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REVIEW ARTICLE

Prognostic Factors in Pediatric Classical Hodgkin Lymphoma: Concepts, Questions and Perspectives

Mário Henrique M. Barros[1], Adriana Morais[2], Vera Morais[2], Rocio Hassan[1,3], MariaTereza Cartaxo Muniz[2]

Abstract:
Hodgkin´s Lymphoma (HL) is a disease typically affecting children and young adults, with more than 80% of patients being cured. The other side of high cure rate is that a fraction of patients will receive excessive antineoplastic radiochemotherapy resulting in the well-recognized late effects of HL treatment. Current clinical and radiological characteristics used for risk stratification in most treatment centers lead to mistaken stratification in almost one third of patients. Prognostic factors in HL are, mostly, crude direct measures of tumor burden and activity (stage, number of involved lymph nodes, bulky disease, B symptoms) or indirect surrogate measures of tumor burden and activity based on laboratory parameters (hemoglobin, s-albumin levels). Clinical characteristics at presentation, as well as protein immunoexpression and Epstein-Barr virus (EBV) association, have also been identified as prognostic factors in several studies. However, when sufficiently intensive treatment for advanced stages is employed, adverse prognostic factors tend to disappear. Thus, the identification of clinical and biological factors that allow discrimination of patients who may undergo a reduction in treatment intensity is a current goal to reduce late effects in HL.
***Keywords*:** *Hodgkin lymphoma, childhood, adolescents, prognostic factors, EBV, microenvironment*

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Introduction

Hodgkin lymphoma is a lymphoid neoplasm characterized by the presence of a few tumor Hodgkin and Reed-Sternberg (H-RS) cells, mostly originated from geno and phenotypically aberrant B cells, amidst an intense inflammatory infiltrate. Based on morphological and phenotypic characteristics and composition of the cellular infiltrate, Hodgkin lymphoma is subdivided into classical and nodular lymphocyte-predominant Hodgkin lymphoma (cHL and NLPHL, respectively) [1].

cHL is one the most frequent lymphomas in the Western world. In the United States, it is the 9th most frequent in children until 15 years and the most frequent in adolescents (16 to 18 years) [2]. Since the introduction of high voltage radiotherapy and polychemotherapy (MOPP-regimen), HL became a curable disease [3]. Nowadays, more than 80% of patients are cured, but this significant improvement in the survival is accompanied by the late effects of treatment [4]. The challenge for pediatric oncologists today is minimize the late toxicity while maintaining the excellent survival; the identification of risk factors is essential to this objective. Those prognostic factors are used to try minimize the over exposition to the anti-neoplastic treatment, reserving the most toxic and presumably more active

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treatments for patients with the worst prognosis and the least toxic, but possibly less effective treatments, for patients with the best prognosis [5, 6].

Prognostic factors in HL are, mostly, crude direct and indirect measures of tumor burden and activity [7,8]. Clinical characteristics at presentation [9,10] as well as protein immunoexpression [1, 9, 11, 12] and Epstein Barr Virus (EBV)-association [13, 14,15] have also been identified as prognostic factors in several studies. However, prognostic indices have been developed mostly for adult cHL, but the number of studies in childhood cHL is still limited [16]. Understanding the relationship of clinical and biological characteristics of the disease with clinical response is essential to therapeutic decision making and drawing of new treatments pointed to the decrease of the late effects in pediatric cHL.

Epidemiological and Demographical Aspects

cHL is subdivided in four histological subtypes: nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich (LR) and lymphocyte depletion (LD) [1]. MC and NS subtypes exhibit different prevalence in cHL, in respect to age and socio-geographical distribution. Three epidemiologic patterns of cHL, according to the level of socioeconomic development, have been described. In pattern I, seen in underdeveloped countries, cHL incidence shows an early childhood peak, and the predominant cHL subtype is MC. Pattern II, observed in developing or transitional economies, displays both a childhood and a second decade peak, and equal frequencies of MC and NS subtypes. Finally, in pattern III, observed in developed countries, cHL shows a third decade peak and a predominance of NS over other subtypes [17]. MC is the most frequent subtype in underdeveloped countries and in childhood cHL [17,18]. However, in developing countries, a predominance of NS subtype can be observed in pediatric cHL, which does not conform to any of the previously described patterns [19].

