Clinico-pathological significance of immunohistochemically marked tumor-associated macrophage in classic Hodgkin lymphoma

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Abstract

Background: Tumor-associated macrophages (TAM) are pivotal in remodeling of the tumor immune microenvironment and clinical outcome. Herein, we aim to evaluate the impact of immunohistochemical (IHC) expression of CD68 and CD163 in TAM on clinico-pathological features, patients’ response to therapy and the overall survival (OS).

Results: This retrospective study was performed on paraffin-embedded tissue blocks of 100 classic Hodgkin Lymphoma (cHL) cases diagnosed and treated at our Institution. Immunohistochemical scores of CD68 and CD163 were statistically related to bulky disease (p value = 0.005 for both), tumor stage (p value = 0.02 for both), International Prognostic Score (IPS) (p value = 0.04 and 0.02 respectively), and the overall response rate (ORR) (p value = 0.001). Additionally, CD163 was also statistically related to gender (p value = 0.02), serum albumin level (p value = 0.03), and B symptoms (p value = 0.04). HCV seropositivity did not relate to either CD68 or CD163 score. Using univariate analysis revealed that B symptoms, bulky disease, IPS ≥ 3, and CD163 > 25% were associated with lower OS (p values = 0.003, 0.006, 0.001, and < 0.001 respectively), while after multivariate cox regression analysis, B symptoms, IPS ≥ 3, and CD163 > 25% were related to inferior OS (p values 0.02, 0.02, and 0.003).

Conclusion: CD163 expressing TAM is a powerful predictor for OS in cHL, unlike CD68.

Keywords: Classic Hodgkin lymphoma (cHL), Tumor-associated macrophage (TAM), CD68, CD163, Disease free survival (DFS), Overall survival (OS)

Background

Hodgkin lymphoma (HL) is a unique neoplasm of B cell that represents about 10% of all diagnosed lymphomas in the USA [1]. The crude incidence of HL in the European Union represents about 2.3, and its mortality rate is about 0.4 cases/100.000/year [2]. In Egypt, the incidence of HL is 1.5 per 100.000 populations [3].

Classic Hodgkin’s Lymphoma (cHL) represents about 95% of all cases of HL and is characterized by the presence of Hodgkin Reed-Sternberg cells (HRS) with many reactive cells in tumor microenvironment such as macrophages, lymphocytes, neutrophils, histiocytes, eosinophils, and plasma cells [2].

cHL microenvironment is unique as the tumor cells (HRS) normally constitute less than 1% of the tumor cellularity and are surrounded by an abundant and heterogeneous inflammatory infiltrate. The interaction between HRS and the microenvironment reactive cells sustain tumor growth and survival [4]. The constituent cells of this microenvironment may expect the clinical outcomes in cHL [5, 6].
Although, it is a potentially curable disease, about 20% of patients still die from progressive disease and another proportion may be over treated leading to solid tumors and end organ dysfunction as sequel [7]. Thus, it is mandatory to predict those cases with a likelihood of treatment failure at the time of diagnosis. International Prognostic Score (IPS) is the most commonly used prognostic stratification system, although, this model is less suitable for patients with limited stage disease and it fails to identify a group of patients whose probability of cure is less than 50% [8].

A recent meta-analysis found that tumor-associated macrophages (TAM) which were identified by immunohistochemistry (IHC), CD68 or CD163, in chL microenvironment could be a predictor of adverse outcome, but this result was limited by poor accessibility to negative studies and variation in the included studies regarding follow-up duration and endpoints [9]. Another large study was carried on 265 patients concluded no association between TAM and prognosis of chL [10].

Regarding the debatable prognostic role of TAM in chL, we evaluated in this study the IHC staining for both CD68 and CD163 to assess the TAM in Egyptian patients diagnosed with chL. Also, the relation of TAM with the clinico-pathological characteristic, patients’ response to therapy and their impact on patients’ outcome and survival were studied.

