Circulating tumor cells in Hodgkin’s lymphoma—A review of the spread of HL tumor cells or their putative precursors by lymphatic and hematogenous means, and their prognostic significance

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Abstract

About 15% of patients diagnosed with classical Hodgkin’s lymphoma (cHL) are considered high risk with unfavorable prognosis. The biology of the disease bears a direct relationship to its clinical course. However, some aspects of the disease are still being debated. Related topics include origin of neoplastic cells as circulating precursor versus germinal center B cell, and disease metastasis via hematogenous routes and the effect of HL circulation on relapse potential and further spread of the disease. The terminally differentiated giant neoplastic Hodgkin Reed–Sternberg (HRS) cells (HRSCs) have limited proliferation and lack mobility. Therefore, they are unable to penetrate epithelium. Thus, the clinical aggressiveness of HRSCs that disseminate via both lymphatic and hematogenous may be determined by their molecular composition. This review discusses in detail the historical perspectives on scientific and clinical evidences of precursors of circulating HL cells and the prognostic importance of these circulating cells for predicting outcome.

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Keywords: Hodgkin lymphoma; Circulating tumor cells; Metastasis; Clinical outcome; Biomarkers; Prognosis

1. Introduction

It has been almost 180 years since Thomas Hodgkin presented seven autopsy cases in his now famous paper “On some morbid appearances of the absorbent glands and spleen” to the Royal Medical and Chirurgical Society of London on January 10 and 24, 1832 (the text of which was published in the Transactions of the Medical and Chirurgical Society of London) [1]. Since then, although our understanding of the disease has increased astronomically, Hodgkin Lymphoma (HL) continues to be an enigma. For several decades, the cellular origin of HL neoplastic cells has been debated. However, within the last two decades or so, the B cell ancestry of the pathognomonic Hodgkin and Reed–Sternberg cells (HRSCs) has been established. The giant Reed–Sternberg (RS) cells are believed to be derived from Hodgkin’s (H) cells by a process similar to endomitosis. Thus, H cells may be the precursors of RS cells, which exhibit limited proliferation and may be terminally differentiated. It has further been speculated that the HRSC population is maintained by a putative circulating precursor (CP) or circulating cancer stem cell (CSC), a concept proposed in the late 1980s [2], but has recently received renewed attention [3], yet remains a subject of debate. The putative CP/CSC may also, in part, account for the spread of the disease. Although extranodal involvement occurs in 10–15% of all cases, metastasis by hematogenous means remains controversial. In addition, some differences in the molecular composition of HRSCs arise from genomic imbalances that resulted in either gain or loss of gene copy number in different subsets of HL patients [4]. In some instances, these changes seem to have a direct impact on the relative aggressiveness of the disease and patient’s response to treatment [4]. For example, since the multi-drug resistant gene MRPI (ABCC1) confers drug resistance, HRSCs that express MRPI may not respond well to ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine) treatment [4]. In this review, we examine historical and current materials that support the hypothesis that a putative CP maintains the HRSC population, and discuss the clinical and prognostic relevance of the putative precursors. We also examine evidence suggesting that dissemination occurs by both lymphatic and hematogenous spread. In addition, we discuss the mechanism(s) of chemo-resistance, putative mechanism(s) of metastasis, and the prognostic significance of extranodal disease.

2. Formation of RS-like cells

2.1. In vitro formation of RS-like cells from PBMC of HL patients

Historically, only a handful of investigators have suspected or expressed an interest in the idea that there was a putative CP that fed the population of HRSCs. Two studies provide insight into a putative precursor [5,6]. Zucker-Franklin and colleagues cultured peripheral blood mononuclear cells (PBMC) from HL patients on soft agar and observed, in 12 of 33 cases, colonies containing multinucleated giant cells, some of which were “indistinguishable” from RS cells [5]. No giant cells formed from PBMC of NHL, mycosis fungoides, or from control cells, suggesting that giant cell formation from PBMC is limited to HL cases, or more specifically, a subset of HL cases. The authors speculated that that “circulating precursors of RS cells do not always find an environment hospitable for their development into RS cells in normal lymph nodes. It is possible that factors extrinsic to the cells may inhibit or enhance their differentiation.” This study also suggested that putative precursors may adapt well to a semi-solid environment of soft agar and may differentiate into RS cells, indicating that the precursor may not easily differentiate in the liquid environment [5]. Therefore, HRSCs are not likely to be detected frequently in peripheral blood in most patients. From a prognostic standpoint, 8 of 12 cases that produced RS-like cells were diagnosed at stage I or II HL, suggesting that putative CPs can occur even at early stages of HL [5].

