ORIGINAL RESEARCH PAPER

Pathology

KEY WORDS: classic Hodgkin lymphoma; rate of tumor cells; abundant Hodgkin/Reed-Sternberg cells; measles virus expression; apoptotic index

TUMOR CELL ABUNDANCE IN CLASSIC HODGKIN LYMPHOMA

Daniel Benharroch* M.D., Department Of Pathology, Soroka University Medical Centre And Faculty Of Health Sciences, Ben-gurion University Of The Negev, Beer-sheva, Israel. * Corresponding Author

Yael Tsenter M.D., Department Of Pathology, Soroka University Medical Centre And Faculty Of Health Sciences, Ben-gurion University Of The Negev, Beer-sheva, Israel.

Yarden Kezerle M.D., Department Of Pathology, Soroka University Medical Centre And Faculty Of Health Sciences, Ben-gurion University Of The Negev, Beer-sheva, Israel.

Karen Nalbandyan M.D., Department Of Pathology, Soroka University Medical Centre And Faculty Of Health Sciences, Ben-gurion University Of The Negev, Beer-sheva, Israel.

ABSTRACT

OBJECTIVES: Study the relevance of a high proportion of tumor cells to the clinical and biological properties of classic Hodgkin lymphoma.

METHODS: Tumor cells were counted in sections of the lymphoma stained with CD30, as this highlights the tumor cells of this neoplasm. We assessed abundant malignant cells (≥99 cells/10 high power fields) as the median count of tumor cells.

RESULTS: A wealth of tumor cells was detected in 61 (52.1%) patients, in contrast with 56 (47.9%) patients with a low count. No clinical variance was found between cases rich or poor in malignant cells, except for a statistically significant preference of a high rate of tumor cells for female patients as well as for a high expression of measles virus antigens. The apoptotic tumor cell distribution showed no major disparity with the neoplasm characteristics.

CONCLUSIONS: The entire type spectrum of Hodgkin lymphoma as evaluated by the tumor cell count has no direct bearing on the course of the lymphoma. But there might be indirect evidence for an association of a high tumor cell count, female preference, a strong expression of measles virus antigens and a poor prognosis.

INTRODUCTION

A text on classic Hodgkin lymphoma (CHL) will invariably begin with the statement that this lymphoma stands out owing to a limited population of Hodgkin/Reed-Sternberg (H/RS) tumor cells, accounting for 0.1-10%, of the cellular population in the lymph node section. The remainder being composed of reactive cells and elements. But the relatively small number of tumor cells is not, as a rule, considered a justification for the favourable response to treatment prevailing in the majority of CHL patients [1].

Since computing the percentage of tumor cells among the wide variance of reactive cells in CHL seemed to us difficult to replicate, a different strategy was adopted. Our method consisted of counting the number of H/RS cells found in tumor cell aggregates in 10 high power fields in sections stained with CD30. In these sections, the malignant cells stand out clearly, consideration being given to CD30+ immunoblasts.

As pathologists, we come across cases of CHL which display an unusually large number of H/RS cells. A query has been raised whether the latter predicts a course of the disease that is more rapid or more aggressive. Since the 1970s, this question has seemed to be of exclusive concern with nodular sclerosis CHL grade II [2, 3], as this variant was believed to carry a worse prognosis [2, 4, 5]. More recently, it has been shown that the markedly increased number of tumor cells in this condition does not carry a more aggressive course [6-8]. Instead, advanced age, advanced stages and male gender are among the factors responsible for a poor prognosis, while a shift towards uniformity in the outcome of nodular sclerosis CHL is mainly associated with modern therapy [1]. To our knowledge, other types of CHL have not been evaluated previously regarding the tumor cell mass.

In this study, our goal was to evaluate the importance of the wealth of H/RS cells in a cohort of patients and its relevance to the clinical and laboratory features of CHL, including all of its histological types. By far the most frequent type in our area, nodular sclerosis CHL, would be investigated to an equal extent with the remaining types.

