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ORIGINAL ARTICLE

Prognostic Value of Tumor Associated Macrophage Markers CD163 and CD68 Immunohistochemistry in Classical Hodgkin Lymphoma

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SUMMARY

Background: **Tumor associated macrophages have been implicated in the pathogenesis of classical Hodgkin's lymphoma and have been suggested to have a negative impact on outcome. The aim of this study is to determine the expression and the prognostic impact of CD163 and CD68 markers of the tumor associated macrophages, in the initial positively infiltrated bone marrow biopsy specimens of our subjects by immunohistochemistry and to correlate their expression with other clinical and laboratory prognostic factors.**
Methods: **This study was conducted on fifty-one patients with de novo classical Hodgkin's lymphoma, presenting to the Clinical Pathology Department at the National Cancer Institute, Cairo University. CD163 and CD68 were detected in the initial bone marrow biopsy specimens from our subjects by immunohistochemistry.**
Results: **The present study included 51 patients with CHL. They comprised 24 males (47.1%) and 27 females (52.9%) with an age of 32.9 ± 14.5 years. After treatment, 33 patients (64.7%) achieved complete remission while 18 patients (35.3%) failed. Comparison between patients with CR and patients without revealed significantly lower CD68 expression [median (IQR): 30.0 (15.0 - 47.5%) versus 55.0 (43.8 - 55.0%), p = 0.003] and CD163 expression [25.0 (10.0 - 37.5%) versus 45.0 (0.35 - 55.0%)] in CR patients. Binary logistic regression analysis identified CD68 and CD163 expressions as significant predictors of CR in univariate and multivariate analyses.**
Conclusions: **The expressions of both tumor-associated markers, CD68 and CD163, are significant predictors of CR in patients with CHL.**
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KEY WORDS

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INTRODUCTION

Within the World Health Organization (WHO) classification of Hodgkin's Lymphoma (HL), there are two entities: nodular lymphocyte predominant HL (NLPHL) and classical HL (CHL). The latter has four histological subtypes: nodular sclerosis CHL, lymphocyte-rich CHL, mixed cellularity CHL, and lymphocyte-depleted CHL differing from each other with respect to clinical features, immunohistochemistry, and molecular genetics. Approximately 90% of all HL cases belong to the

CHL type with only 10% being diagnosed with the NLPHL subtype [1].

In the United States, HL is listed as a rare disease with an age-adjusted annual incidence rate of 2.8 cases per 100,000 population [2]. In Egypt, HL was diagnosed in 0.7% of newly diagnosed female and 1.29% of newly diagnosed male cancer patients [3].

Although the prognosis of HL is relatively good, cases with poor prognosis still exist; hence, the need for proper early risk stratification of cases. Over recent years, the study of the components of the tumor microenvironment has proven to be of significant value in achieving this quest with macrophages regarded as a particularly valuable component [4].

Tumor associated macrophages (TAMs) have been implicated in the pathogenesis of CHL and have been suggested to have a negative impact on disease outcome. Most studies addressing the role of macrophages in CHL have relied on the identification of macrophages by generic macrophage antigens [4]. Of these antigens CD163 and CD68 are of notable importance. Both are scavenger pattern recognition receptors expressed on the cells of the monocyte/macrophage lineage, they have a role in the innate immunologic functions of macrophages and are thought to have a role in the development of the atheromatous plaque [5].

The present study aimed to determine the expression and the prognostic impact of CD163 and CD68 markers of the tumor associated macrophages in the initial positively infiltrated bone marrow biopsy specimens of our subjects by immunohistochemistry and to correlate their expression with other clinical and laboratory prognostic factors, to assess their role in the response to treatment for the possibility to use modifying drugs and improve the response in HL patients.

MATERIALS AND METHODS

The present study was conducted at the National Cancer Institute (NCI), Cairo University, Egypt. Patients were recruited from January 2014 through December 2015 and were followed up until the end of December 2018. The study protocol was approved by the local IRB and a written informed consent was obtained from all patients/patients' guardians before the study entry.

The study included 51 Egyptian patients with lymph node biopsy confirmed stage IV CHL with malignant cell positivity for CD30 and CD15 and initial bone marrow positivity for infiltration and immunohistochemically confirmed with CD30 positivity.