In a second study demonstrating the generation of RS-like cells from PBMC of HL patients, the authors cocultured mononuclear cells isolated from autologous peripheral blood of HL patients with a single cell suspension of tumor cells in a system that separated the cell populations by a microporous membrane that allowed only the passage of viruses
and cytokines [6]. In this experimental setup, various types of giant cells formed in the PBMC fractions, some of which were “indistinguishable” from RS-cells and H-cells. In all cases, 10% of the giant cells stained for CD30, indicating the presence of HRSCs in the mix. The remaining giant cells formed in this experimental set up, the CD30-negative cells, may be a consequence of EBV infection, since EBV can also induce HRSC-like morphology and phenotype on some infected cells (as is often seen during acute EBV infection in infectious mononucleosis). Also, in this same study, in situ hybridization showed that some of the resulting giant cells were positive for Epstein–Barr virus (EBV+), suggesting a role for EBV in the transformation of the giant cells from their mononuclear precursors. It was suggested that HL lymph node-derived factors may promote the formation of giant cells, including RS-like cells, from a PBMC precursor [6].

Even though the study by Sitar et al. showed that some of the intravitro PMBC-derived HRS-like cells were CD30+ [6], neither this study nor the study by Zucker-Franklin et al. [5] have provided by genetic means, evidence of a clonal relationship between putative PMBC-derived HRSCs and HRSCs of HL tumors. Vockerodt and colleagues analyzed multiple peripheral blood (PB) and bone marrow samples from two HL patients using a highly sensitive HRS cell clone-specific PCR assay, but the authors did not detect any HRS cell-specific amplicons [7]. This suggests that HRSC clonotopic relatives were either infrequent or absent in these patients. Although the study by Vockerodt et al. included only two clinical subjects [7], one of its main strengths was that the subjects were relapsed patients in whom the disease may have spread to multiple sites; thus, it is reasonable to speculate that dissemination occurred via lymphatic and hematogenous spread. Yet there was no indication of residual HRSCs in PB. In contrast, Jones et al. found striking similarities (e.g., similar immunoglobulin gene rearrangements) between putative CPs (clonotypic CD20+ B cells) in PB and HRSCs in lymph node [3]. However, this study was called into question for technical reasons [8]. For example, the fragment length analysis of Vκ light chain rearrangements was considered not well suited to determining clonal identity because the V-J joints of light chain rearrangements showed little length variation (66% of polyclonal V-J joints have an identical CDR3 length of 27 base pairs) [8]. Other studies however, suggested that length analysis of Vκ light chain rearrangements is a standard and commonly used method for the identification of relationships among B-cell clones [9]. The HL cell line L1236 was raised from primary peripheral blood mononuclear cells isolated from an advanced stage cHL patients. Interestingly, L1236, like the circulating clonotypic CD20+ B cells [3], appears to have strong clonal relationship to lymph node HRSCs [10].

2.2. Generation of RS cells from HL cell lines

Other studies suggested that giant RS cells can be generated from precursors in HL cell lines. A sub-population of B cells that expressed CD20 in HL cell lines gave rise to HRSCs in vitro [2,3]. Cells with similar immunophenotypes were also observed in subsets of HL patients, but these cells failed to generate HRSCs in culture [3]. Earlier studies showed that mononuclear Hodgkin cells can give rise to multinuclear RS cells by endomitosis [2,6,11]. The resulting giant RS cells display limited proliferation, a characteristic that supports the hypothesis that the pool of HRSCs is maintained by putative precursors [11,12]. These studies suggest that putative precursors, present in cell lines and in PBMC, can give rise to giant RS cells or RS-like cells, and also suggest that a precursor may be responsible for feeding the HRSC population in subsets of HL cases.