Age has been demonstrated to be a prognostic indicator of outcome in HL, with low risk children having an OS of more than 90%, but older adults having a poor outcome [20,21]. These distinct outcomes have been attributed to differences in disease biology between young and old patients [21], and/or differences in the tumor microenvironment composition, secondary to underlying differences in the immune response. Of course, it is possible that the co-morbidity in older patients has some contribution to the outcome differences [21] .

cHL is the most common neoplasm among adolescents [2,22] and some results are pointing to a worse outcome in adolescents with cHL when compared to young adult patients [20,22,23] and young children [22]. An important point of discussion about the differences in survival between adolescents and young adults is the lack of uniformity in treatment criteria, since adolescents are treated with adult or pediatric protocols depending on center specific policies and referral patterns. It is possible that the poor outcome of adolescents observed in the past studies may be attributable to their treatment with adult regimens, rather than the risk-adapted combined modality treatment that is used nowadays in many pediatric centers [20].

In fact, some present studies showed no differences in the outcome between adolescents and young adults when treated with the same protocols [24,25]. In our 2 different Brazilian groups of pediatric cHL (Brazilian National Cancer Institute, and Centro de Oncohematologia Pediátrica), we did not observe differences in the outcome between young children and adolescents [26,27].

Tumor Burden

Tailoring of cancer treatment is classically based on the estimation of the total number of tumor cells: as higher the tumor burden, more intensive should the treatment be. The Ann Arbor staging is a simple method to stratify cHL patients and it has been shown to be of prognostic significance for disease free survival (DSF) and overall survival (OS) estimation in several pediatric studies [26, 28, 29,30,31]. However, Ann Arbor staging is unable to accurately predict tumor burden, because it does not take into account the number of involved anatomic sites. For example, a stage IIA patient with cervical and axillary nodes, both on the same body side, would have a lower tumor burden than another patient with no symptoms and cervical and axillary bilateral involvement, who would also be staged as IIA. Therefore, it is possible that a fraction of advanced Ann Arbor stage HL children are over- or under-treated [26,32].

The combination of Ann Arbor staging and presence/absence of B-symptoms are used to stratify cHL patients into two risk groups: low risk-group (or early stage) and high risk-group (or advanced stage). There is no consensus about the best grouping, but the IIB, IIIB and IV are uniformly considered as advanced stages, compared to I, IIA and IIIA stages [16,26, 28,33,34]. An intermediate stage is incorporated by the German-Austrian group [31], which includes patients with I_E, II_EA, IIB, IIIA stages; but most of the studies consider these children as having advanced disease [35].

The number of involved anatomic areas (IAA) may be a good marker of tumor burden, a strong adverse prognostic factor for cHL. Vassilakopoulos et al. showed for the first

time a significant influence of this variable on DFS prediction in adolescents and adults with advanced stage [33]. However, the effect of the number of IAA on DFS could not be replicated in a pediatric study [16]. The discordance between the results of both studies might be due to the fact that the latter only considered the number of involved nodal sites. In a pediatric HL retrospective series studied by us, children with more than 4 IAA (nodal and extranodal) had a 6.4 fold increased risk of unfavorable outcome (p= 0.0001) [26]. We also observed that a sub-group of children with better prognosis can be identified among the unfavorable risk patients, based on tumor burden as defined by number of IAA, which suggested that the main negative consequence of tailoring chemotherapy based only on clinical stage and/or risk-group is the overtreatment of a group of patients stratified as advanced-disease, without having a high tumor burden [26] .

Computed tomography (CT) is the principal technique to assess the distribution of the disease, however this imaging modality also has several limitations given that interpretation of nodal involvement is based only on anatomic criteria such as size and shape, making it often impossible to discriminate lymphoma lesions from benign CT abnormalities [36].

The fluorodeoxyglucose (FDG) positron emission tomography (PET) scan is the best method to staging pediatric cHL, this technology may improve the staging in until one third of pediatric patients [37]. This is a functional whole-body imaging method, which allows for the visualization and quantification of the glucose uptake in tissues (typically increased in HRS cells). It provides both functional metabolic data from FDG-PET and structural anatomic information from CT in one examination. The combination of the high sensitivity and specificity of FDG-PET with the high anatomical resolution of CT improves the diagnostic accuracy for detection of malignant lymph nodes (even the ones with a size of less than 1 cm) [38].

