

Research Article

## Lymphangiogenesis in Hodgkin Lymphoma and in Indirectly Related Conditions – A Review

Daniel Benharroch<sup>1\*</sup>, Isebrand Prinsloo<sup>2</sup>, Jacob Gopas<sup>3</sup>, Benzion Samueli<sup>1</sup>

<sup>1</sup>Soroka Medical Center and FOHS, Ben Gurion University of the Negev, Beer Sheva, Israel

<sup>2</sup>Deceased and buried at Beer-Sheva, Israel

<sup>3</sup>Head of the Shraga Segal Department of Immunology, Microbiology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, and Department of Oncology, Soroka Univ. Med. Centre, Beer-Sheva, Israel

**\*Corresponding Author:** Dr. Daniel Benharroch, Soroka Medical Center and FOHS, Ben Gurion University of the Negev, Israel, E-mail: [danielbenharroch1@gmail.com](mailto:danielbenharroch1@gmail.com)

**Received:** 18 March 2020; **Accepted:** 01 April 2020; **Published:** 03 April 2020

**Citation:** Daniel Benharroch, Isebrand Prinsloo, Jacob Gopas, Benzion Samueli. Lymphangiogenesis in Hodgkin Lymphoma and in Indirectly Related Conditions – A Review. Journal of Cancer Science and Clinical Therapeutics 4 (2020): 078-085.

### Abstract

The significance of lymphangiogenesis in malignant tumors and specifically in classic Hodgkin lymphoma has not been properly evaluated, in contrast with that of angiogenesis in these neoplasms. In the study reviewed herein, the relevance of lymphatic vessel proliferation was explored in 19 cases of classic Hodgkin lymphoma stained with the D2-40 (anti-podoplanin) antibody. In each case, three lymphatic vessel hot spots were analyzed twice. Of the 57 hot spots thus investigated, 15 were chosen at random for image acquisition and analysis and microvessel counting. The mean perimeter, major axis length, surface area and complexity factor for each hot spot were established by morphometry, and associations with clinical and laboratory

traits of classic Hodgkin lymphoma were identified. No relationships were found with the clinical features or immunostaining for typical markers of this lymphoma. However, significant inverse relations were found with BAX, pRb and IκB-α expression in tumor cells, genes believed to play roles in apoptosis in this lymphoma. The mean lymphatic major axis length was inversely correlated with the complexity factor. Clinicopathological associations were further obtained for the expression of BAX, pRb, and IκB-α in a large cohort of classic Hodgkin lymphoma patients that was previously published. Since the issue was poorly explored at this point, we have reviewed the significance of lymphangiogenesis in indirectly associated conditions.

**Keywords:** Lymphatic vessels; Complexity factor; Hodgkin lymphoma; pRb; IκB-α; BAX

## 1. Introduction

Recently, it has been suggested that lymphatic vessel proliferation (lymphangiogenesis - LAG) may provide a better target for immunotherapy and biological therapy than angiogenesis, where such therapies have mainly failed [1-4]. Classic Hodgkin lymphoma (cHL) is a primary malignant neoplasm of the immune system. For practical purposes, it almost exclusively involves lymph nodes (LNs) at diagnosis. Whereas LAG in the LNs has been detected mainly in metastatic tumors, LAG has occasionally been described in primary malignant lymphomas [5-7]. Angiogenesis has rarely been detected in cHL. Such an unusual description has been investigated by morphometric methods, microvessel density or the hot spot technique [8]. Lymphangiogenesis and angiogenesis have been explored by evaluating the expression of VEGF and variants in cHL [9].

We have previously attempted to clarify the features of LAG in cHL by using a morphometric method and looking for clinicopathological correlations [10]. A review of the previously published work on this topic is presented herein. The possible relevance of LAG is investigated, and when highlighted, a search is carried out to demonstrate the genes and growth factors associated with the role of LAG in cHL in an expanded population of cHL patients. Since studies on LAG in lymphoma are scarce, we conclude the present paper with a review of the literature published recently on LAG in conditions related directly or indirectly to lymphoma.