METHODS

Of a cohort of 251 patients confirmed as CHL by two hematopathologists, we investigated 117 who were included, as only these cases met the technical conditions of the experiment and only in these, was a complete analysis performed. For each of these patients, we used a lymph node neutral formalin-fixed, paraffin-embedded section stained for CD30 to count the number of H/RS cells in 10 high power fields (HPF), from areas highlighting tumour cell aggregates. This approach was selected since H/RS cells are not dispersed uniformly in a lymph node section, but have the propensity to form collections of scattered tumor cells. Segregation of CD30+ H/RS cells from immunoblasts was possible due to their larger size, as well as membrane and Golgi area CD30 staining. In cases with weak or negative CD30 expression, tumor H/RS cells were also counted in H&E sections.

The cut-off point in the distribution of the H/RS cell count in 10 HPF was calculated to represent the median split of the total count. For this purpose, we described graphically the H/RS cell distribution representing a Gaussian curve and we determined the median value of the cell count thereof (data not shown).

In parallel, but this time on H&E sections, the number of apoptotic H/RS cells in 10 HPF in the above mentioned aggregates was calculated as described previously [9]. The results were then compared with prior analysed apoptotic indices.

For statistical analysis, contingency tables were addressed by the χ^2 and Fisher exact tests as appropriate. A Kaplan-Meier analysis used the Log Rank test.

Submitted : 09th June 2019 | Accepted : 11st August 2019 | Publication : 15th October, 2019

RESULTS

The H/RS cell count among the 117 patient cohort ranged from 6 to 403 H/RS per 10 HPF. Based on the median value, a cut-off was calculated to be 99 H/RS cells per 10 HPF. Unexpectedly, the count was superior to the cut-off in 61 (52.1%) cases. A comparison with the consensual proportion of the H/RS cells of the total cellular population of the CHL lymph node was not available, since we did not assess the background (reactive) compartment of the lymph node [10].

The patients' age was less than 45 in 86 patients (73.5%). Nodular sclerosis CHL was diagnosed in 75 patients (64.1%) and 42 were either mixed cellularity or lymphocyte depleted CHL.The stage was I-III in 85 (72.6%) and IV in 13 (11%) patients.The tumor was bulky in 24 patients (20.5%).Systemic symptoms were evident in 46 patients (39.3%). Relapse occurred in 31 patients (26.6%). On their last visit, 75 (65%) patients were alive without evidence of the disease. And notification had been given that 25 (21.4%) had died of the tumor.

The immunophenotype included strongly positive CD30 in 101 patients, while in 14, CD30 showed negative to weak expression. CD15 was positive in 104 patients. A case was MV-positive if it expressed any two or more measles virus (MV) antigens (87 patients – 74.4%), but it expressed MV hemagglutinin antigens in only 32 patients; p53 expression was positive in 102 patients; and MDM2 was positive in 58 patients.

Except for a significant association of a high share of tumour cells with the female gender, no statistically significant association between the rate of H/RS cells and the clinical features in our CHL patients was found (Table 1), including outcome and relapse rate. In contrast, among the laboratory features, a statistically significant relationship between the positive expression of several MV antigens and a high count of H/RS cells was evident (p=0.031). This was not the case with LMP1/EBV expression (p=0.055). MDM2 was also found to be related to a high tumor cell count (p=0.028;Table 2).

The rate of apoptotic H/RS cells was scrutinised and a single clinical association was found with relapsed CHL (Table 3; p=0.03). Regarding the biological features, a positive expression of MDM2 was significantly correlated with a high count of apoptotic tumor cells (Table 4;p=0.047).

A single significant association was found with the apoptotic index. This was an inverse link between an apoptotic index superior to the median, with a negative expression of BCL-2 (Table 5 and 6;p=0.036).

No statistically significant difference was apparent between the types of CHL, regarding the proportion of H/RS cells, of apoptotic H/RS cells, or relating to the apoptotic index (data not shown).