Early PET-responsive disease is associated with an excellent prognosis and can be used to modify therapy [29,37,39]. Likewise, persistent FDG uptake after front-line chemotherapy is associated with relapse [37,40]. In adults, the analysis of the HD15 trial (German-Austrian group) has shown that consolidation radiotherapy could be omitted in PET-negative patients after effective chemotherapy [41]. PET-oriented therapy appears to be a promising approach to reduce toxicity for patients undergoing chemotherapy.

Data on pediatric HL patient are rare, showed discordant results, and were mainly obtained retrospectively [42,43]. A recent prospective multicenter trial [44] assessed early and late therapy response in 40 pediatric HL patients and concluded that it helps to identify patients with excellent prognosis, which might benefit from de-escalation of antineoplastic therapy.

The erythrocyte sedimentation rates (ESR), lactic dehydrogenase level (LDH), β2-microglobulin level, haemoglobin level and number of leukocytes/lymphocytes are used routinely in the pediatric oncology practice, but nowadays with the risk-adapted treatment, the majority of these variables are incapable to predict the outcome of pediatric cHL [32]. Among the serological markers considered to evaluate prognosis in HL, several studies, none with a focus in pediatric cHL that showed an adverse prognostic impact of the high level of serum IL10 [33,45,46]. IL10 is an immunoregulatory cytokine with pleiotropic activity, produced by macrophages, dendritic cell DC, B cells and various subsets of CD4 and CD8 T cells [47]. IL10 inhibits cytokine production by both T cells and NK cells via inhibition of accessory cells function. It is possible that high serum levels of IL10 reflects a systemic response to a high tumor burden; however, IL10 production may be a marker of immune dysfunction and have consequences at the systemic as well as at the microenvironmental levels (see below).

Treatment Overview

The successful treatment of HL has been one of the most significant accomplishments in cancer therapy over the last century. Since the introduction of extended field radiotherapy and MOPP combination chemotherapy, more than 60% of patients can be cured [48]. Nowadays, six to eight courses of ABVD are considered as the standard treatment for patients with advanced stage HL. On the contrary, the optimal treatment strategy for early stage HL is still the subject of intense debate [48].

In concern to intermediate and high-risk HL, multiple clinical questions remain, such as the ideal CT regimen and the optimal extent of radiation therapy. Despite late squelae (infertility, second malignancy, cardiopulmonary toxicity) and knowledge that total dose and rate of drug delivery impact treatment efficacy, the adequacy of low-intensity regimens deterred efforts to improve them. The goals are to achieve excellent treatment efficacy with reduced cumulative therapy, thereby limiting the potential for long term toxicity.

Previous trials in advanced HL [28] showed that rapid early response (RER) was predictive of event free survival (EFS), whereas later response (at completion of chemotherapy) was not predictive. This supported the premise that RER was a measure of chemosensitivity. On this basis, Schwartz et al described a risk-adapted,

response based approach using a dose-dense chemotherapy regimen, ABVE-PC (doxorubicin, bleomycin, vincristine, etoposide, predinisone, cyclofosfamide) has been used with the goal of enhancing tumor cytotoxicity and inducing rapid tumor responses. This dose-dense early-response-based treatment minimizes cumulative therapy and offers 84% 5-year EFS. Current Children Oncology Group (COG) trials evaluate the elimination of radiation therapy in early responders, considering the hypothesis that early response to chemotherapy can identify the patients with tumors that are sufficiently chemo sensitive.

A prospective, randomized, multicenter trial [49] compared ABVD and Stanford V (mechloretamine, doxorubicin, vimblastine, prednisone, vincristine, bleomycin and etoposide) for the treatment of advanced HL. In this trial, ABVD was no bettered by Stanford V (SV) regimen, despite the use of RT in the majority of instances. ABVD offers the potential to avoid radiotherapy in patients who experience complete remission. However, for some patients, SV can be the first choice regimen because of the brief duration of treatment and reduced risk of acute pulmonary toxicity.

For many, ABVD still represents the standard initial treatment regimen for advanced HL; however, excellent responses and progression free survival have been documented with standard and escalated regimens of bleomycin, etoposide, doxorubicin, cyclofosfamide, vincristine, procarbazine and prednisone (BEACOPP) [35]. Several groups have compared BEACOPP with ABVD in randomized trials, but neither has been established yet as definitely superior [50,51].