Methods
Patients and clinico-pathological characteristics
We performed a retrospective study on formalin-fixed paraffin-embedded tissue blocks of 100 cases diagnosed with chL which were collected from the archive of pathology laboratory at our institution from 2008 to 2016 where the patients were diagnosed according to the World Health Organization (WHO) criteria. All included patients were subjected to comprehensive clinical history, physical examination, CT scan, and laboratory and radiological investigations. Patients were staged according to the Ann Arbor staging system and were categorized into stages I, II, III, and IV. Relying upon several risk factors including patient’s age, gender, stage, and presence of hypoalbuminemia and lymphopenia, the International Prognostic Score (IPS) was categorized as: low-risk IPS if presented with up to two risk factors and high-risk IPS if presented with three or more risk factors [11].

Most patients (90%) were treated with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) protocol, and only 10 patients (10%) were treated with combined modality as 1st line therapy. The attained response was defined as complete remission (CR) when the patient became completely free from nodal enlargement by CT or PET/CT scans as well as normalization of the initially abnormal laboratory tests and/or biopsies for at least 1 month. On the other hand, partial response (PR) was defined as there was remaining nodal enlargement which is decreased in size and number by at least 50%. Relapse was considered when there was appearance of new lymph nodes after previous remission, either complete or partial response to therapy, This is confirmed by abnormal hilum by CT and/or lymphoma infiltration with biopsy, or only bone marrow involvement without nodal enlargement.

Recorded follow-up data was included to define the disease-free survival (DFS) as the time between the date of CR to time of progression, relapse, or death, while the overall survival (OS) was recognized as the time between date of diagnosis to the date of death from any reasons or last date of follow-up for still alive patients. The exclusion criteria include:

1. Patients with previous history of any other malignancy,
2. Relapsed or refractory chL and
3. Insufficient recorded laboratory, radiological, follow-up data or paraffin blocks.

The current study was reviewed and approved by our Institutional review board (IRB) of our faculty of medicine (Code number: R/18.02.27).

Immunohistochemistry
Immunohistochemistry (IHC) for CD68 and CD163 were done according to the manufacturer’s data sheet. Paraffin blocks of the selected cases were sectioned 4-μm thick, deparaffinized, and rehydrated then incubated for about 30 min with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity followed by epitope retrieval using heat-induced epitope retrieval (HIER) for 30 min in EDTA buffer solution, pH 8.0 together with a pressure cooker followed by rinsing in distilled water and phosphate-buffered saline (PBS). The prepared tissue sections were then incubated for 30 min at room temperature with primary antibodies against monoclonal mouse CD68/Macrophage Marker Ab-3 Antibody (catalog #: MS-397-R7 [7.0 ml], Thermo Fisher Scientific, UK) and monoclonal mouse CD163 Antibody (catalog #: MS-1103-S0,-S1 or -S [0.1 ml, 0.5 ml, or 1.0 ml supernatant], Thermo Fisher Scientific, UK). Immunoperoxidase method was done using ImmunoPure Ultra-Sensitive ABC Peroxidase (catalog #: TL-060-PHJ, Thermo-scientific, UK), using diaminobenzidine as chromogen (catalog #: TA-125-HDX, Thermo-scientific, UK). After that, tissue sections were washed in distilled water then counterstained with hematoxylin followed by
dehydration in ascending grades of alcohol and xylene and finally covered with coverslips.

Evaluation of the IHC
Both CD68 and CD163 expressions were semi-quantitatively assessed by the pathologist for each case. The percentage of positive cells was considered in the evaluation. Both CD68 and CD163 positive macrophage showed membrane-associated reactivity. The percentage of CD68 and CD163 positive macrophages was determined in relation to negative Reed-Sternberg cells and reactive inflammatory cells in the background. The staining was detected in the micro-environment in Reed-Sternberg rich areas and was evaluated regardless if it is a hot-spot or not and scored as: I (< 5%), II (5–25%), and III (> 25%) [12]. For quality control, an internal positive control (the endothelial cells lining the adjacent vascular spaces) as well as negative control (slides which were not incubated by primary antibodies) were used.

Statistical analysis
Data were tabulated then statistically analyzed using Statistical Package for Social Sciences (SPSS), version 22. \( \chi^2 \) (Chi-square) or Fisher exact tests were used to test significant differences between groups. Chi-Square \( \chi^2 \) test was used for comparison of 2 or more groups. Fisher Exact test was used as correction for Chi-Square test when more than 25% of cells have count less than 5 in 2 × 2 tables.