Initial assessment

Clinical assessment included thorough clinical examination and history taking including personal history. Routine laboratory work-up included complete blood count (CBC) with differential count, serum lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), full biochemical panel including liver, renal function tests and virology scan for hepatitis viruses B and C and HIV virus.

Radiological assessment for all patients was performed using CT with contrast of the neck, chest, abdomen and pelvis to visualize enlarged non-palpable lymph nodes, spleen and liver enlargement or foci etc., for accurate diagnosis and staging. Echocardiography was performed for all patients to determine the cardiac functional status prior to the initiation of therapy with adriamycin-based protocols. Other radiological procedures were performed when needed including abdominal ultrasonography, chest X-ray, and mammogram.

Histopathological assessment of the biopsied lymphomatous tissue included gross examination, formalin-fixed, paraffin embedded tissue sections, stained smears, microscopic examination for the diagnosis of CHL, denoting its histological subtype.

Assessment of tumor tissue metabolism was done by whole body [^{18}F] 2-fluro-2-deoxy-D-glucose positron emission tomography (FDG-PET-CT) for all patients to determine response to therapy using the Deauville 5-point scoring system [6].

Immunohistochemistry

Bone marrow specimens from patients with CHL were subjected to morphological examination using hematoxylin and eosin preparations. Specimens found to be morphologically positive for infiltration with mononuclear HD cells and/or RS cells were in turn subjected to confirmatory immunohistochemistry (IHC) for CD30. Specimens found to be positive for CD30 were subjected to IHC for the markers of interest in our study, CD163 and CD68.

IHC was performed per the manufacturer's instructions using Antihuman CD163 (Novus biologicals™, Centennial, Colorado, USA, Entrez gene ID: 9332, Clone: 10D6, Code: NB110-59935) monoclonal mouse, IgG1, tissue culture supernatant and Antihuman CD68 (Dako™, produktionsvej 42, DK-2600 Glostrup, Denmark, FLEX, Ready-to-use, Entrez gene ID: 968, Clone: KP1, Code: IR609) monoclonal mouse, IgG1, kappa, both obtained from tissue culture supernatants, positive and negative controls were done in every run. Microscopic examination was performed using Olympus light microscope (CX 21 series). The most cellular area of the tumor, with the minimum necrosis or inflammatory cell infiltration and having the highest cytoplasmic staining density, was selected and the number of positively stained cells was recorded in consecutive fields at x 40 magnification. The percentage of tumor cells expressing both CD163 and CD68 was determined by counting 1,000 cells per slide for each marker [7]. The four hematopathologists then independently graded the extent of tumor-infiltrating macrophages by both CD68 and CD163 assessing all neoplastic tissue on the slide. The estimates were performed in 5% increments, and the percentage of staining macrophages was reported in relation to overall cellularity. The mean value of CD68+ and CD163+ cells was calculated from the individual

grades provided by all four hematopathologists involved in the study and reported as "mean macrophage staining" in order to minimize inter-observer variability [6] (Figures 1 and 2).

Treatment protocol

All patients received the standard ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) HL treatment protocol or 4 - 6 cycles with a maximum of 12 cycles. Some patients with localized bulky disease received involved-site/field radiation therapy (ISRT/IFRT). Other patients received second-line chemotherapy regimens (DHAP, ICE, and GDP), while selected cases underwent autologous hematopoietic stem cell transplantation according to the NCI combined clinic verdict.

Response evaluation and follow up

Evaluation of response was done according to revised response criteria for malignant lymphoma (Cheson criteria) [8]. Response was classified into complete response (CR), partial response (PR), progressive disease (PD), stable disease (SD) or unable to evaluate (UE). Patients were followed up according to standard NCI protocols which entailed: 1) Medical history and physical examination every 2 - 4 months for 2 years, then every 3 - 6 months for the next 3 - 5 years. 2) CT chest, abdomen, and pelvis every 6 - 12 months for first 2 - 3 years.

Statistical analysis

Data obtained from the presented study were expressed as number and percent, mean and standard deviation or median and interquartile range. Numerical variables were compared using *t*-test or Mann-Whitney U test while categorical variables were compared using chi-square test. Logistic regression was used to identify predictors of CR. P value less than 0.05 was considered statistically significant. All statistical calculations were computed using SPSS 26 (IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA).