Over the last decade, some trials have explored the possibility of treating HL patients with CT alone [52], [53]. There is now increasing evidence that early stage HL patients are likely to be cured by four to six courses of ABVD alone, thus avoiding RT altogether [35,54]. The rationale for reduced radiation therapy field size is to further improve the therapeutic ratio. In patients treated with combination therapy, reducing the radiation therapy field size from extended-field radiation therapy (EFRT) to involved-field radiation therapy (IFRT) is associated with a reduction in radiation induced toxicity rates, without compromising overall survival (OS) or freedom from treatment failure (FFTF) [55,56].

The involved-nodal radiation therapy (INRT) has been proposed as a means to further improve the therapeutic ratio by reducing the risk of acute radiation-induced toxicity and potentially reducing the rate of long-term complications, including second malignancies [57]. The rationale for this approach is based on the observation that after chemotherapy alone, most relapses of HL occur in previously involved nodes; therefore, it has been extrapolated that the addition of INRT should be equivalent to IFRT in preventing local relapse [58]. Based on this premise, it follows that the success of INRT will be dependent on the sensitivity of detecting and localizing sites of HL for radiation therapy (when FDG-PET is strongly recommended). So, this reduction in RT field size does not appear to be associated with an increased risk in local regional relapse or marginal recurrence [59]. With the use of more modern imaging modalities and radiation therapy techniques which permit for tighter radiation therapy margins, further reductions in field size might be safely undertaken [59].

Histological and Immunoexpression Characteristics

Among the four histological subtypes of cHL (NS, MC, LR and LD), NS is the more heterogeneous subtype, and can be stratified according to the WHO classification in NS grade I and grade II [1]. In this grade system, the NS-GII is characterized by various nodules with high number of H-RS cells. In the past, NS-GII was associated with a worst outcome [60], but with the current treatments this histological grading lost prognostic significance [61].

A new NS-graduation system was proposed by von Wasielewski et al [62] based on eosinophil count, cell atypia and lymphocyte depletion; cases with eosinophilia (> 5% of all cells or clusters in at least 5 high-power fields) and/or lymphocyte depletion (< 33% of all cells in the whole section) and/or atypia in the neoplastic cells (>25% of H-RS cells bizarre and highly anaplastic appearing with pleomorphic nuclear features, hyperchromatism, and highly irregular nuclear outlines) are considered as NS-high risk and cases without none of these factors are called NS-low risk [62]. This study was conducted predominantly with adult patients. In our pediatric group, we did not observe prognostic significance of this new NS-graduation system, however we observed that high-risk NS was associated with features of a more aggressive disease, such as presence of mediastinal mass, higher number of neoplastic cells and p53 accumulation [27].

In adults, as well as in children patients, the cHL histological subtypes have not shown prognostic significance. Histological characteristics, such as interfollicular pattern, number of H-RS cells, number of mitotic H-RS cells, type and number of inflammatory cells are not frequently investigated in pediatric HL. In our pediatric HL group, we observed an association between MC subtype and low aggressive disease at diagnosis, including low risk patients, low number of mitotic H-RS cells, B cell differentiation and EBV-association,

suggesting that, even though not associated with clinical response, histological characteristics may reflect disease activity and aggressiveness [27]

The prognostic significance of the immunophenotype of H-RS cells is controversial. In the past decade it was demonstrated that H-RS cells almost always derive from pre-apoptotic B-lymphocytes [63]. Despite this B cell origin, one of the characteristics of H-RS cells is the loss of B cell markers [63]. In fact, the pan-B cell marker CD20 is expressed only in 20-40% of cHL [1]. In adult series, the prognostic role of CD20 expression is not clear, with some studies showing association with unfavorable outcome and the majority showing no prognostic association [64, 65,66]. There are few reports on pediatric series, and in all of them, CD20 was not associated with outcome [67,68].

Most of cHL cases express CD30, a 120 kDa transmembrane glycoprotein which is part of the nerve growth factor/TNF superfamily and a marker of lymphoid activation. The absence of CD30 expression seems not to be associated with prognostic significance in pediatric cHL [64,65,67,68,69].

CD15 is a group of fucosylated molecules that may function in cell adhesion and regulation of signaling cascades, pointing to an activation rather than a survival role in H-RS cells [70] and its expression is observed in 75-85% of cHL [1]. In adult HL, the prognostic value of CD15 is controversial [64,69,71,72,73], with only two studies showing clinical impact [64,69]. In pediatric series, Dinand et al, showed that CD15-negativity was associated with low OS, high stages (III/IV) and p53-negativity [68]. Conversely, in our pediatric group, CD15 was not associated with outcome [67].

