochemical markers in the TME and on HRS cells

is associated with treatment outcome [4-7]. However, most studies have been performed in adult patients, and studies based on pediatric populations are scarce and inconsistent [8-10]. Previous studies have shown differences in TME composition, PD-L1 expression, and the role of Epstein Barr Virus (EBV) between pediatric and adult cHL patients [10-14]. Therefore, it is uncertain whether outcomes of studies in adults with cHL are applicable to children with cHL.

Therefore, we investigated the prognostic value of eight different immunohistochemical markers in pediatric cHL. PD-1, PD-L1, CD15, CD30, CD68 and CD163 have previously been analyzed in pediatric studies and show conflicting outcomes [8-10,15]. PAX5 and TARC were chosen based on their prognostic impact in adult studies[6,16,17].

MethodsA detailed description of our study method is provided in the supporting information. An event-enriched cohort was created to increase the number of patients with relapsed disease and consequently the statistical power of this study. We collected 73 samples of children (23 relapses/50 complete remission) diagnosed with cHL at the Erasmus Medical Center - Sophia Children's Hospital. We analyzed CD15, CD30, PAX5, TARC and PD-L1 expression levels on the HRS cells, and PD-1+, PD-L1+, CD68+ and CD163+ cell counts in the TME. Primary outcome was the event free survival (EFS). The secondary outcome was the achievement of complete remission at interim PET scan and/or computed tomography (CT).

Results Sixty-seven of 73 patients met the inclusion criteria (Supplemental Figure S1). Baseline characteristics are presented in Supplemental Table S1. The mean follow-up time was 5.4 years (range 1.6 – 7.2 years). Twenty-two patients experienced treatment failure (32.8%), five of these patients (7.5%) passed away. We found no association between the expression of any of the immunohistochemical markers and EFS (Supplemental Table S2). All markers besides CD163 had an area under the curve (AUC) of < 0.5 and CD163 had an AUC of 0.613, suggesting a poor discrimination ability (Figure 1). Additional stacked histograms showed that none of the markers had discriminative power for EFS (Figure 1). There was also no association between any of the markers and early versus late relapse (Supplemental Table S3)

Thirty patients (44.8%) did not achieve complete remission (CR) at interim PET scan. Significantly more females than males achieved CR (Odds Ratio (OR) 3.60, 95% CI 1.31 – 9.90, $p = 0.012$). Patients with lower stage were more likely to achieve CR at interim PET-scan (OR 2.97, 95% CI 1.08 – 8.18, $p = 0.033$). For the total group, there were no statistically significant differences in achievement of CR at the interim PET scan for expression of any of the markers (Supplemental Table S4). A sub-analysis, only including patients treated according to the EuroNet-PHL protocols (n=45), showed that low expression of PD-L1 in the TME was significantly associated with complete remission ($p = 0.04$) (Table 1).

Discussion

The results of our study add significantly to the limited data available for pediatric patients. This is due to the high percentage of relapsed patients in our cohort and to the high number of markers analyzed[8-10,15]. We found an association between PD-L1 expression and interim remission status, which is not reported in the literature before. PD-L1 plays an important role in the pathogenesis of cHL. It is upregulated through amplification of chromosome 9p24.1 and may support cHL proliferation [18]. PD-L1 also contributes to the immune escape of the tumor [19]. This may explain why patients with cHL respond so well to PD-1/PD-L1 checkpoint inhibition [19-21]. Our findings imply that PD-L1 expression in pediatric cHL is correlated with outcome. This is an important finding that should be further investigated in a larger cohort of pediatric patients.

Remarkably, there are some differences between our data and previously published findings in children with cHL (See Supplemental Table S5 for a summary of the findings of previous studies). Barros et al. found a significant association between the expression of CD163 and the progression-free survival (PFS) [8], however, our study and the study of Gupta et al. found no significant association. Gupta et al. and Dinand et al. found a significant association between CD30 and CD15 expression and EFS [9,15]. We were not able to confirm this finding. These differences may be caused by differences in treatment protocols between the different studies. Second, our study contained a much higher percentage of events than the study of Barros

and Dinand [8,15]. Third, expression patterns in our study were based on lager areas and exact percentages were calculated.