RESULTS

The present study included 51 patients with CHL. They comprised 24 males (47.1%) and 27 females (52.9%) with an age of 32.9 ± 14.5 years. After treatment, 33 patients (64.7%) achieved complete remission while 18 patients (35.3%) failed. These included 6 patients (11.8%) with partial response (PR), 5 patients (9.8%) with progressive disease (PD), 5 patients (9.8%) with stable disease (SD), and 2 patients (11.1%) were unable to evaluate (UE).

Comparison between patients with CR and patients without revealed significantly lower CD68 expression [median (IQR): 30.0 (15.0 - 47.5%) versus 55.0 (43.8 - 55.0%), p = 0.003] and CD163 expression [25.0 (10.0 - 37.5%) versus 45.0 (0.35 - 55.0%)] in CR patients. No statistically significant differences were found between patients with CR and patients without regarding age (32.2 ± 17.6 versus 34.4 ± 17.8 years, p = 0.67), gender distribution (male/female: 18/15 versus 6/12, p = 0.76), positive family history (6.1% versus 5.6%, p = 0.94), B symptoms (36.4% versus 50.0%, p = 0.34), lymphadenopathy (84.9% versus 94.4%, p = 0.31), splenomegaly (21.2% versus 22.2%, p = 0.93), and hepatomegaly (18.2% versus 33.3%, p = 0.22). Also, no significant differences were found between patients with CR and patients without regarding laboratory parameters including Hb (10.9 ± 2.2 versus 10.3 ± 2.7 gm/dL, p = 0.4), TLC (median (IQR): 6.3 (5.0 - 8.4) versus 6.3 (5.2 - 8.1) x 10^3/mL, p = 0.95), platelets (293.9 ± 107.0 versus 274.3 ± 129.6 x 10^3/mL, p = 0.56), ESR (median (IQR): 18.0 (13.5 - 28.5) versus 20.5 (12.3 - 45.5) mm/hour, p = 0.71), LDH (median (IQR): 243.0 (186.0 - 357.0) versus 241.5 (204.8 - 406.8) U/L, p = 1.0).

Furthermore, no significant differences were found between patients with CR and patients without regarding pathological subtypes (p = 0.31), BM cellularity (p = 0.21) and BM fibrosis (p = 0.47) (Table 1).

Comparison between patients with moderate and high CD68 expression revealed significantly higher CD163 expression in patients with high CD68 expression [10.0 (7.5 - 20.0%) versus 45.0 (26.3 - 50.0%), p = 0.001]. No significant relationship was found between CD68 expression and other clinical, laboratory, and pathological data (Table 2). Comparison between patients with moderate and high CD163 expression revealed significantly higher CD68 expression in patients with high CD163 expression [10.0 (7.5 - 20.0%) versus 45.0 (35.0 - 55.0%), $p \leq 0.001$]. No significant relationship was found between CD163 expression and other clinical, laboratory, and pathological data (Table 3).

Binary logistic regression analysis identified CD68 and CD163 expressions as significant predictors of CR in univariate and multivariate analyses (Table 4).

DISCUSSION

Whereas previous studies sometimes examined sections from positively infiltrated bone marrow with CHL in conjunction with lymph node and splenic sections, which constituted the main bulk of tumor tissue specimens for their work, the present study solely examined the markers in positively infiltrated bone marrow sections in an effort to investigate the possibility of adding an extra diagnostic insight to routinely performed investigations done for patient staging.

In concordance with our study, in 2010 Tzankov and colleagues utilized immunohistochemistry to analyze the prognostic importance of the CD68-positive macrophage number compared to other cellular environmental components in an unselected series of 105 HL patients. They found that cases with increased numbers showed worse overall survival compared to patients with lower numbers [9].

Again in 2010, another study by Steidl and colleagues,

Table 1. Comparison between patients with CR and patients without regarding the clinical and laboratory data.