One of the characteristics of cHL is the constitutive activation of the NFκB pathway [74], apoptosis resistance [75] and alterations in the cell cycle machinery [76]. Many immunohistochemical studies were designed to search for prognostic markers based in the biology of H-RS cells [7, 73,75,76,77], most of them with adult patients, being required the validation in the pediatric population.

Bcl2 is an inner mitochondrial membrane protein which inhibits apoptosis, so extending cell survival [78]. In adult cHL, its expression may be associated with clinical resistance to drug-induced apoptosis and poor outcome. Rassidaskis et al, in the largest study of the literature, showed that Bcl2 expression by H-RS cells had a worst prognosis in adults treated with ABVD or equivalent regimens, [65] and the same was observed by others [77], [75]. However, in some other studies, Bcl2 expression was not associated with survival [7,73]. In the few studies with pediatric cHL, Bcl2-expression was not associated with survival [27,79]. It is important to mention that in our series, although we have not observed prognostic association with Bcl2-expression, children expressing Bcl2 in $\geq$10% of H-RS cells were in a cluster characterized by a more aggressive disease [27]. Similar to other immunohistochemical studies, the observed differences could be associated with the different cut-offs adopted to consider a case Bcl2-positive.

p53 is a nuclear phosphoprotein involved in the regulation of transcription and cell growth. Mutations of p53 are the most commonly detected genetic abnormality in human neoplasms [80]. Those mutations induce a conformational change in the protein, rendering it stabilized; for this reason, nuclear overexpression of p53 by immunohistochemistry is meant to be correlated with p53 mutations. However, in some cases, p53 positivity occurs without detectable mutations [81,82], especially in cHL [83]. As dysfunctional p53 is associated with accumulation of errors in DNA, many studies were conducted to evaluate if this alteration could bear some prognostic impact. Like Bcl2, the association between p53 and outcome is controversial in adult cHL. Some studies showed an independent association of p53 accumulation with shorter survival, while some others failed to find such association [7,73,75,77,84]. A major problem in all of these papers is the lack of consensus about an ideal cut-off to consider p53-accumulation, making the data difficult to compare. Some authors use a low p53 cut-off because they believe that even few cells expressing p53 are sufficient to enable disease progress [73,75,77]. Others favor the use of a high cut-off (>50% of cells with p53 over-expression) [7,84], since p53 nuclear overexpression is a well-known phenomenon in HL, and low levels of expression are usually unrelated to the presence of gene alterations [85]. At this respect, we have used 50% as threshold to consider positivity for p53, because it is similar to that found to reflect p53 mutations in non-HL and HL [86,87].

In the few pediatric cHL series focusing on this molecule, p53 expression was not associated with outcome [79,88], even when p53 is evaluated in combination with p21 [27,79]. The combined expression of p53 and its downstream effector p21 is used to classify cases according to p53 functionality, because some cases with p53 over expression may maintain p21-functionality [89]. It is possible that either p53 over-expression is losing its prognostic value with the present efficiency of HL treatment, similar to what is occurring with other classical prognostic factors [32] or in children, p53 really do not contribute to a worst outcome. Since p53, like Bcl2, are important prognostic factors in adults, to answer these questions would be necessary a large prospective study with pediatric cHL. In our children, we have observed that

p53-positivity is associated with NS GII and a high number of mitotic H-RS cells, suggesting that loss of functional p53, and consequently the perpetuation of cell-cycle, is important in the composition of this NS histological subtype [27].

Cell proliferation markers, such Ki67, PCNA and Topoisomerase-IIα, have been evaluated as prognostic factor in several adult cHL series [73, 77, 90,91], some studies showing an adverse outcome for patients with high cellular proliferation index (PI) [73,77, 91]. The interest by these markers increased after a gene-profiling study showed a cell cycle regulatory signature, containing genes related to mitotic checkpoint, as differentially expressed between cases with good and poor outcome [90]. However, other studies were not able to confirm the prognostic impact of the cell proliferation markers [77], [92]. There are few studies including only pediatric cHL. Tiemann et al did not observe differences in the outcome of children and adolescents with high Ki67 expression by H-RS cells [93]; while Dinand et al. showed good failure free survival (FFS) in children with high PI [68]. Accordingly, we found a high FFS in children belonging to the unfavorable groups (IIB, IIB, and IV), when Ki-67 was expressed in >50% H-RS cells [67. cHL with high PI may represent a disease more responsive to chemotherapy, considering that drug sensitivity is proportional to the proliferating cell fraction.