3. Putative roles of EBV and proinflammatory cytokines in transformation events

3.1. Potential role of EBV in the transformation of putative circulating HRS precursors

EBV has long been suspected in the etiology of HL; however, only 40–60% of HL cases are EBV+. HRSCs in a large subset of patients are clonally EBV-infected as evident by the detection of a clonal viral genome by Southern blot and in situ hybridization [13,14], suggesting that EBV infection may be an early event in HL pathogenesis, perhaps an event that involves EBV-induced dysregulation of regulatory pathways of a putative HRSC precursor. Proteins encoded by the EBV genome include EBNA1, LMP1, and LMP2a. In HL, LMP1 mimics CD40 ligand (a central costimulatory molecule for B cells) binding to the CD40 receptor, thereby activating NF-κB signaling, which is important for HRSC survival, proliferation, and escape from immune detection [15]. It is not known if this is an early transforming event in HL, although several studies have shown that B cell precursors can be transformed by EBV [16,17], some of which lead to the development of B cell lymphomas [18]. CD30+ RS-like cells that developed from PBMC of HL patients were found to be EBV+, suggesting a role of EBV in the transformation of a putative CP for HRSCs [6]. The transformation event may occur well before the lymph node, because mature nodal HRSC share key features (e.g., immunoglobulin gene rearrangements) with their putative CPs, and also because mature HRSCs are clonally EBV infected. In some ways, transcription of EBV genes by HRSCs [19] is similar to that of nasopharyngeal carcinoma (NPC), in which both LMP1 and LMP2A induce precursors, which are then involved in the development of NPC [20,21]. These observations suggest a role of EBV in the transformation of precursors of tumors cells, including HRSCs of HL.

3.2. Inflammation and the role of lymph node in transformation and maintaining putative HRS precursor

Inflammation of the lymph node has been considered a contributing factor in the dysregulation that leads to generation of cancer stem cells (CSC) or transformation
of tumor precursor cells. Inflamed lymph nodes and tumor lymph nodes are known to produce multiple growth factors, cytokines, and chemokines, which are frequently detected in blood. These molecules exert systemic and endocrine effects on circulating precursors. In vitro studies using autologous HL PBMC and tissues provide some insight into the role of the HL lymph node in the formation of RS-like cells from PBMC [6,22]. Proinflammatory chemokines and cytokines such as IL-1 and IL-6 are found at detectable levels in serum of subsets of HL patients, and are associated with disease activity and clinical symptoms [23,24]. IL-1 appears to play a role in partly stimulating and generating CSCs in different types of solid tumors [25,26]. Lymphocytopenia and presence of B symptoms, two negative prognostic factors in HL, were accurate predictors of IL-6 serum levels before treatment, and higher pretreatment levels of IL-6 were observed in patients with treatment failure [27]. IL-6 signaling may be responsible for CSC stimulation. In breast cancer, IL-6 has been shown to be a direct regulator of breast CSC self-renewal [28]; a process that is mediated by the IL-6 receptor/GP130 complex through activation of Stat3, which is also activated in HL. In inflammatory cells, IL-6-mediated Stat3 signaling selectively induces a tumorigenic microenvironment [29]. Stat3 activation, in turn, leads to transcriptional activation of NF-κB in inflammatory cells that secrete additional IL-6 and IL-8, which act on tumor cells. Although breast cancer is an epithelial tumor and HL is a lymphoid malignancy, proinflammatory cytokine-induced transformation of putative precursors may be a shared molecular feature. Stat3 and NF-κB are both constitutively active in subsets of HRSCs in HL. It will be of interest to determine if IL-6 plays a role in the differentiation of putative CPs of HRSCs.

4. HRSCs in peripheral blood

The possibility of hematogenous dissemination and the resulting presence of HRSCs in PB were first suggested by Dorothy Reed in her classic description of the morbid pathology of Hodgkin’s disease in 1902. “These giant cells occur in great numbers in the large lymph sinuses of the gland and occasionally occur in blood vessels.” One of the earliest reports of RS cells in PB came from a study of Jeanselme and Marchal (1926) [30,31]. They observed RS cell emboli within blood vessels in biopsied and postmortem organs of a patient who had died from Hodgkin’s disease. In addition, these giant cells were described as being frequent in perivascular locations, and in some sections appeared to be passing through a vessel into the parenchyma [32]. Table 1 summarizes a list of studies that report RS cells in PB. As indicated, prognosis of this manifestation was ominous in the pre-MOPP/ABVD era (death less than 2 years after either diagnosis or treatment), but appeared much improved with either MOPP or ABVD. One case treated with ABVD went into remission [33], and of those treated with MOPP, one had a CR but was treated with second line treatment by radiation for relapse [34]. A second had a CR, but relapsed and was treated successfully with chemotherapy and radiation, yet died within 3 years [35]. In addition, subsets of present day high risk, unfavorable HL (progressive disease, primary refractory/early relapse) still have a poor prognosis [36].