	All patients n = 51	CR n = 33	No CR n = 18	p-value
Age (years) mean ± SD	32.9 ± 14.5	32.2 ± 17.6	34.4 ± 17.8	0.67
Male/Female n	24/27	18/15	6/12	0.76
Positive family history n (%)	3 (5.9)	2 (6.1)	1 (5.6)	0.94
B symptoms n (%)	21 (41.2)	12 (36.4)	9 (50.0)	0.34
Lymphadenopathy n (%)	45 (88.2)	28 (84.9)	17 (94.4)	0.31
Splenomegaly n (%)	11 (21.6)	7 (21.2)	4 (22.2)	0.93
Hepatomegaly n (%)	12 (23.5)	6 (18.2)	6 (33.3)	0.22
Laboratory data				
Hb (gm/dL) mean ± SD	10.7 ± 2.4	10.9 ± 2.2	10.3 ± 2.7	0.4
TLC (x 10^3/mL) median (IQR)	6.3 (5.0-8.3)	6.3 (5.0-8.4)	6.3 (5.2-8.1)	0.95
Platelets (x 10^3/mL) mean ± SD	287.0 ± 114.6	293.9 ± 107.0	274.3 ± 129.6	0.56
ESR (mm/hour) median (IQR)	18.0 (13.0 - 32.0)	18.0 (13.5 - 28.5)	20.5 (12.3 - 45.5)	0.71
LDH (U/L) median (IQR)	243.0 (198.0 - 379.0)	243.0 (186.0 - 357.0)	241.5 (204.8 - 406.8)	1.0
CD68 (%) median (IQR)	40.0 (20.0 - 55.0)	30.0 (15.0 - 47.5)	55.0 (43.8 - 55.0)	0.003
CD68 expression grades				
1	-	-	-	0.002
2	17 (33.3)	16 (48.5)	1 (5.6)	
3	34 (66.7)	17 (51.5)	17 (94.4)	
CD163 (%) median (IQR)	35.0 (15.0 - 50.0)	25.0 (10.0 - 37.5)	45.0 (0.35 - 55.0)	0.002
CD163 expression grades				
1	-	-	-	0.009
2	23 (45.1)	20 (60.6)	3 (16.7)	
3	28 (54.9)	13 (39.4)	15 (83.3)	
Pathological subtypes n (%)				
Nodular sclerosis	25 (49.0)	14 (42.4)	11 (61.1)	0.31
Mixed cellularity	21 (41.2)	14 (42.4)	7 (38.9)	
Lymphocyte rich	3 (5.9)	3 (9.1)	-	
Lymphocyte depletion	2 (2.9)	2 (6.1)	-	
BM cellularity n (%)				
Normocellular	35 (68.6)	20 (60.6)	15 (83.3)	0.21
Hypocellular	3 (5.9)	2 (6.1)	1 (5.6)	
Hypercellular	13 (25.5)	11 (33.3)	2 (11.1)	
BM fibrosis n (%)				
Grade 1	12 (23.5)	8 (24.2)	4 (22.2)	0.47
Grade 1 - 2	1 (2.0)	1 (3.0)	-	
Grade 2	14 (27.5)	9 (27.3)	5 (27.8)	
Grade 2 - 3	3 (5.9)	3 (9.1)	-	
Grade 3	3 (5.9)	(3.0)	2 (11.1)	

using gene-expression profiling also ran in accordance with our findings. In an independent cohort of patients, it was found that an increased number of CD68+ macrophages was correlated with a shortened progression-free survival and an increased likelihood of relapse after autologous hematopoietic stem-cell transplantation, resulting in shortened disease-specific survival [10].

A study in 2012 examined one hundred pediatric CHL

Table 2. Relationship between CD68 expression grades and the clinical and laboratory data.