Tumor Microenvironment

The tumor microenvironment in cHL has been considered to be a manifestation of host immune reactions to malignant cells [87]. The immune response in HL is likely to be inadequate because of the poor immunogenicity of H-RS cells, the immunosuppressive effect exerted by the tumor cells, or the poor response of the host immune system [94, 95]. The functional status of the reactive microenvironment was found to be associated with the number, subset type, and activation state of the reactive immune cells, specifically the cytotoxic (CTL) and regulatory T (Treg) cells [95,96,97,98,99,100]. In cHL, CD4 T cells are the largest population of infiltrating non-tumor cells [101]. A subset of these cells is Treg cells, characterized by a CD4+ CD25+ FoxP3+ phenotype, which are actively attracted to the microenvironment by H-RS cells [102], [94]. Treg cells can inhibit both interleukin (IL) 2 production and the up-regulation of IL-2Rα-chain (CD25) expression, thus delaying or blocking the activation of CD8 and natural killer (NK) cells [103], [104]. These immunosuppressive properties of Treg cells may be important in cHL, and may contribute to immune evasion by H-RS cells and, consequently, theirs survival [105].

From this, one would anticipate that a high number of Treg cells associated with low numbers of CTL would be associated with poor outcome, as observed in solid tumors [106]. However, in cHL the opposite is observed: cases with high number of CTL and low number of Treg cells are associated with poor survival and cases with low number of CTL and high number of Treg cells are related to better survival [98,102,105,107]. The full significance of infiltrating immune cells in the pathogenesis of cHL and the explanation to this prognostic difference continues to be obscure. In all cited studies, the determination of Treg cells was realized by immunohistochemistry for the detection of FoxP3, a protein expressed mainly in Treg cells [108]. However, recent reports in humans demonstrated FoxP3 expression also in activated conventional T cells without suppressive activity [109], [110,111]. Nevertheless, FoxP3 is currently considered the best single marker for the detection of Treg cells.

It is possible that CD4-positive Th2 cells may have anti-tumor activity in cHL. A recent paper [100] showed that low Th2/Treg cells ratio was associated with an adverse clinical outcome, suggesting a possible role of Th2-mediated anti-tumor immunity controlled by Treg cells in HL. This hypothesis is compatible with the worse survival of cHL patients that have a higher number of CTL.

Unfortunately, all prognostic studies based in the tumor microenvironment of cHL were realized with adults or a mix the old children/adolescents and adults, showing the importance of validating these prognostic factors in the pediatric population.

As an important immunoregulatory cytokine IL10 can have local effects directly on CD4 T cells, inhibiting proliferation and production of IL12 (a key cytokine for the differentiation of the Th1 cell subset), IFN-γ, IL4, IL5 and IFN-α [112]. Thus, IL10 can directly regulate innate and adaptive Th1 and Th2 responses by limiting T cell activation and differentiation in lymph nodes, as well as suppressing proinflammatory responses in tissues [47] IL10, as well as TGFb, can induce DC to change into a specialized tolerogenic DC (tDC) subset that is able to induce Treg cells. IL10 also inhibits production of both CC and CXC chemokines by activated monocytes; these molecules are implicated in the recruitment of monocytes, DC, neutrophils and T cells [112]. On the other side, H-RS cells can secrete IL10, contributing to an immunosupressive microenvironment.

Epstein-Barr Virus Association

EBV is a gamma-herpesvirus, which asymptomatically infects more than 90% of the human population and is implicated in the pathogenesis of several lymphoid and

epithelial neoplasms, such as undifferentiated nasopharyngeal carcinoma, Burkitt's lymphoma, posttransplant lymphoproliferations, and Hodgkin's lymphoma [113], [114].

The latent membrane protein 1 (LMP1) is the major viral oncogene, and is expressed in the tumour cells of virtually all cHL EBV-positive [115], It is proposed that LMP1 is involved in the pathogenesis of this disease, by rescuing H-RS precursors from apoptosis [116], [117].