## 2. Methods

For the original study on LAG in cHL, nineteen cases of mixed cellularity cHL, primarily involving the LNs, were selected from our archives of formalin-fixed, paraffin-embedded tissue samples. Five-micron-thick sections were

submitted for immunohistochemistry (IHC) (avidin-biotin complex method) with the D2-40 antibody (1:10, Dako to identify lymphatic vessels (LVs) and an anti-CD34 antibody to identify the blood vessel (BV) endothelium. For each case, three lymphatic vessel hot spots were analyzed (57 hot spots in total). The hot spots were imaged at 200X magnification and then printed with black ink on white paper. The vessels were underlined in the images: the LVs were traced in brown, and the BV endothelium was traced in red.

Fifteen of the 57 hot spots, which were selected at random, were subjected to morphometry twice. For each case, mean LV perimeter, major axis length, surface area and mean (shape) complexity factor analyses used the microvessel count and image analysis approaches described by Korkopoulou et al. [8]. The complexity factor was computed as equal to  $5\pi \cdot \text{area}/\text{perimeter}^2$ . The above parameters were correlated with clinical characteristics and were also related to CD30, EBV/LMP1, EBER, CD20, p53, MDM-2, pRb, BAX and IκB-α expression and to the apoptotic index [11]. In 10 distinct sections of the hot spots, double staining of LVs was performed with D2-40 (counterstained brown with a DAB substrate) and an anti-CD34 antibody (counterstained red with an AEC substrate). For this analysis, we used the method reported by Kyras et al. [12]. Statistical analysis employed the Mann-Whitney test for nonparametric values and Spearman's analysis for nonparametric correlations. Finally, we carried out a review of the recent (2014-2019) relevant literature on LAG and lymphomas, but included also conditions akin to lymphomas.

## 3. Results

### 3.1 Lymphangiogenesis in cHL

No statistically significant associations were found between the four lymphatic parameters and clinical characteristics. Statistically significant inverse relationships were obtained

between the LV parameter mean lymphatic area and mean complexity factor and pRb expression. BAX was inversely and significantly linked with the mean surface area and the mean LV length. IκB-α expression was inversely and significantly related to the mean LV perimeter. Of all the LV parameters, only the LV major axis length was inversely correlated with the shape factor, and this correlation was significant ( $r=-.6$ ;  $p=.03$ ). The only significant association of the mean lymphatic perimeter was an inverse relationship with IκB-α expression ( $p=.015$ ). A direct and significant relation was found between the shape factor and pRb expression [10].

### **3.2 Relevance of BAX, pRb and IκB-α expression in an expanded (n=178) cHL cohort**

As these genes were shown to present significant relations with LAG in cHL, confirmation in a larger population was deemed necessary. BAX was inversely linked with bulky cHL ( $p=.040$ ). No other significant clinical associations were found in this large cohort. pRb expression was inversely related to the expression of a sialylated CD15 antigen. The relationships between BAX expression and the biological markers of cHL, included direct links with MCL1, p53 and MDM-2 expression, and inverse links with BAK and BCL-2 expression [10].

A significant inverse association between IκB-α and LMP1 of EBV was demonstrated. Kaplan-Meier analysis showed no significant difference in overall survival among patients stratified by BAX expression. In addition, there was no relationship between IκB-α expression and overall survival in cHL (data not shown).

## **4. Discussion**

Lymphangiogenesis in primary epithelial malignancies as well as in related lymph node metastasis differs from angiogenesis both for characteristics and significance [13-

15]. However, we are mainly concerned here with the issue of LAG in cHL, which develops primarily in the LNs. Moreover, the mode of dissemination of cHL, at least in the early stages of disease, is migration from one group of LNs to the adjacent group. A single article investigated LAG in cHL by evaluating VEGF expression in cHL-HRS cells. The authors showed limited expression of VEGF-C in HRS cells in patient serum samples and gene expression analysis as these cells relate with LAG [9].