	Expression grade 2 n = 17	Expression grade 3 n = 34	p-value
Age (years) mean ± SD	31.6 ± 17.2	33.6 ± 17.9	0.71
Male/Female n	7/10	17/17	0.55
Positive family history n (%)	-	3 (8.8)	0.21
B symptoms n (%)	7 (41.2)	14 (41.2)	1.0
Lymphadenopathy n (%)	16 (94.1)	29 (85.3)	0.36
Splenomegaly n (%)	1 (5.9)	11 (32.4)	0.054
Hepatomegaly n (%)	2 (11.8)	10 (29.4)	0.16
Laboratory data			
Hb (gm/dL) mean ± SD	11.3 ± 1.9	10.4 ± 2.5	0.19
TLC (x 10^3/mL) median (IQR)	6.1 (5.2 - 7.5)	6.4 (5.0 - 8.5)	0.62
Platelets (x 10^3/mL) mean ± SD	308.0 ± 101.0	276.5 ± 120.8	0.36
ESR (mm/hour) median (IQR)	18.0 (14.5 - 39.5)	19.0 (13.0 - 31.5)	0.92
LDH (U/L) median (IQR)	233.0 (167.0 - 429.5)	245.5 (212.0 - 364.5)	0.92
CD163 (%) median (IQR)	10.0 (7.5 - 20.0)	45.0 (26.3 - 50.0)	0.001
CD163 expression grades n (%)			
1	-	-	< 0.001
2	14 (82.4)	7 (20.6)	
3	3 (17.6)	27 (79.4)	
Pathological subtypes n (%)			
Nodular sclerosis	7 (41.2)	18 (52.9)	0.85
Mixed cellularity	8 (47.1)	13 (38.2)	
Lymphocyte rich	1 (5.9)	2 (5.9)	
Lymphocyte depletion	1 (5.9)	1 (2.9)	
BM cellularity n (%)			
Normocellular	11 (64.7)	24 (70.6)	0.45
Hypocellular	2 (11.8)	1 (2.9)	
Hypercellular	4 (23.5)	9 (26.5)	
BM fibrosis n (%)			
Grade 1	4 (23.5)	8 (23.5)	0.44
Grade 1 - 2	-	1 (2.9)	
Grade 2	6 (35.3)	8 (23.5)	
Grade 2 - 3	2 (11.8)	1 (2.9)	
Grade 3	-	3 (8.8)	

cases, but unlike our study, CD68-positive cells did not show an effect on outcome. Worse overall survival was observed in cases with CD163/CD8 ratio ≥ 2. However, in agreement with our study, high numbers of CD163 (+) cells were associated with worse progression-free survival (PFS) [4].

Also, in 2012, Harris and colleagues examined 44 cases of CHL. Their study failed to identify a correlation between staining for CD68 or CD163 and disease recurrence and, hence, did not prove their prognostic significance, in discordance with our study [7].

Yet again in 2012, Tan and colleagues investigated the prognostic significance of TAMs in the E2496 Intergroup trial. In line with our study, CD68 and CD163 expression appeared to be associated with a more adverse outcome, where increased CD68 and CD163 expression was significantly associated with inferior failure-free survival and OS in the validation cohort [11]. Similar to our findings, in 2013, Greaves and colleagues found that increased CD68 confers inferior freedom from first-line treatment failure (FFTF) and overall survival (OS) [12].

Table 3. Relationship between CD163 expression grades and the clinical and laboratory data.

	Expression grade 2 n = 21	Expression grade 3 n = 30	p-value
Age (years) mean ± SD	33.6 ± 16.0	32.5 ± 18.8	0.82
Male/Female n	13/8	14/16	0.28
Positive family history n (%)	1 (4.8)	2 (6.7)	0.78
B symptoms n (%)	8 (38.1)	13 (43.3)	0.71
Lymphadenopathy n (%)	19 (90.5)	26 (86.7)	0.68
Splenomegaly n (%)	2 (9.5)	9 (30.0)	0.08
Hepatomegaly n (%)	3 (14.3)	9 (30.0)	0.19
Laboratory data			
Hb (gm/dL) mean ± SD	11.2 ± 2.3	10.3 ± 2.4	0.16
TLC (x 10^3/mL) median (IQR)	6.3 (5.2 - 8.0)	6.5 (5.0 - 8.4)	0.65
Platelets (x 10^3/mL) mean ± SD	322.7 ± 103.2	262.0 ± 117.1	0.062
ESR (mm/hour) median (IQR)	21.0 (13.0 - 33.0)	18.0 (13.0 - 31.0)	0.9
LDH (U/L) median (IQR)	257.0 (168.0 - 425.0)	239.0 (218.8 - 364.5)	0.91
CD68 (%) median (IQR)	10.0 (7.5 - 20.0)	45.0 (35.0 - 55.0)	< 0.001
CD68 expression grades			
1	-	-	< 0.001
2	14 (66.7)	3 (10.0)	
3	7 (33.3)	27 (90.0)	
Pathological subtypes n (%)			
Nodular sclerosis	8 (38.1)	17 (56.7)	0.55
Mixed cellularity	11 (52.4)	10 (33.3)	
Lymphocyte rich	1 (4.8)	2 (6.7)	
Lymphocyte depletion	1 (4.8)	1 (3.3)	
BM cellularity n (%)			
Normocellular	13 (61.9)	22 (73.3)	0.56
Hypocellular	2 (9.5)	1 (3.3)	
Hypercellular	6 (28.6)	7 (23.3)	
BM fibrosis n (%)			
Grade 1	8 (38.1)	4 (13.3)	0.16
Grade 1 - 2	-	1 (3.3)	
Grade 2	5 (23.8)	9 (30.0)	
Grade 2 - 3	2 (9.5)	1 (3.3)	
Grade 3	-	3 (10.0)	

Table 4. Predictors of CR in the studied patients.