Using the Pearson's correlation ratio, a direct correlation was found between the H/RS cell count and the apoptotic H/RS cell count in 10 HPF ($r=0.426$; $p<0.001$). A direct correlation was found between the rate of apoptotic H/RS cells and the apoptotic index ($r=0.318$; $p<0.001$). In contrast, an inverse correlation between the tumor cell count in 10 HPF and the apoptotic index did not reach statistical significance.

A Kaplan-Meier (K-M) analysis was carried out to test the equality of survival distribution of the different levels of the H/RS cell count per 10 HPF (Figure 1). No significant variance was found by Log Rank test. The K-M analysis regarding the apoptotic H/RS cell distribution was not statistically significant (p=0.534; data not shown). The K-M analysis relating the apoptotic index showed equally non-significant results (p=0.757; data not shown).

DISCUSSION

The proportion of H/RS cell abundant CHL cases was suprisingly predominant, but not to a major extent. Thus the two subsets of patients were near equal and therfore, no statistically significant variation was found, regarding most clinical and biological characteristics.

The impact of the H/RS cell mass on the course of the disease in nodular sclerosis CHL has led in the past to disparate conclusions. More recently, it has been agreed that its grade II variant does not behave more aggressively than grade I subtype [1]. This generalisation did not include the variant classification suggested by von Wasielewski et al., as it has not been of wide use [11].

Classic HL usually features a rich and varied background, the remaining space being occupied by H/RS cells. The tumour cell mass was highlighted in our computing of the H/RS cells. *A priori,* there is no rationale for the restriction of the tumor cell counting only to nodular sclerosis CHL since abundance of tumor cells has also been found in mixed cellularity- and lymphocyte depleted CHL. For our purpose, a counting technique was needed which was as precise as possible. The CD30 staining is considered to highlight >90% of H/RS cells [12]. In the presence of a positive CD30 stain, the H/RS cells stand out distinctly. In this cohort, 101 of the patients showed tumor cells strongly positive for CD30 (87.7%). For 14 patients the H/RS cells were negative to weakly positive for this marker. In addition, the tumor cells have a propensity to accumulate into vague and non-compact collections. We therefore carried out the enumeration of H/RS cells with a CD30 stain by assessing these aggregates and computing these cells in 10 HPF.

A limitation of our paper was the definition of the cut-off point. Textbooks will often underline the standard H/RS cell load as representing 0.1-10% of the cellular population in the lymph node section. These texts do not account for the technique which allowed these authors to reach these figures, essentially of the reactive components of the CHL lymph nodes.The counting method which was employed hereby was different and probably more precise, and sets aside systematically the characterisation of the background, including the evidence of necrosis.

One of the few positive associations disclosed by our analysis was between the H/RS cell count/10 HPF and the positive expression of several MV antigens. We have previously found a link between the expression of MV antigens and several clinical and laboratory variables in CHL, which *a priori* should carry favourable prognostic significance and which included young age, early stages and female gender [13]. However, the mere presence of MV antigens in a case of CHL will carry a poorer prognosis.. A striking association is found presently between a high tumor cell count and the female gender. This supports the significance given previously to a positive expression of measles virus antigens as a probable mechanism mediated by the modulation of apoptosis in this cancer. Further findings have implied an effect for the MV expression on the H/RS cell apoptosis modulation [14]. An indirect connection is possible through the Pearson's correlation ratio computed above. The relationship is between a high H/RS cell count/10 HPF and a superior apoptotic H/RS cell count/10 HPF, highlighting a strong link between the two parameters.

An association between a high apoptotic H/RS cell count/10 HPF and an increased rate of relapse is also significant. The apoptotic cell count was also related with positive MDM2 expression. The product of this oncogene has a strong affinity for the wild-type p53 product, causing its inhibition. It has been suggested that, in the presence of a high rate of apoptotic H/RS cells, this p53 inhibitor, if expressed, will

reduce the number of apoptotic H/RS cells in the CHL lymph node [14].