5. Origins of HRSCs

5.1. Cellular origin of HRSCs

There appears to be two types of morphologically distinct but immunophenotypically similar neoplastic cells that are characteristic of HL: the mononuclear Hodgkin’s cells and the multinuclear Reed–Sterberg cells, collectively known as HRSCs. Molecular analysis of microdissected HRSCs from solid cHL tumors showed that these cells harbored clonal immunoglobulin gene rearrangements similar to that of B cells, suggesting a B-cell ancestry [37,38]. In the majority of cases, the immunoglobulin gene rearrangements showed somatic hypermutation, suggesting that the HRSCs arose from germinal center or post-germinal center B cells. In some instances, cHL showed loss of function mutations, including nonsense mutations, in their V genes [37]. Normally, cells that inherit such crippling mutations will rapidly undergo programmed cell death. However, post-germinal center HRSCs are able to escape apoptosis, perhaps via an advantageous mechanism(s) inherited from their precursors. In very rare instances, cHL contains HRSCs that are descendants of T cells as they (the HRSCs) carry clonal T cell receptor gene rearrangements [39,40]. Manifestation of HRSCs has been reported for other hematological malignancies including peripheral T cell lymphomas [41,42], multiple myeloma (MM) [43,44], chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) [45,46], and follicular lymphoma [47,48]. And at least one study reported the coexistence of HRSCs with mantle cell lymphoma (MCL) tumor cells in MCL [49]. It will be interesting to determine whether HRSCs in different lymphomas are lineal descendants of common precursors, and whether the characteristic(s) of each variant is determined by the microenvironment of each lymphoma subtype.

5.2. Loss of B cell programming by HRSCs

HRSCs rarely express immunoglobulin or other B-cell markers [50–52]. Occasionally, their failure to express immunoglobulin appears to be a consequence of crippling mutations in immunoglobulin genes. However, such crippling mutations occur only in a minority of HL cases, and usually only in those that are EBV-positive [52]. A lack of functional immunoglobulin (and other B cell-specific gene expression) has been attributed to epigenetic (e.g., demethylation) silencing of the immunoglobulin (and other B cell-specific gene expression) has been attributed to epigenetic (e.g., demethylation) silencing of the immunoglobulin heavy chain and/or master transcription factors [53,54]. One consequence of epigenetic silencing is the upregulation of NOTCH1, a negative
regulator of the B cell program [55], and also upregulation of non-B cell lineage proteins [56]. Demethylation studies of cultured RS cells showed that the B cell program is irreversible [57]. In spite of this, subsets of HRSCs retain some aspects of a B cell signature, expressing CD20, SDC1/CD138, or CD79a [58–62]. There is not a clear consensus on the prognostic significance of these molecules in HL. Nonetheless, differences in the B cell signature of these tumor cells, and therefore, may have prognostic importance.

6. Chemoresistance

6.1. Mechanism(s) of chemoresistance: involvement of primary HRSCs and putative precursors

Chemoresistance, an increasing problem with conventional therapy for multiple cancer types, may be due to primary tumor cells or CPs that develop resistance to therapeuetic drugs. Either way, the responsible cells can be regarded as circulating tumor cells, HL may be one such neoplasm. Resistance to the doxorubicin used in the ABVD regimen has been demonstrated in HL cell lines [4], other human lymphoma cells [63], and in cases of multiple myeloma and non-Hodgkin’s lymphoma [64]. Increases in the copy number of the doxorubicin-resistance gene ABCC1 were frequently detected in HRSCs in pre-treated and relapsed biopsies of patients who failed primary treatment [4]. In another study, 0.2–6.5% of cells with an HRS-like phenotype present in primary HL tumors showed the ability to increase efflux of Hoechst 33342 dye, were resistant to gemcitabine (a commonly used drug for the treatment of radioresistant-HL), and expressed other multidrug resistance genes including ABCG2 and MDR1 (ABCB1, P-glycoprotein, pp) [65]. Overexpression of ABCB1 by HRSCs of HL is associated with primary progressive disease, presence of B symptoms, and shortened disease-free survival [66]. These observations suggest that subsets of HRSCs employ several different multidrug resistance proteins to overcome the effects of cytotoxic drugs.