	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Age	1.0	0.98 - 1.04	0.66	-	-	-
Gender	1.2	0.38 - 3.8	0.76	-	-	-
LDH	0.99	0.99 - 1.0	0.63	-	-	-
CD68	1.06	1.02 - 1.1	0.003	1.03	1.01 - 1.07	0.032
CD163	1.06	1.02 - 1.1	0.003	1.04	1.02 - 1.12	0.012

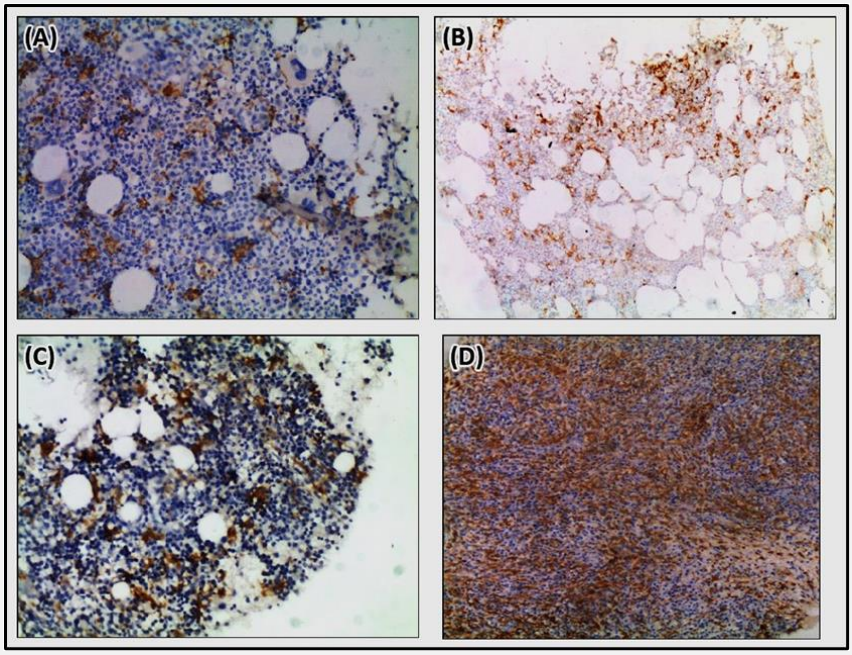

Figure 1. Micrograph of BMB IHC showing an average CD163 expression of: (A) 5% (200X), (B) 20% (100X), (C) 45% (200X), (D) 80% (400X).