The Pearson's correlation also indicates a significant and direct correlation between the rate of apoptotic tumour cells and the apoptotic index.

The only association of the apoptotic index was with the BCL-2 expression. As expected, it was inversely related with an apoptotic index higher than the median value.

CONCLUSIONS:

This study sustains the now generally accepted lack of relevance of H/RS cell abundance to the course of nodular sclerosis CHL. This conclusion was presently expanded from the nodular sclerosis grade II to the other histological CHL types, except for the lymphocyte-rich CHL, which was not identified in the present cohort. Our computing method seems adequate, but it ignores the evaluation of the background population. Our avoiding the reactive features in CHL might not be completely justified, since a very complex interaction takes place between the tumour cells and the cellular and interstitial milieu in this malignant tumor. Unusual findings were the association of a high H/RS cell portion (≥99/10 HPF) with female gender and with the positive expression of several MV antigens. This combination might be the only, though indirect, link with a poor prognosis. But, one notable finding is that, unexpectedly, the tumor cell rich CHL represent a majority of the cases, and this represents a novel finding in our study.

ACKNOWLEDGEMENTS:

Thanks to Kibbutz Sde Boker for their suggestions.

FIGURE LEGENDS

Figure 1. Kaplan-Meier analysis of the overall survival of CHL by the count of H/RS cell superior versus inferior to 99 cells/10 HPF.

Table 1. Wealth of Hodgkin/Reed Sternberg cells per 10 HPF and classic Hodgkin lymphoma clinical properties.

	=>99 (%)	<99 (%)	Total	p value
Gender				
F	31 (60.8)	20 (39.2)	51	
M	30 (45.5)	36 (54.5)	66	
total	61 (52.1)	56 (47.9)	117	p=0.023
Stage:				
I-III	43 (50.6)	42 (49.4)	85	
IV	6 (46.1)	7 (53.9)	13	
total	49 (50)	49 (50)	98	p=0.766
Outcome:				
AWD+DOD	13 (46.4)	15 (53.6)	28	
NED	43 (57.3)	32 (42.7)	75	
total	56 (54.3)	47 (45.7)	103	p=0.323
Relapse:				
None	39 (59.1)	27 (40.9)	66	
Yes	15 (48.4)	16 (51.6)	31	
total	54 (55.7)	43 (44.3)	97	p=0.322

99/10 HPF - the cutoff of the rate of H/RS cells/10 HPF.

AWD - alive with disease; DOD - dead of disease; NED - no evidence of tumor.

Table 2. Percentage of Hodgkin/Reed-Sternberg cells per 10 HPF and classic Hodgkin lymphoma biological properties.

	=>99 (%)	<99 (%)	Total	p value
CD30:				
Weak-Neg	11 (78.6)	3 (21.4)	14	
Strongly-Pos	49 (48.5)	52 (51.5)	101	
total	60 (52.2)	55 (47.8)	115	**P=0.038** Mid-P exact
MV:				
Neg	9 (34.6)	17 (65.4)	26	
Pos	51 (58.6)	36 (41.4)	87	
total	60 (53.1)	53 (46.9)	113	**p=0.031**
H14:				
Neg	25 (43.9)	32 (56.1)	57	
Pos	36 (61)	23 (39)	59	
total	61 (52.6)	55 (47.4)	116	p=0.064
LMP1:				
Neg	47 (58)	34 (42)	81	
Pos	14 (38.9)	22 (61.1)	36	
total	61 (52.1)	56 (47.9)	117	p=0.055
MDM2:				
Neg	20 (40.8)	29 (59.2)	49	
Pos	36(62.1)	22 (37.9)	58	
total	56 (52.3)	51 (47.7)	107	**p=0.028**

LMP1 - EBV antigen; MV - two or more MV are expressed; H14 - MV nucleoprotein antibody; MDM2 - oncogene.

Table 3. Proportion of apoptotic Hodgkin/Reed-Sternberg cells per 10 HPF and classic Hodgkin lymphoma clinical features.

