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The prognostic impact of tumour-associated macrophages and Reed-Sternberg cells in paediatric Hodgkin lymphoma ☆

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Abstract ***Background:*** Tumour-associated macrophages (TAM) are associated with treatment failure in adults with Hodgkin lymphoma (HL). Equivalent data in paediatric HL are sparse. We aimed to determine the prognostic significance of TAM and Reed-Sternberg (RS) cells in paediatric HL.

Methods: All children aged 0–18 with HL between 1980 and 2009 with available diagnostic biopsy material were identified. A treatment failure-enriched cohort was assembled. Demographic, disease and outcome data were abstracted. Tissue microarrays with duplicate cores were constructed from diagnostic biopsy material and stained with immunohistochemical markers for TAM (CD68, CD163) and RS (CD30). A high score was defined as >5% positive cells relative to overall cellularity in any core. The association of candidate variables with event-free survival (EFS) was determined using Cox proportional hazards.

Results: The final study cohort comprised 96 patients with a median age of 14 years (interquartile range 11–15). Agreement on scores between cores from the same biopsy revealed weighted kappas of 0.60, 0.68 and 0.73 for CD30, CD68 and CD163 respectively, indicating moderate tumour heterogeneity. In univariate analysis, a high CD30 score was significantly associated with treatment failure (hazard ratio (HR) 2.27; 95th confidence interval 1.01–5.11; $p < 0.05$). High CD68 and CD163 scores were not associated with EFS.

Conclusions: Unlike adult HL, a higher percentage of RS cells was associated with poor outcome, while a higher percentage of TAM was not. Adult HL findings may not extend to paediatric HL. Cooperative group trials of paediatric HL should prospectively determine the association of different components of the tumour microenvironment with outcome.

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1. Introduction

Hodgkin lymphoma (HL) is commonly encountered by paediatric oncologists.[1] Multimodality treatment with chemotherapy and radiation has led to cure rates in excess of 80%, but at the cost of significant therapy-related late effects including infertility, cardiac and respiratory dysfunction and second malignancies.[2,3,4] Novel prognosticators available at the time of diagnosis may have significant impact by identifying children in whom treatment de-intensification is warranted. Alternatively, such prognosticators could also identify the children who are likely to fail treatment, allowing for upfront intensification.

Recently, several studies have examined whether various aspects of the tumour microenvironment may play such a role. Uniquely among malignancies, the malignant cells in HL, Reed-Sternberg (RS) cells, can comprise less than 1% of the tumour cell population. The composition of the remaining 99%, called the tumour microenvironment, is determined by the complex expression of various cytokines and chemokines by both RS and reactive cells.[5] The most promising potential prognosticator seems to be the presence of tumour-associated macrophages (TAM), which multiple investigators have found to be associated with inferior survival among adults with HL.[6,7,8] As of yet, equivalent data for paediatric HL patients are sparse. The prognostic value of the burden of RS cells is relatively unexplored in both populations.

Our primary objective was therefore to determine the prognostic significance of the percentage of both TAM and RS cells using the immunohistochemical markers CD68 and CD163, and CD30, respectively, in diagnostic biopsy specimens among children with HL.

2. Methods

2.1. Study population

The study followed a retrospective cohort design. In order to increase statistical power, a cohort enriched with cases of treatment failure was assembled. This technique has been used in past studies of tumour microenvironment.[6] All patients aged 0–18 years and diagnosed at The Hospital for Sick Children between 1980 and 2009 were identified using a local electronic patient database. Patients were excluded for the following reasons: (1) nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), (2) post transplant lymphoproliferative disease, (3) prior immunodeficiency, (4) synchronous additional malignant diagnoses and (5) initial diagnostic biopsy material insufficient or unavailable for immunohistochemical studies. In cases where the initial biopsy was performed at an outside hospital, every effort was made to obtain the original biopsy material.

Using the same electronic database, all patients experiencing disease progression, relapse or death were identified and were added to the study cohort. The remaining patients (i.e. those who experienced long-term progression-free and relapse-free survival) were then identified. Patients from this remaining population were then randomly selected using a computer-generated random number sequence generator and added to the study cohort until a total sample size of 100 patients was reached; this total was determined *a priori* based on feasibility and study resources.

2.2. Immunohistochemistry

Tissue microarrays (TMAs) were constructed from duplicate 1.0 mm cores from formalin-fixed paraffin-embedded blocks of diagnostic biopsy tissue. Cores were obtained from representative areas containing RS cells as evaluated by morphology and immunohistochemical stains that were performed for routine diagnosis. A total of 22 samples had insufficient material to be included in the TMA but sufficient material for whole section immunohistochemical staining. These 22 patients were retained in the study cohort, with immunohistochemical staining performed on whole sections. Three arrays were built from the remaining 78 cases in duplicate with cases arranged randomly across the TMAs. Both four micron sections from the TMAs and whole sections were cut and stained with haematoxylin and eosin (H&E) and the immunohistochemical markers CD68 (predilute, clone KP1, Ventana), CD163 (1:100, clone 10D6, Leica) and CD30 (1:50, clone BerH2, Dako Canada). The immunohistochemical stains were performed on an automated stainer, Ventana Benchmark XT (Ventana Medical Systems, Tucson, Arizona), using a multimer detection kit (ultraView Universal DAB, Ventana Medical Systems, Tucson, Arizona).

Two paediatric pathologists (C.C. and R.C.) independently scored the TMA and whole section slides. Two areas of whole section slides were scored. Both pathologists were blinded to patients' clinical characteristics and treatment outcomes. Immunoreactivity was scored based on the percentage of cells showing cytoplasmic (CD68, CD163) and membranous and golgi (CD30) staining, relative to overall cellularity as follows: 1 = <5%, 2 = 5–25%, 3 = 26–50%, 4 = >50%. Discrepancies between the two reviewers were discussed until consensus was reached.

2.3. Data abstraction

The charts of patients were obtained and data relating to patient characteristics, disease, treatment and outcome were abstracted. Patients were classified as belonging to the early time period if diagnosed between 1980 and 2000, and to the late period if diagnosed

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