The significance of LAG in cHL was scrutinized, since evidence of LAG in cancer is generally controversial [16-18]. Here, we favored the morphometric approach. Lymphatic vessels were defined by IHC with the D2-40 antibody, with CD34 expression evaluated when necessary to identify BVs. We defined four morphometric parameters for an LV: a mean perimeter, a mean surface area, a mean major axis length and a computed factor of complexity [8]. Our work represents the morphometry of 15 hot spots.

pRb was considered to play roles in two morphometric parameters of LAG. This tumor suppressor gene has been proposed to be a regulator of angiogenesis in various neoplasms [19-21], but it has not been related to LAG to date. BAX showed inverse associations with two other morphometric parameters of LVs. On the other hand, we previously noted a direct relationship between BAX expression and VEGF-C expression in urinary bladder carcinoma [22]. IκB-α was found to be inversely and significantly related to the mean LV perimeter. This gene is believed to modulate angiogenesis but, to date, not LAG [5]. Therefore, restriction of IκB-α expression may promote NF-κB and consequently angiogenesis. At this stage, we are not aware of LAG activation by this combined regulation.

In the original work, we demonstrated an inverted correlation between the mean LV shape factor and LV major

axis length. This may indicate that in the process of LV maturation, vessels may progress from small and complex figures to long and simple shapes. However, most clinical or biological variables were not associated with either of these morphometric parameters. The use of antiangiogenic therapy has not met with significant clinical success [4]. For LAG, we are not aware of any therapeutic relevance. Of note, the significance of angiogenesis in cHL has been poorly clarified [7, 23-25].

#### 4.1 Analysis of the three genes in an enlarged cohort

Links between these three genes were investigated in depth in the second part of the present paper. For that purpose, a larger cohort of cHL patients (n=178) was examined. This cohort had been the subject of a previously published article on cHL [13]. One aspect of the prior study concerned BAX and  $\text{I}\kappa\text{B}-\alpha$  expression, which, on the one hand, linked with LAG and, on the other hand, linked with the putative role of the measles virus in cHL [26]. Another aspect of the older study linked BAX expression with apoptosis regulation. The interactions between different members of the BCL-2 family of proteins are not straightforward [23]; their associations with cell survival are also complex [24]. It seems that, overall, the distributions of different members of this family are more significant than the status of each member. Their specific levels of activation may also be critical [27].

The association of HRS cell apoptosis with LAG, although indirect, is identifiable in the first part of this review. A further part of the larger sample population work identified associations between pRb and sialylated CD15 expression and between  $\text{I}\kappa\text{B}-\alpha$  and nonsialylated CD15 expression. These new relations between CD15 and LAG require further investigation. Although the correlations between the LV parameters and the three genes are mostly contrary; they suggest, however, roles for these factors in LAG in cHL.

#### 4.2 Recent literature review of LAG in lymphomas and variously related conditions

To review the recent medical literature regarding the role of LAG in cancer in general and in lymphoma specifically, we performed the following search in PubMed: lymphangiogenesis AND lymphoma AND ("2014"[Date - Publication]: "2019"[Date - Publication]). The search disclosed 20 results. One article was excluded because it was published in Polish, another represented our own previous article on the topic [10] and was reviewed above, and five additional articles were excluded due to lack of relevance to the subject in question. The remaining 13 articles are reviewed here. Of these, three discuss the role of lymphangiogenesis in cancer in general but relate peripherally to lymphoma, five discuss LAG as it relates to lymphoma specifically, and four address mechanisms in human immunodeficiency virus (HIV)-infected patients.

The role of LAG in HIV is relevant here because so-called opportunistic malignancies represent a notable complication of AIDS, and in fact, several hematological and non-hematological malignancies are AIDS-defining illnesses [28]. The mechanisms underlying these diseases are currently being elucidated, but several studies have directly linked HIV proteins with LAG. Several authors [29-32] have found that the HIV matrix protein p17 induces angiogenesis and LAG within the lymph nodes via activation of the MAPK/ERK and PI3K/AKT pathways *in vivo*. This is significant because, although AIDS patients are at a great risk of developing lymphoma, HIV itself does not enter these lymphoid cells, and therefore, other mechanisms for initiating lymphomagenesis must be present, with the role of LAG being the currently favored mechanism [31, 32], and in fact, lymph node-derived lymphatic endothelial cells probably directly release prolymphomagenic factors into the microenvironment [32]. We postulate that these findings, as they pertain to the role of LAG in lymphoma, may be

significant not only in AIDS-related lymphomas but also more generally in lymphomagenesis.