	>=14.5 (%)	<14.5 (%)	Total	p value
Age:				
<45	41 (47.7)	45 (52.3)	86	
>=45	18 (58.1)	13 (41.9)	31	
total	59 (50.4)	58 (49.6)	117	0.321
Stage:				
I-III	43 (50.6)	42 (49.4)	85	
IV	6 (46.1)	7 (53.9)	13	
total	49 (50)	49 (50)	98	0.766
Outcome:				
AWD+DOD	17 (60.7)	11 (39.3)	28	
NED	37 (49.3)	38 (50.7)	75	
total	54 (52.4)	49 (47.6)	103	0.305
Relapse				
None	29 (43.9)	37 (56.1)	66	
Yes	22 (71)	9 (29)	31	
total	51 (52.6)	49 (47.4)	91	**0.013**

<14.5 - less than the median value of the count of apoptotic H/RS cells in 10 HPF.

Table 4. Count of apoptotic Hodgkin/Reed-Sternberg cells per 10 HPF and classic Hodgkin lymphoma biological properties.

	>=14.5 (%)	<14.5 (%)	Total	p value
LMP1:				
Neg	42 (51.9)	39 (48.1)	81	
Pos	17 (47.2)	19 (52.8)	36	
total	59 (50.4)	58 (49.6)	117	0.644
MV:				
Neg	14 (53.8)	12 (46.2)	26	
Pos	42 (48.3)	45 (51.7)	87	
total	56 (49.6)	57 (50.4)	113	0.618
H14:				
Neg	29 (50.9)	28 (49.1)	57	
Pos	29 (49..2)	30 (50.8)	59	
total	58 (50)	58 (50)	116	0.853
Al3922:				
Neg	16 (44.4)	20 (55.6)	36	
Pos	35 (53.8)	30 (46.2)	65	
total	51 (50.5)	50 (49.5)	101	0.365
MDM2:				
Neg	21 (42.9)	28 (57.1)	49	

Pos	36 (62.1)	22 (37.9)	58	
total	57 (53.3)	50 (46.7)	107	**0.047**

Table 5. Apoptotic index of Hodgkin/Reed-Sternberg cells and classic Hodgkin lymphoma clinical characteristics.

	>=8.3 (%)	<8.3 (%)	Total	p value
Age:				
<45	41 (47.7)	45 (52.3)	86	
>=45	18 (58.1)	13 (41.9)	31	
total	59 (50.4)	58 (49.6)	117	0.321
Stage:				
I-III	43 (50.6)	42 (49.4)	85	
IV	4 (30.8)	9 (69.2)	13	
total	47 (48)	51 (52)	98	FE - 0.200
Outcome:				
AWD+DOD	14 (50)	14 (50)	28	
NED	37 (49.3)	38 (50.7)	75	
total	57 (50.4)	56 (49.6)	113	0.952
Relapse:				
None	32 (48.5)	34 (51.5)	66	
Yes	17 (54.8)	14 (45.2)	31	
total	49 (50.5)	48 (49.5)	97	0.559

<8.3 - less than median value of apoptotic index.

Table 6. Apoptotic index of Hodgkin/Reed-Sternberg cells and classic Hodgkin lymphoma biological characteristics.

	>=8.3 (%)	<8.3 (%)	Total	p value
LMP1:				
Neg	37 (45.7)	44 (54.3)	81	
Pos	22 (61.1)	14 (38,9)	36	
total	59 (50.4)	58 (49.6)	117	0.123
MV:				
Neg	15 (57.7)	11 (42.3)	26	
Pos	41 (47.1)	46 (52.9)	87	
total	56 (49.6)	57 (50.4)	113	0.344
H14:				
Neg	29 (50.9)	28 (49.1)	57	
Pos	29 (49.2)	30 (50.8)	59	
total	58 (50)	58 (50)	116	0.853
AL3922:				
Neg	16 (44.4)	20 (55.6)	36	
Pos	35 (53.8)	30 (46.2)	65	
total	51 (50.5)	50 (49.5)	101	0.365
BCL-2:				
Neg	36 (60)	24 (40)	60	
Pos	23 (40.4)	34 (59.6)	57	
total	59 (50.4)	58 (49.6)	117	**0.036**

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	.355	1	.551

Test of equality of survival distributions for the different levels of rate of Hodgkin/Reed-Sternberg cells per 10 HPF.