Other genes that are upregulated by HRSCs following exposure to cisplatin, etoposide, or melphalan include the cytokine receptors IL5RA and IL13RA1, antigen-presenting cell markers CD40 and CD80, and genes with known associations with chemoresistance such as myristoylated alanine-rich protein kinase C substrate and PRAME (preferentially expressed antigen in melanoma) [67]. A number of recent clinical trials targeting these molecules or their cognate pathways resulted in only modest improvement in patient outcome, perhaps because of exposure to heavy pre-treatment. For example, in a phase II clinical trial, the anti-CD80 monoclonal antibody Galiximab was administered to 30 radioresistant-HL patients resulted in a 6.9% overall response rate [68].

7. Metastasis

7.1. Molecular mechanism(s) of metastasis by HRSCs and their putative precursors

Although HRSCs are rarely observed in PBMCs, the anatomic view of predictable, contiguous spread is consistent with lymphatic or hematogenous dissemination. However,
the molecular mechanism(s) by which HRSCs escape the tumor microenvironment is not clear, although subsets of these cells express genes known to promote aggressive tumor metastases: FGF2, SDC1, TGFβ1, MMP2, MMP9. From a clinical standpoint, MMP9 is associated with an adverse outcome [69]; elevated serum SDC1 has been reported for subsets of HL patients [70], and high levels of serum FGF2 in HL is associated with an increased erythocyte sedimentation rate, a factor associated with poor prognosis [71]. TGFβ1 is expressed by HRSCs and other cell types, and it has been detected in urine of patients with active disease [72]. The particular combination of these markers upregulated by subsets of HRSCs may determine their metastatic aggressiveness. In myeloma and other cancers, shedding of SDC1 can facilitate growth, angiogenesis, and metastasis [73–76]. Shedding is in part driven by heparinase, which influences syndecan-1 localization by upregulating expression of enzymes that accelerate its shedding from the cell surface. Elevated levels of heparinase observed in the blood of HL patients including children [77,78] may be responsible for shedding of SDC1 from the surface of malignant HRSCs, resulting in the high levels of soluble SDC1 observed in serum of HL patients [70]. As a consequence, the shedding may promote the escape of HRSCs from their microenvironment.

These metastatic molecules may also promote metastatic spread of HRSCs via activation of the NF-κB pathway. In HL, proliferation and survival of HRSCs is promoted by constitutive activity of the NF-κB pathway [79]. Indeed, several studies have focused on the requirement of the NF-κB signaling pathway for MMP-9 expression [80–82]. The NF-κB pathway is also important in tumor invasion and metastases in multiple cancer types. It has been reported that MMP-9 expression is regulated transcriptionally through NF-κB elements within the MMP-9 gene [83]. Using an adenovirus that overexpressed the inhibitory subunit IκBα, it was found that NF-κB activation was an absolute requirement for upregulation of MMP-9. Although not demonstrated in hematological malignancies, constitutive activity of NF-κB and upregulation of MMP9 were found to be strongly influenced by the upregulation of SDC1 in endometrial cancer, resulting in increased cancer cell proliferation and invasiveness [84]. NF-κB was also shown to participate in upregulation of SDC1 in malignant glioma cells [85]. These studies suggest that SDC1 and NF-κB are involved in a feedback loop, possibly driving the expression of downstream targets including MMP9 [84,85].

The expression of SDC1 may also be under the influence of FGF2. A cis-acting FGF2-inducible response element (FiRE) located upstream of the SDC1 gene [86] may allow FGF-2-induced upregulation of SDC1 in NIH3T3 fibroblasts [87,88]. A further transcriptional role of either or both FGF2 and SDC1 has been suggested by their nuclear localization [76,89–91]. In addition, the overexpression of nuclear SDC1 resulted in increased accumulation of nuclear FGF2 in mesenchymal tumor cells [89]. As well, LMP-1 induces release of FGF2, which is involved in activation of NK-κB signaling [92]. In addition, in vitro studies showed that TGFβ1 and FGF2 together promote SDC1 upregulation and its shedding from the surface of 3T3 cells [87]. Thus, it appears that TGFβ1 and FGF2 activate SDC1 expression, which in turn drives the NF-κB pathway to upregulate MMP9, thereby promoting cellular invasion and metastasis (Fig. 1A). In
addition, multiple signaling involving some of these molecules in aggressive HRSCs may be responsible for extranodal disease, and poor clinical outcome in HL (Fig. 1B).