Another important oncogenic virus is human herpes virus-8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV). In addition to its causal role in Kaposi's sarcoma, HHV-8 is important in the pathogenesis of several B-cell lymphomas and that of multicentric Castleman disease (MCD). In their review of HHV-8 oncogenesis, Purushothaman et al. [33] discussed the role of LAG in both lymphoproliferative disorders and Kaposi's sarcoma. Notably, among the pathways affected by HHV-8, there is upregulation of MAPK and PI3K expression, which have been found to induce lymph node LV proliferation and additional oncogenic effects. Additional studies [34, 35] have specifically examined the role of mast cells in cancer, and it is becoming clear that these cells have a significant role in LAG, which in turn promotes tumor spread and progression. The authors of relevant studies note that in several cancers (including both solid tumors and some hematological malignancies), increased mast cell numbers correlate with tumor progression and a poor prognosis. This association between increased mast cell numbers and a poor prognosis is pronounced in HL. A recent review of LAG by Stacker et al. [36] did not discuss lymphoma at all, but this review is mentioned here because it provides a thorough explanation of the mechanisms discussed so far. Furthermore, the authors highlight the significance of these findings because immunotherapy targeting LAG is currently in clinical trials for several nonhematological malignancies.

As we turned our attention to the recent literature focused on the role of LAG in lymphomas, we found five articles that provided striking elucidation of the mechanisms in a variety of malignancies, encompassing both HL and NHL, and three of these reports focused on the role of vascular endothelial growth factor C (VEGF-C). It has been shown [37] that 1)

miRNA-155 exhibits upregulated expression in NK/TCL cells compared to normal NK cells, 2) miRNA-155 is correlated with alterations in the expression of genes related to LAG, and 3) targeting miRNA-155 decreases the ability to promote LAG *in vivo*. More generally, a study of transgenic mice [38] found that LAG within the lymph nodes was an essential precursor of neoplastic B cell growth and spread. Similarly, it was found [39] that increased expression of VEGF-C in neoplastic mycosis fungoides (MF) cells correlates with increased numbers of LVs in the area of the tumor and worse clinical outcomes. In three studies published in a single article [40] elucidating the role of VEGF-C in HL in humans, the authors found that 1) increased serum VEGF-C levels correlated with tumor spread, progression, and negative clinical outcomes and that 2) Reed-Sternberg cells could express VEGF-C, particularly in those patients with elevated serum levels.

Taken together, these findings suggest that LAG plays a significant role in the progression of malignant disease, both in hematological and nonhematological malignancies, and that certain pathways and signaling molecules, namely, MAPK, PI3K, and VEGF-C, play special roles in lymphomagenesis. While clinical investigations into the pharmaceutical relevance of these pathways in several nonhematological malignancies are ongoing, perhaps increased attention should also be given to some NHLs and HLs as well. Our original study [10] suggested roles for BAX, pRb and I $\kappa$ B- $\alpha$  in LAG in cHL, which need further clarification.

### Acknowledgements

In Memoriam: Dr. Isebrand Prinsloo was the initiator and driver of this study. Unfortunately, he passed away at the age of 96 before he could finalize his work. The authors dedicate the review of his work to his prized memory.

### Author Contributions

Original study preparation: I.P., D.B., and J.G.; Review conception: D.B. and B.S.; Primary review writing and editing: D.B.; Recent (secondary) review and writing: B.S.; Visualization and supervision: D.B. and J.G.; Final approval of the manuscript: D.B., J.G., and B.S.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### References