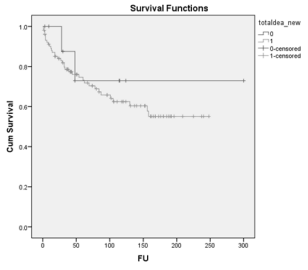

FIGURE 1

REFERENCES

1. van Spronsen DJ, Vrints LW, Hofstra G, et al. Disappearance of prognostic significance of histopathological grading of nodular sclerosing Hodgkin's disease for unselected patients, 1972-92. Brit J Haematol. 1997;96:322-327.
2. Bennet MH, Tu A, Vaughan Hudson G. Analysis of grade 1 Hodgkin's disease. Clin Radiol. 1981;32:491-498.
3. Bennet MH, Maclennan KA, Easterling MJ, Vaughan Hudson B, Jelliffe AM, Vaughan Hudson G. The prognostic significance of cellular subtypes in nodular sclerosing Hodgkin's disease: an analysis of 271 non-laparotomized cases (BNLI report). Clin Radiol. 1983;34:497-501.
4. Jairam R,Vrints LW, Breed WPM, Wijihuizen TJ, Wijnen JT. Histological classification of the nodular sclerotic form of Hodgkin's disease. Netherlands J Med. 1988;33:160-167.
5. Maclennan KA, Bennet MH, Tu A, et al. Relationship of histopathological features to survival and relapse in nodular sclerosing Hodgkin's disease: a study of 1659 patients. Cancer. 1989; 64:1686-1693.
6. Masih AS,Weisenburger DD, Vose JM, et al. Histologic grade does not predict prognosis in optimally treated, advanced stage nodular sclerosing Hodgkin's disease. Cancer. 1992;69:228-232.
7. d' Amore ESG, Lee CKK, Aeppli DM,et al. Lack of prognostic value of histopathologic parameters in Hodgkin's disease, nodular sclerosis type. A study of 123 patients with limited stage disease who had undergone laparotomy and were treated with radiation therapy. Arch Pathol Lab Med. 1992;116:856-861.
8. Hess JL, Bodis S, Pinkus G, et al. Histopathological grading of nodular sclerosis Hodgkin's disease: lack of prognostic significance in 254 surgically staged patients. Cancer. 1994;74:708-714.
9. Benharroch D, Levy A, Prinsloo I, et al. Apoptotic index as a prognostic factor in Hodgkin's disease. Leuk Lymphoma. 1999;33:351-359.
10. Stein H, Pileri SA, Weiss LM, et al. Hodgkin lymphoma introduction. in: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Arber DA, Hasserjian RP, Le Beau MM, Orazi A, Siebert R (Eds). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 2017, IARC, Lyon, p. 426.
11. von Wasielewski S, Franklin J, Fischer R, et al. Nodular sclerosing Hodgkin disease: new grading predicts prognosis in intermediate and advanced stages. Blood. 2003;101:4063-4069.
12. Fend F. Classical Hodgkin's lymphoma and related lesions. in: Jaffe ES, Arber DA, Campo E, Harris NL, Quintanilla-Martinez L, eds. Hematopathology, 2nd Edit., 2017, Elsevier, Philadelphia. p. 535.
13. Benharroch D, Ariad S, Tadmor N, et al. Relevance of the measles virus expression in cancer - an update. Pathol Oncol Res. 2016;22:661-666.
14. Benharroch D, Einav I, Feldman A, et al. Apoptosis of Hodgkin-Reed-Sternberg cells in classical Hodgkin lymphoma revisited. APMIS. 2010; 118: 339-345.