The classic metastatic marker in many cancer types, CD44, is also upregulated in HL [93], and plays an important role in hematogenous dissemination of non-Hodgkin’s lymphoma [94]. Serum levels of CD44 are elevated in untreated HL patients. In patients with complete response, CD44 decreases to levels comparable to that in normal controls, but remains increased in those with progressive disease [95]. In addition, the expression of CD44 splice variant v10 by large numbers of HRSCs is associated with aggressive behavior and high risk of relapse in nodular sclerosis HL [96]. The overexpression of CD44 in HL may be related to demethylation [97].

An alternative view of HL metastasis is related to putative circulating HRSC precursors. Recent studies have implied a role for the microenvironment, the so-called “premetastatic niche,” in determining the pattern of metastatic spread [98]. The premetastatic niche in extranodal sites may permit the homing of CPs, which subsequently develop into mature HRSCs within a permissive niche that favors their development and survival. In other neoplasms, tumor precursor cells are known to release exosomes with the potential to promote the formation of a premetastatic niche [99], a process that is also CD44-dependent [100]. Although no direct data on exosome involvement in HL is available, the manner by which HRSCs secrete FGF2, which lacks a secretion signal sequence, may occur via exosome release [101]. In addition, pro-inflammatory cytokines [23] and EBV [6] may be important players in the early stages of a multi-step transformation event of these putative precursors. Fig. 2 summarizes HL metastasis by putative CPs for HRSCs. The occurrence of putative CPs that feed the occult tumor cell population implies that metastatic spread is random. However, the evidence is overwhelming that spread of the disease is contiguous, the expectation of which is integral to the current staging system. Direct evidence implicating contributions of a premetastatic niche in HL pathogenesis may be hindered by the rarity of the putative HRS precursors and the difficulty of growing these cells in culture [3]. However, immunohistochemical analysis of tissue biopsies from primary and secondary tumors, as well as primary extranodal HL tumors, may provide clues regarding the nature of the premetastatic niche.

8. Routes of dissemination

8.1. Lymphatic and hematogenous spread

Early work resulted in a better understanding of the characteristic nonrandom and predictable spread of HL via lymphatic channels to contiguous lymph node chains and other lymphoreticular structures [102,103]. However, spread to distant tissues is expected to involve either lymphatic or hematogenous routes. Fig. 3 summarizes the routes of spread of HL. Once a migratory cell has detached from its primary tumor site, it may intravasate into blood vessels or lymphatics. Either route of dissemination can lead to venous circulation, as lymphatics drain into blood, most commonly through the left lymphatic duct (thoracic duct) or the right lymphatic duct, and subsequently into the subclavian veins. Along the way, lymphatic fluid is filtered through lymph nodes, a process that will normally clear abnormal cells such as tumor cells from the circulation. When immunity is reduced, as occurs in lymphocytopenia, a negative prognostic factor in HL [104], aggressive HRSCs may metastasize more rapidly. The exact cause of lymphocytopenia in HL is not known, but it may be
a consequence of immunosuppressive cytokines produce by HL tumor cells.

9. Prognosis of extranodal disease

9.1. Prognostic significance of extranodal sites commonly involved in primary nodal HL

Extranodal HL can arise either by dissemination from a primary tumor site, or as a primary tumor in non-nodal, non-lymphatic tissue. Extranodal invasion of adjacent and distant tissues via hematogenous spread occurs in 5–10% of HL cases [105]. Roughly 6–12% of cases of lung parenchyma involve spread of HL in origin [106–109]. Lung parenchyma involvement may or may not involve direct extension of a large mediastinal mass in the lungs. Patients with limited stage disease having a large mediastinal mass that extends into the lung parenchyma exhibit inferior disease-free and total survival rates after treatment with radiation alone [110]. Pleural effusion is relatively common at presentation [109] occurring in about 13% of cases [111]. The main cause of effusion in HL, which may be unilateral or bilateral, is obstruction of lymphatic drainage by enlarged mediastinal lymph nodes. Although pleural effusion does not appear to be of significance in HL prognosis, frequent analysis of pleural fluid for the presence of HRSCs may be useful in follow-up to identify relapse [112]. Heart and pericardial effusion in HL is estimated to occur in 5–18% of cases [105,113–115]. This may be partly due to retrograde lymphatic spread, hematogenous spread, or direct extension from an intrathoracic tumor mass. Heart involvement, which represents a late manifestation of the disease and indicates a poor prognosis, is asymptomatic and in most cases is determined only at autopsy [105,113]. However, because of improved imaging techniques for early detection of pericardial effusion or heart failure by computed tomography, pediatric patients with imaging data experienced greater long term survival than the group without imaging data and resumed normal cardiac function after chemotherapy [114,116]. Chest wall involvement accounts for 6.4% of cases [111,117,118] including pediatric cases [117]. Early stage (I and II) HL patients with chest wall invasion exhibited inferior outcomes (progressive disease, relapse, inferior disease-free survival, worse cause-specific survival, and shorter overall survival) [118].