1. Orecchioni S, Gregato G, Martin-Padura I. et al. Complementary populations of human adipose tissue CD34+ progenitor cells promote growth, angiogenesis, and metastasis of breast cancer. *Cancer Res* 73 (2013): 5880-5891.
2. Bing Z, Jian-ru Y, Yao-quan J, et al. Evaluation of angiogenesis in non-small cell lung carcinoma by CD34 immunohistochemistry. *Cell. Biochem. Biophys* 70 (2014): 327-331.
3. Wong ML, Prawira A, Kaye AH, et al. Tumor angiogenesis: its mechanisms and therapeutic implications in malignant gliomas. *Clin Neurosci* 16 (2009): 1119-30.
4. Jeong HS, Jones D, Liao S, et al. Investigation of the lack of angiogenesis in the formation of the lymph node metastasis. *J Natl Cancer Inst* 107 (2015): 155.
5. Alitalo A, Detmar M. Interaction of tumor cells and lymphatic vessels in cancer progression, *Oncogene* 31 (2012): 4499-4508.
6. Oki Y, Copeland A, Younes A. Clinical development of panobinostat in classical Hodgkin lymphoma. *Expert Rev Hematol* 4 (2011): 245-252.
7. Koh YW, Park C, Yoon DH, et al. Prognostic significance of Cox-2 expression and correlation with Bcl-2 and VEGF expression, microvessel density and clinical variables in classical Hodgkin lymphoma. *Am J Surg Pathol* 37 (2013): 1242-1251.
8. Korkolopoulou P, Thymara I, Kavantzias TP, et al. Angiogenesis in Hodgkin lymphoma: a morphometric approach in 286 patients with prognostic implications. *Leukemia* 19 (2005): 894-900.
9. Rueda A, Olmos D, Vicioso L, et al. Role of vascular endothelial growth factor-C in classical Hodgkin lymphoma. *Leuk Lymphoma* 56 (2015): 1286-1294.
10. Benharroch D, Prinsloo I, Gopas J, et al. Lymphangiogenesis in classical Hodgkin lymphoma *J Cancer* 77 (2016): 2117-2123.
11. Benharroch D, Levy A, Prinsloo I, et al. Apoptotic index as a prognostic factor in Hodgkin's disease. *Leuk Lymphoma* 33 (1999): 351-359.
12. Kyzas PA, Geleff S, Batistatou A, et al. Evidence of lymphangiogenesis and its prognostic implications in head and neck squamous cell carcinoma. *J Pathol* 206 (2005): 170-177.
13. Benharroch D, Einav I, Feldman A, et al. Apoptosis of Hodgkin-Reed-Sternberg cells in classical Hodgkin lymphoma revisited. *APMIS* 118 (2010): 339-345.
14. Fujii T, Sutoh T, Morita H, et al. Vascular invasion, but no lymphatic invasion, of the primary tumor is a strong prognostic factor in patients with colorectal carcinoma. *Anticancer Res* 34 (2014): 3147-3151.
15. Kannigurn S, Thongsukai P, Chewatanakornkul S. Chalkey microvessel, but no lymphatic density correlates with axillary lymph node metastasis in primary breast cancers. *Asian Pac J Cancer Prev* 14 (2013): 583-587.
16. Kostis G, Ioannis L, Helen K, et al. The expression of vascular endothelial growth factor-C correlates with lymphatic microvessel density and lymph node metastasis in prostate carcinoma. An immunohistochemical study. *Urol Ann* 6 (2014): 224-230.