Interestingly, our results are in contrast with previous findings in adult patients. They mostly reported an adverse association between OS and EFS and the expression of PAX5 on the HRS cells, and CD68+, CD163+, PD-1+, and PD-L1+ in the TME [4-7]. These differences between pediatric cHL and adult cHL may be due to differences in pathogenesis, resulting from differences in the composition of the TME[10-14]. Moreover, differences may be due to the use of different antibodies and/or scoring methods.

We found high expression of CD30 and PD-L1 on HRS cells, and PD-1 and PD-L1 in the TME in all patients. CD30, PD-L1 and PD-1 are all markers that can be targeted therapeutically (e.g. by brentuximab vedotin binding to CD30). The expression in pediatric patients underscores the need for further investigations of these novel therapies in first line treatment in children.

Our study is the first to examine eight different markers simultaneously. Another strength of our data is the event-enriched setting particularly in pediatric patients. However, due to this event-enriched setup, we included patients diagnosed over a period of 18 years with different treatment regimens. Despite this event-enriched cohort, the study still lacked the statistical power to perform a sub-analysis per treatment protocol for the primary outcome.

A possible limitation of the study is the use of immunohistochemical staining. This can lead to inconsistent results depending on differences in tissue fixation, the type of antibody and staining method, the scoring method, and the observer's interpretation. To minimize these inconsistencies, we based our scoring method on published previous studies [4,7,14,22,23] and we tried to overcome the interobserver variability by using a dual-head microscope and reviewing tissue samples together.

In conclusion, we found an association between PD-L1 expression and interim remission status, which should be further investigated. Furthermore, our data demonstrate differences in the expression patterns and prognostic impact of immunohistochemical markers between pediatric and adult patients with cHL. Further research into these differences may lead to specific prognostic factors in pediatric cHL, indispensable for improvement of treatment in this population.

Conflict of Interest statement

The authors have no conflict of interest to disclose.

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References

1. Aldinucci D, Gloghini A, Pinto A, De Filippi R, Carbone A. The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape. *J Pathol.* 2010;221(3):248-263.

2. Jacobson CA, Longo DL. Hodgkin's Lymphoma. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J, eds. *Harrison's Principles of Internal Medicine, 20e.* New York, NY: McGraw-Hill Education; 2018.

3. Cuccaro A, Bartolomei F, Cupelli E, Galli E, Giachelia M, Hohaus S. Prognostic factors in hodgkin lymphoma. *Mediterr J Hematol Infect Dis.* 2014;6(1):e2014053.

4. Guo B, Cen H, Tan X, Ke Q. Meta-analysis of the prognostic and clinical value of tumor-associated macrophages in adult classical Hodgkin lymphoma. *BMC Med.* 2016;14(1):159.

5. Hollander P, Kamper P, Smedby KE, et al. High proportions of PD-1(+) and PD-L1(+) leukocytes in classical Hodgkin lymphoma microenvironment are associated with inferior outcome. *Blood Adv.*2017;1(18):1427-1439.

6. Nguyen TT, Frater JL, Klein J, et al. Expression of TIA1 and PAX5 in Classical Hodgkin Lymphoma at Initial Diagnosis May Predict Clinical Outcome. *Appl Immunohistochem Mol Morphol.* 2016;24(6):383-391.

7. Steidl C, Lee T, Shah SP, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med.*2010;362(10):875-885.

8. Barros MH, Hassan R, Niedobitek G. Tumor-associated macrophages in pediatric classical Hodgkin lymphoma: association with Epstein-Barr virus, lymphocyte subsets, and prognostic impact. *Clin Cancer Res.* 2012;18(14):3762-3771.

9. Gupta S, Yeh S, Chami R, Punnett A, Chung C. The prognostic impact of tumour-associated macrophages and Reed-Sternberg cells in paediatric Hodgkin lymphoma. *Eur J Cancer.* 2013;49(15):3255-3261.