Early studies on staging laparotomy showed that spleen is infiltrated in about 30–40% of patients at presentation [119–121], most of which are marked by splenomegaly [106,119]. However, diagnostic laparotomy with splenectomy in HL has been rarely performed in recent years due to concerns over impact on survival, delay in onset of treatment, and long-term consequences of splenectomy [122,123]. Nevertheless, spleen involvement is usually more frequent in advanced stage disease, and represents a poor prognostic factor [124]. The involvement of spleen in HL increases the likelihood of hepatic involvement [120], as the splenic and common hepatic arteries share a common hematogenous route. Hepatic involvement at presentation occurs in 6–20% of patients [106,119,125]. Hepatic involvement, like splenic involvement, is an indicator of adverse prognosis [124,126–129]. In extremely rare cases, HL is involved in the gastrointestinal tract [125,130] and the genitourinary system [125]. Gastrointestinal involvement occurs mostly in late stage (IV) disease and may represent an adverse prognostic factor in disseminated HL [130]. Although data for different treatment options for gastrointestinal disseminated HL are not available, in primary gastrointestinal HL, either radical gastrectomy or multi-agent chemotherapy resulted in no recurrent disease on follow-up [131,132], suggesting that primary GI HL may have a better prognosis than gastrointestinal disseminated HL. There has been one report of testicular and epididymal involvement with stage IV-A; the patient remained disease-free three years after diagnosis and treatment with combination therapy, indicating a good prognosis [133].

The incidence of skeletal Hodgkin’s disease varies from 9 to 14% during the course of the disease but reaches as much as 30–50% at post mortem [134]. Skeletal involvement may include invasion of bone marrow and osseous tissue by tumor cells. About 5–38% of patients will develop bone marrow involvement during the course of the disease [135,136] but only 1–4% will exhibit bone marrow involvement at presentation [119,137,138]. Bone marrow involvement in most cases is considered a late stage manifestation, and is associated with poor prognosis [136,139]. Involvement of osseous tissue may arise from either contiguous spread or via hematogenous dissemination [119,138].

Integumentary or cutaneous involvement is also extremely rare, occurring in 0.5–3.4% of HL patients [140,141]. The mechanism(s) of skin involvement in HL remains unknown; however, possibilities include retrograde lymphatic spread from tumor-involved lymph nodes, direct extension into skin by tumor cells in underlying lymph nodes, or hematogenous spread [140,141]. Cutaneous involvement is usually associated with diffuse lymphadenopathy, late stage disease (typically IV), and poor prognosis [140–143], although a good response to standard therapy has been reported [144].

Central nervous system involvement of HL (CNS-HL) is an uncommon but well-known complication of systemic HL, occurring in 0.2–0.5% of all cases in advanced stages [145–147]. Within the CNS, intracranial metastases, metastases to the epidural space of the spinal cord, which causes compression of the spinal root, metastatic leptomeningeal disease, and intramedullary spinal cord metastases are most commonly seen. Hematogenous spread is the putative mechanism of dissemination of HL to the CNS [148]. Although most early studies indicate a poor prognosis of CNS-HL, a recent study showed that complete remission and improved long term survival can be achieved with chemotherapy and radiation [149].
10. Opportunity for brentuximab and rituximab in treatment of cHL