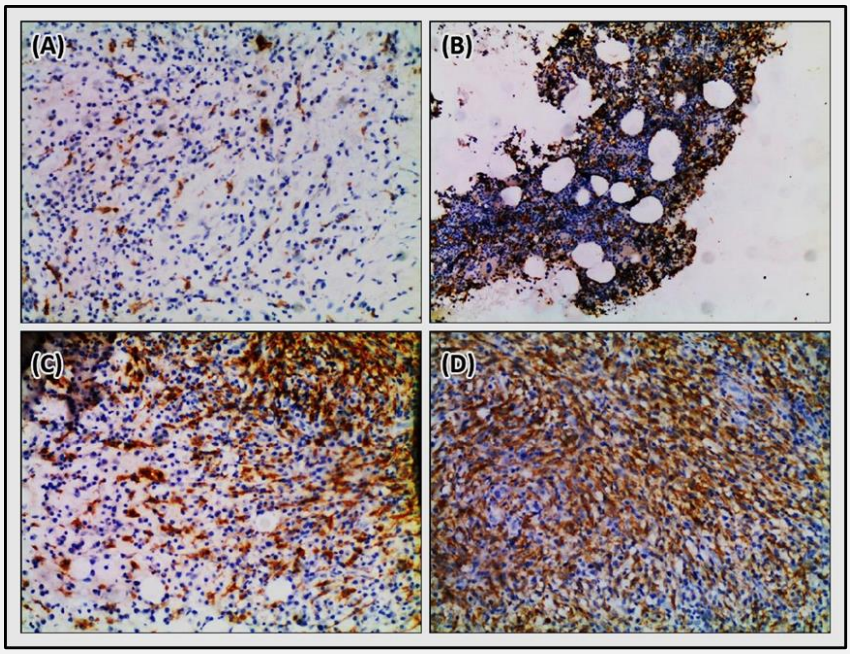

Figure 2. Micrograph of BMB IHC showing an average CD68 expression of: (A) 5% (200X), (B) 35% (100X), (C) 50% (200X), (D) 85% (200X).

In congruence with our results, a study from South Korea in 2014 examined the prognostic relevance of TAMs in relation to vascular endothelial growth factor (VEGF) expression and angiogenesis in uniformly treated cases of CHL. High CD163 expression was strongly associated with inferior event free survival (EFS) and OS [13]. Another study by Klein and colleagues examined immunohistochemistry (IHC) of CD68 and CD163 in a retrospective cohort of 88 patients with CHL and confirmed a prognostic role of tumor-infiltrating macrophage markers in CHL, though only for CD163 (p-value < 0.001) and not for CD68 (p-value = 0.414) [14]. A study carried out in 2014 concluded that CD163 is not an M2 macrophage-specific marker and suggested that in pediatric CHL the polarization of macrophages may depend on EBV status of HRS cells, and that high numbers of M2 macrophages, but not M1 macrophages, is associated with worse PFS (p-value = 0.009) and recommended the use of double-labelling immunohistochemistry [15].

In 2015 a study from France evaluated the relationship between CD68 expression, interim positron emission tomography (iPET) results, and outcome in 158 patients with HL. In congruence with our results, that study confirmed the prognostic value of CD68 in HL. Where a correlation between CD68 and iPET was detected suggesting a potential for a better stratification (p-value = 0.0016) [16].

In 2016, a meta-analysis was reported on the association of CD68 and CD163, as markers of the tumor-associated macrophages (TAM) with the clinical outcome of adult CHL. The analyses suggested that a high density of either $CD68^+$ or $CD163^+$ TAMs is a robust predictor of adverse outcomes in adult CHL and recommended that increased TAMs should be taken into account to further improve prognostic stratification and the planning of appropriate therapeutic strategies. Twenty-two eligible studies with a total of 2,959 patients were identified. In concordance with our study, the analysis indicated that a high density of $CD68^+$ TAMs in the tumor microenvironment of adult CHL predicted poor overall survival (OS) (HR: 2.41; 95% CI: 1.92 - 3.03), shorter progression-free survival (PFS) (HR: 1.78; 95% CI: 1.45 - 2.18), and poor disease-specific survival (HR: 2.71; 95% CI: 1.38 - 5.29). Further in support of our findings, high density of $CD163^+$ TAMs in the tumor microenvironment of adult CHL also predicted poor OS (HR: 2.75; 95 % CI: 1.58 - 4.78) and poor PFS (HR: 1.66; 95% CI: 1.22 - 2.27) [17].

Of note, the present study did not find significant relationships between CD163 and CD68 expression and other clinical, laboratory, and pathological data in accordance with previous studies [12,14].

In conclusion, our study suggests a possible beneficial role for CD163 and CD68 markers of the tumor associated macrophages in the bone marrow biopsy sections of Egyptian patients with classical Hodgkin's lymphoma involving the bone marrow as a tool of risk stratification and prognostic indication of therapy outcome. The level of their expression may help in predicting an unfavorable disease course and prompt preemptive measures early on.

We recommend further extensive studies involving larger sample sizes, longer follow up periods, a combination of more markers for the tumor microenvironment using double-labeling immunohistochemistry for the detection of macrophage markers together with transcription factors to characterize both M1 and M2 macrophages, including the EBV status of the patients, more advanced methodology including computer assisted analysis of micrographs. Relating marker expression in both lymph node and splenic sections with infiltrated bone marrow sections for the same patients, are recommended to better establish a standardized prognostic marker profile and method of reporting intended for routine use in patients with classical Hodgkin lymphoma, that is both feasibly cost-effective and evidence-based**.**

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None.

Declaration of Interest:
None.

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