10. Jimenez O, Colli S, Garcia Lombardi M, Preciado MV, De Matteo E, Chabay P. Epstein-Barr virus recruits PDL1-positive cells at the microenvironment in pediatric Hodgkin lymphoma. *Cancer Immunol Immunother.* 2021;70(6):1519-1526.

11. Barros MH, Vera-Lozada G, Soares FA, Niedobitek G, Hassan R. Tumor microenvironment composition in pediatric classical Hodgkin lymphoma is modulated by age and Epstein-Barr virus infection. *Int J Cancer.*2012;131(5):1142-1152.

12. Keegan TH, Glaser SL, Clarke CA, et al. Epstein-Barr virus as a marker of survival after Hodgkin's lymphoma: a population-based study.*J Clin Oncol.* 2005;23(30):7604-7613.

13. Nagpal P, Descalzi-Montoya DB, Lodhi N. The circuitry of the tumor microenvironment in adult and pediatric Hodgkin lymphoma: cellular composition, cytokine profile, EBV, and exosomes. *Cancer Rep (Hoboken).* 2021;4(2):e1311.

14. Roemer MG, Advani RH, Ligon AH, et al. PD-L1 and PD-L2 Genetic Alterations Define Classical Hodgkin Lymphoma and Predict Outcome.*J Clin Oncol.* 2016;34(23):2690-2697.

15. Dinand V, Malik A, Unni R, Arya LS, Pandey RM, Dawar R. Proliferative index and CD15 expression in pediatric classical Hodgkin lymphoma. *Pediatr Blood Cancer.* 2008;50(2):280-283.

16. Hsi ED, Li H, Nixon AB, et al. Serum levels of TARC, MDC, IL-10, and soluble CD163 in Hodgkin lymphoma: a SWOG S0816 correlative study.*Blood.* 2019;133(16):1762-1765.

17. Peh SC, Kim LH, Poppema S. TARC, a CC chemokine, is frequently expressed in classic Hodgkin's lymphoma but not in NLP Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and most cases of anaplastic large cell lymphoma. *Am J Surg Pathol.* 2001;25(7):925-929.

18. Green MR, Monti S, Rodig SJ, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood.*2010;116(17):3268-3277.

19. Xie W, Medeiros LJ, Li S, Yin CC, Khoury JD, Xu J. PD-1/PD-L1 Pathway and Its Blockade in Patients with Classic Hodgkin Lymphoma and Non-Hodgkin Large-Cell Lymphomas. *Curr Hematol Malig Rep.*2020;15(4):372-381.

20. Shanbhag S, Ambinder RF. Hodgkin lymphoma: A review and update on recent progress. *CA Cancer J Clin.* 2018;68(2):116-132.

21. Ramchandren R, Domingo-Domenech E, Rueda A, et al. Nivolumab for Newly Diagnosed Advanced-Stage Classic Hodgkin Lymphoma: Safety and Efficacy in the Phase II CheckMate 205 Study. *J Clin Oncol.*2019;37(23):1997-2007.

22. Mavili HS, Isisag A, Tan A, Miskioglu M, Baraz LS, Nese N. Relationship of Tumor-Associated Macrophage Population Detected by CD68 PG-M1, CD68 KP1, and CD163 with Latent EBV Infection and Prognosis in Classical Hodgkin Lymphoma. *Turk Patoloji Derg.*2021;37(2):130-138.

23. Tanaka Y, Maeshima AM, Nomoto J, et al. Expression pattern of PD-L1 and PD-L2 in classical Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, and gray zone lymphoma. *Eur J Haematol.*2018;100(5):511-517.

Legends

Figure 1. ROC curves and stacked histograms. ROC were used to calculate the Youden's Index for the individual immunohistochemical markers to determine the optimal cut-off values. The stacked histograms show the expression of the markers among the two different groups. Curves and histograms for the markers PAX5 (A), CD15 (B), CD30 (C), PD-L1 on HRS cells (D), TARC (E), CD163 (F), CD68 (G), PD-1 (H), and PD-L1 in the TME(I).

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