The survival curves were performed using Kaplan Meier method to detect mean and median survival times. The differences between the curves were studied using the log-rank test. Multivariate Cox regression was used to identify predictors and risk factors affecting the survival. In all tests, \( P \leq 0.05 \) was considered to be statistically significant.

Results
Clinico-pathological and histological features of the studied cases (Table 1)
This study included 100 patients diagnosed with cHL. Their median age was 33 (ranged 15–74 years). The male:female ratio was 37%:63%. The hemoglobin level was less than 10.5 g/dl in 36 patients (36%). The white blood cell count was \( \geq 15 \times 10^9/l \) in 15% of patients, and 35 patients (35%) had less than 4 g/dl serum albumin levels. Screening for HIV antibodies, HBs Ag, and HCV antibodies is routinely carried out for patients under treatment in our oncology center unlike, EBV status which was not investigated. No included patients were positive for HIV antibodies or HBs Ag, but HCV antibodies were detected in sera of 14 patients (14%). Ninety-five patients had performance status (0 and 1).

Table 1 Clinico-pathological characteristics of the included 100 patients

| Item                  | Value         | Percentage |
|-----------------------|---------------|------------|
| Age (median)          | 33 (15–74)    | years      |
| Gender                | Male: female  | 37%: 63%   |
| HB level              | \( \geq 10.5 \text{ g/dl} \) | 64%        |
|                       | < 10.5 g/dl   | 36%        |
| WBC                   | \( \geq 15 \times 10^9/l \) | 15%        |
|                       | < 15 \times 10^9/l | 85%        |
| Serum albumin         | \(< 4 \text{ g/dl} \) | 35%        |
|                       | \( \geq 4 \text{ g/dl} \) | 65%        |
| HCV antibodies        | Positive      | 14%        |
|                       | Negative      | 86%        |
| Performance status    | <2            | 95%        |
|                       | \( \geq 2 \)  | 5%         |
| B symptoms            | Absent        | 46%        |
|                       | Present       | 54%        |
| Bulky disease         | No            | 89%        |
|                       | Yes           | 11%        |
| Histopathology subtype| Mixed cellularity | 46%      |
|                       | Nodular sclerosis | 42%      |
|                       | Lymphocyte rich | 12%       |
| Extra nodal disease   | No            | 73%        |
|                       | Yes           | 27%        |
| Splenic involvement   | No            | 78%        |
|                       | Yes           | 22%        |
| Bone marrow involvement| No            | 96%        |
|                       | Yes           | 4%         |
| Ann Arbor stage       | I             | 1%         |
|                       | II            | 27%        |
|                       | III           | 42%        |
|                       | IV            | 30%        |
| Stage                 | Limited       | 21%        |
|                       | Advanced      | 79%        |
| IPS                   | < 3           | 60%        |
|                       | \( \geq 3 \)  | 40%        |
| CD 68                 | I             | 41%        |
|                       | II            | 41%        |
|                       | III           | 18%        |
| CD 163                | I             | 37%        |
|                       | II            | 40%        |
|                       | III           | 23%        |
| Response              | PR            | 12%        |
|                       | PD            | 7%         |
|                       | SD            | 20%        |
| Relapse after CR      | Yes           | 20%        |
| Status                | Alive/Dead    | 82%        |

**Hb** hemoglobin, **WBC** white blood cell count, **HCV** hepatitis C virus, **CR** complete response, **PR** partial Response, **PD** progressive disease, **SD** stationary disease, **IPS** International Prognostic score
The majority of patients (54%) exhibited B symptoms, and 11 patients (11%) presented with bulky disease. Most of the patients had histological features consistent with mixed cellularity subtype (46% of patients) followed by nodular sclerosis 42% while the least in incidence was lymphocyte rich 12%.

Twenty-seven patients (27%) had extra nodal disease; splenic involvement was detected in 22% of cases while the bone marrow was infiltrated only in 4 patients (4%). Using Ann Arbor stage, one patient (1%) was diagnosed as stage I, 27 patients (27%) were stage II, 42 patients (42%) were stage III, and 30 patients (30%) were stage IV. Limited stage was detected in 21 out of 100 patients (21%) whereas 79% of patients were advanced stage. Calculated IPS for included patients was more than or equal to 3 in 40 patients (40%).