17. Zhao YC, Ni XJ, Li Y, et al. Peritumoral lymphangiogenesis induced by vascular endothelial growth factor C and D promotes lymph node metastasis in breast cancer patients. *World J Surg Oncol* 109 (2012): 165.
18. de Sousa SF, Gleber-Netto FO, de Oliveira-Netto HH, et al. Lymphangiogenesis and podoplanin expression in oral squamous cell carcinoma and associated lymph nodes, *Appl Immunohistochem Mol Morphol* 20 (2012): 588-594.
19. Dasgupta P, Sun J, Wang S, et al. Disruption of the Rb--Raf-1 interaction inhibits tumor growth and angiogenesis, *Mol Cell Biol* 24 (2004): 9527-9541.
20. Claudio PP, Stiegler P, Howard CM, et al. Rb2/p130 gene enhanced expression down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in vivo. *Cancer Res* 61 (2001): 462-468.
21. Gaballini C, Del Bufalo D, Zupi G. Involvement of RB gene family in tumor angiogenesis. *Oncogene* 25 (2006): 5326-5332.
22. Mylona E, Magkou C, Gorantonakis G, et al. Evaluation of the vascular endothelial growth factor-C role in urothelial carcinomas of the bladder. *Anticancer Res* 26 (2006): 3567-3571.
23. Brousset, P, Benharroch D, Krajewski S, et al. Frequent expression of the cell death-inducing gene Bax in Reed-Sternberg cells of Hodgkin's disease. *Blood* 87 (1996): 2470-2475.
24. Rassidakis GZ, Medeiros LJ, McDonnell TJ, et al. Bax expression in Hodgkin and Reed-Sternberg cells of Hodgkin's disease: correlation with clinical outcome. *Clin Cancer Res* 8 (2002): 488-493.
25. Coles A.H, Gannon H, Cerny A, et al. Inhibitor of growth-4 promotes IkappaB promotor activation to suppress NF-kappaB signaling and innate immunity. *Proc Natl Acad Sci USA* 107 (2010): 11423-11448.
26. Benharroch D, Gopas J, Ariad S Does the measles virus contribute to carcinogenesis? A review. *J Cancer* 5 (2014): 98-102.
27. Iyer S, Anwari K, Alsop A.E, et al. Identification of an activation site in BAK and mitochondrial BAX triggered by antibodies. *Nat Commun* 7 (2016): 11734.
28. CDC MMRW Recommendations and Reports. December 5, 2008 / 57(RR10); 9. Appendix A, AIDS-Defining Conditions.
29. Basta D, Latinovic O, Lafferty M.K, et al. Angiogenic, lymphangiogenic and adipogenic effects of HIV-1 matrix protein p17. *Pathog Dis* 73 (2015): ftv062.
30. Caccuri F, Rueckert C, Giagulli C, et al. HIV-1 Matrix Protein p17 Promotes Lymphangiogenesis and Activates the Endothelin-1/Endothelin B Receptor Axis. *Arterioscler Thromb Vasc Biol* 34 (2014): 846-856.
31. Liu D, Zeinolabediny Y, Caccuri F, et al. p17 from HIV induces brain endothelial cell angiogenesis through EGFR-1 mediated cell signaling activation. *Lab Invest* 99 (2019): 180-190.
32. Mazzuca P, Marsico S, Schulze K, et al. Role of Autophagy in HIV-1 Matrix Protein p17-Driven Lymphangiogenesis. *J Virol* 91 (2017): e00801-e00817.
33. Purushothaman P, Uppal T, Sarkar R, Verma SC. KSHV-Mediated angiogenesis in tumor progression. *Viruses*. 8, (7): . pii: E198.
34. Varricchi G, Galdiero MR, Loffredo S, et al. (2017): Are Mast Cells MASTers in Cancer? *Front Immunol* 8 (2016): 424.
35. Varricchi G Galdiero MR, Marone G, et al. Controversial role of mast cells in skin cancers. *Exp Dermatol* 26 (2017): 11-17.
36. Stacker S, Williams SP, Karnesis T, et al. Lymphangiogenesis and lymphatic vessel remodeling in cancer. *Nat Rev Cancer* 14 (2014): 159-172.

37. Chang Y, Cui M, Fu X, et al. MiRNA-155 regulates lymphangiogenesis in natural killer/T-cell lymphoma by targeting BRG1. *Cancer Biol Ther* 20 (2019): 31-41.
38. Habenicht L, Kirschbaum SB, Furuya M, et al. Tumor regulation of lymph node lymphatic sinus growth and lymph flow in mice and in humans. *Yale J Biol Med* 90 (2017): 403-415.
39. Jankowska-Konsur A, Kobierzycki C, Grzegolka J, et al. Podoplanin expression correlates with disease progression in mycosis fungoides. *Acta Derm Venereol* 97 (2017): 235-241.
40. Yakimchuk K, Hasni MS, Guan J, et al. Inhibition of lymphoma vascularization and dissemination by estrogen receptor  $\beta$  agonists. *Blood* 123 (2014): 2054-2061.



This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC-BY\) license 4.0](https://creativecommons.org/licenses/by/4.0/)