Three populations of cells in HL are potential targets for therapy: H and RS cells, which express CD30, and CD20+ clonotypic B cells, which may represent putative CPs of HRSCs. CD30+ HRSCs have been discussed at length by many authors. CD20+ clonotypic B cells and their putative role in HL pathology have only recently come to attention. As indicated, CD20+ clonotypic B cells are postulated to be CSCs that feed the HRSC population [3]. However, the existence of CSCs is still a hotly debated issue in multiple cancer types [150] including HL [8]. Should more solid evidence prove the existence of circulating clonotypic CD20+ B cells in cHL, they may become potential targets for anti-CD20 therapy. A phase II clinical trial assessing the combination of Rituximab (which targets CD20) plus ABVD on the behavior of CD20+ clonotypic B cells in stage IIB-IV HL patients resulted in actuarial 3-year event-free and overall survival rates of 83% and 98%, respectively [151]. EBV copy number in plasma fell dramatically during cycle 1 in patients with EBV+ tumors [151]. In this same study, the authors also found that the persistence of detectable circulating clonotypic B cells was associated with a greater relapse frequency [151]. In addition, the results reported that the levels of clonotypic CD20+ B cells became undetectable in a subset of patients after Rituximab-ABVD treatment [151]. However, the biology and the role of circulating clonotypic CD20+ B cells in HL pathogenesis are not completely understood. Prior to the study by Kasamon et al. [151], two independent studies found that peritumoral CD20-expressing background B cells were associated with favorable outcome [152,153]. However, anti-CD20 antibody therapy with rituximab has resulted in objective responses in a subgroup of relapsed cHL patients [154]. The response to rituximab therapy may be due to a direct effect on subsets of HRSCs that occasionally express CD20 [61]. An alternative but simultaneous effect of rituximab therapy may be the eradication of reactive CD20+ B cells from the tumor microenvironment, a point that seems consistent with one of the main findings of the study by Kasamon et al. (decreased circulating clonotypic CD20+ B cells) [151], despite differences in microenvironment.

Rituximab has also exhibited success in the treatment other neoplasms [155]. However, the Rituximab plus ABVD combination, although well tolerated by HL patients [151], may result in more side effects and greater risk of long term complications than either brentuximab vedotin or rituximab alone. A combination of brentuximab, vedotin, and rituximab, however, may produce superior or comparable outcomes as ABVD, but with less severe side effects. The main advantage of this combination is that both CD20+ and CD30+ malignant cells are targeted, thus removing larger numbers of occult tumor cells, along their putative CPs. This treatment may especially benefit HL patients in whom the HRSCs express multidrug resistance genes, a group of patients who will most likely experience primary treatment failure with a conventional therapeutic regimen. Success of this protocol, however, will depend on identification of patients before conventional treatment, because heavily pretreated patients who fail frontline therapy continue to do poorly with alternative/second line treatments.

11. Prognostic significance of a putative precursor for HRSCs

Currently, there is strong suspicion that circulating CSCs or circulating precursor cells are largely responsible for refractory and relapsing disease in both solid and liquid tumors. The persistence of circulating clonotypic CD20+ B cells presumed to include putative CPs for HRSCs [3] has been shown to be associated with greater frequency of relapse in rituximab-ABVD treated HL patients [151]. The drug resistant HL cell line KM-H2 contains a similar subset of cells, but these cells appear to extrude Hoechst stain in a manner similar to the expulsion of drugs by drug resistant cells. Although KM-H2 and primary HRSCs of subsets of HL patients who experience primary treatment failure harbor elevated expression of drug resistant genes [4,156], it is not clear if the clonotypic CD20+ B cells also overexpress such genes. This information may be important in developing alternative therapies for relapsing and refractory HL, as most current treatments for high risk HL continue to have little impact.

12. Technical limitations and considerations for analysis of putative HRS precursors in vitro

The putative HRSC precursors isolated from HL patients are difficult to culture in the laboratory setting [3]. Since RS-like cells can develop from patient PBMCs when cocultured with autologous HL lymph node extract [6], it may be useful to use a similar system to study the in vitro behavior putative HRS precursors.

13. Conclusion

The evidence indicates that HL dissemination can occur either by lymphatic or hematogenous means, resulting in extranodal disease and ultimately poor prognosis. There appear to be three occult cells that are responsible: H cells, which are considered more aggressive, RS cells, which have limited proliferation, and putative circulating precursors that feed the HRSC population. The molecular phenotypes of subsets of HRSCs may determine prognosis. Putative circulating precursor cells of HL or of other tumor types represent an ominous indicator, and are frequently associated with chemoresistance, treatment failure, and relapse. Genetic profiling of putative HRSC precursors and similar cells in other lymphomas should provide insight into common origins, and perhaps will lead to better treatment options.
Conflict of interest

The authors have no conflict of interest to declare.

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References


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