Regarding EBV association, a 3-disease model was proposed for cHL, on the basis of age at diagnosis and EBV status [118]. The first entity is largely a disease of childhood, EBV-associated, with higher incidence in developing countries and usually of MC subtype. Development of cHL is probably associated with an early exposure to EBV infection, which occurs at a particularly young age in less economically developed countries. The second entity, predominantly affecting older adults, is also EBV-associated, usually of MC subtype, and likely to be related to EBV reactivation events. The third entity predominantly affects young adults. It is more prevalent in developed countries, usually of NS subtype, and not EBV-associated. In developed populations, tumor H-RS cells are infected by EBV in about 40% of cases of classic cHL [119]. On the other hand, a very high association of EBV with pediatric cHL from Latin America has been reported, where nearly all cases are EBV positive [120]. Notably, we have found the presence of EBV in only 54% and 48% in Argentine and Southeastern Brazil HL, respectively, and in Southeastern Brazilian pediatric HL, we observed a higher association of NS with EBV [79].

The prognostic value of EBV presence in H-RS cells is being investigated and the results are still inconclusive. In some studies, EBV was associated with unfavorable outcome [13,15], while others show the opposite [77,121,122], and still others do not show any impact of the virus on the survival [123,124,125]. It is important to note that the majority of these studies included adult patients whose median ages are variable. This is an important detail, especially in the studies where EBV is associated with unfavorable outcome, because old patients undergo EBV-reactivation with a higher frequency due to the progressive decay of their health status and impaired immunosurveillance. In these cases, the presence of EBV might be an indirect marker of co-morbidities and not the element causing the bad prognosis per se.

In pediatric cHL, this question has been less described. Engel et al (Engel et al, 2000) have established a significantly longer median survival in EBV-positive pediatric HL [126] . Keegan et al [127] showed that EBV-positive status was associated with a more favorable survival than EBV-negative status in a small subgroup of children, postulating that EBV might serve as a useful indicator of prognosis. Claviez et al, in contrast, reported that latent EBV expression has no influence on failure-free survival [128] as well as Dinand et al [18]. We observed in a collaborative study between Brazil and Argentina that EBV infection was not associated with outcome [79].

Prognostic Factors: What is next?

With the current risk-stratified treatment for cHL, long-term disease-free survivals were 85% to 100% in patients with early-stage disease, and of more than 60% in those with advanced disease, are obtained [18,68]. The other side of this high cure rate is that a fraction of patients will receive excessive anti-neoplastic radio-chemotherapy resulting in the well-recognized late effects of cHL treatment [26,61,129,130].

The current clinical and radiological characteristics used for risk stratification in most treatment centers lead mistaken stratification in almost one third of patients [7]. However, when sufficiently intensive treatment for advanced stages is employed, adverse prognostic factors tend to disappear [32]. Thus, the identification of clinical and biological factors that allow discrimination of patients who may admit a reduction of treatment intensity is a current goal to reduce late effects in cHL.

It is important to note, as already described, that many prognostic factors in cHL were derived from adult or adolescent/adult studies and it is necessary to validate these factors in pediatric population. A good example of this is the number of IAA that our group validated in pediatric population [26]. Our results indicated that a sub-group of patients with better prognosis can be identified among the unfavorable risk patients, based on tumor burden as defined by number of IAA and suggest that the main negative consequence of tailoring chemotherapy based only in the stage and/or risk-group (stage combined with presence of B-symptoms) is the overtreatment of a group of patients stratified as advanced-disease, without having a high tumor burden [26].

A sibling question would be the planning of treatment reduction for selected pediatric cHL groups aiming not only to the reduction of late effects but also including relevant information on clinical and biological characteristics of the tumor. Otherwise, the past prognostic factors could gain clinical relevance again. One multivariate study performed at the Brazilian National Cancer Institute in pediatric cHL disclosed that some histological, immunophenotype, cell cycle factors and EBV status cluster with aggressive disease at diagnosis [27]. Even when these variables cannot predict clinical

response at the present status of HL treatment, they can be relevant for planning the decrease of treatment.

What comes next? About pediatric cHL, certainly the validation of prognostic factors found in the adult studies is a current goal. New protocols aiming reduce treatment intensity in select children group (and consequently decreased the late effects chances) is another objective in pediatric oncology. The association of molecularly targeted therapies, for instance focusing on microenvironment specific targets [131], with traditional chemotherapy would help to achieve this objective without losing efficiency.

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