Ninety patients (90%) were treated with ABVD protocol, and 10 patients (10%) were treated with combined modality ABVD + IFRT as 1st line therapy; 61 out of 100 patients (61%) showed CR, whereas 12 patients (12%) revealed PR, 20 patients (20%) showed stable disease (SD), and 7 patients (7%) revealed progressive disease (PD). Twenty patients (20%) relapsed after achieving 1st CR. Fifty-four patients (54%) received 2nd line chemotherapy. The follow-up duration ranged from 0.29 to 10.2 years. Eighty-two patients (82%) were still alive so the median OS was not reached.

Relation of CD68 and CD163 expression with clinico-pathological parameters

Using IHC staining for CD68, 41 patients had score I (< 5%), 41 patients had score II (5–25%), and 18 patients had score III (> 25%) (Fig. 1a, b, and c). CD68 score was statistically related to bulky disease ($p = 0.005$), stage ($p = 0.02$), IPS ($p < 0.04$), and the overall response rate (ORR) ($p = 0.001$), so that higher CD68 score was statistically related to the presence of bulky disease, advanced stage, IPS score, and lower ORR. On the other hand, there was no statistically significant difference between IHC expression of CD68 and

Fig. 1 Immunohistochemical expression of CD68 and CD163 (× 400). a CD68 immunoreactivity in < 5% of cells (score I), b CD68 immunoreactivity in 5–25% of cells (score II), c CD68 immunoreactivity in > 25% of cells (score III), d CD163 immunoreactivity in < 5% of cells (score I), e CD163 immunoreactivity in 5–25% of cells (score II), and f CD163 immunoreactivity in > 25% of cells (score III)
patient’s age, gender, hemoglobin, WBC, albumin levels, presence or absence of B symptoms, histopathology subtype, and presence or absence of extra nodal disease as illustrated in Table 2.

Additionally, using IHC staining for CD163, 37 patients had score I, 40 patients had score II, and 23 patients had score III (Fig. 1d, e, and f). CD163 score was statistically correlated to patient’s age ($p = 0.05$), gender ($p = 0.02$), serum albumin level ($p = 0.03$), B symptoms ($p = 0.04$), bulky disease ($p = 0.005$), stage ($p = 0.02$), IPS ($p = 0.02$) and ORR ($p = 0.001$), such that higher CD163 score was statistically related to older age, male gender, lower serum albumin, presence of B symptoms, bulky disease, advanced stage, and lower ORR. But there was statistically insignificant difference between IHC expression of CD163 and hemoglobin categories, WBCs categories, histopathology subtype, and presence or absence of extra nodal disease as illustrated in Table 2.

### Relation of CD68 and CD163 to OS

Kaplan Meier curve for patients’ overall survival (OS) revealed decrease in OS insignificantly with increasing CD68 and significantly with increasing CD163 expression (inverse relationship) (Fig. 2a, b). The univariate analysis of OS showed that OS was statistically affected by B symptoms ($p = 0.003$), bulky disease ($p = 0.006$), IPS ($p = 0.001$), and CD163 score ($p < 0.001$) but not affected by CD68 score ($p = 0.1$) as illustrated in Table 3.

As no consensus on optimal cutoff of CD68 and CD163 is well recognized, we studied impact of CD163 at cutoff > 5% (score 2) as well as CD163 at cutoff > 25% (score 3) on OS in multivariate analysis. This multivariate Cox regression

### Table 2 Clinico-pathological characteristics in relation to IHC expression of CD68 and CD163

|                         | CD68       | P value | CD163       | P value |
|-------------------------|------------|---------|-------------|---------|
| Age category            |            |         |             |         |
| < 45 years              | 31%        | 0.5     | 29%         | 0.05*   |
| ≥ 45 years              | 10%        |         | 8%          |         |
| Gender                  |            |         |             |         |
| Male                    | 10%        | 0.5     | 9%          | 0.02*   |
| Female                  | 31%        |         | 28%         |         |
| HB category             |            |         |             |         |
| < 10.5 g/dl             | 17%        | 0.6     | 16%         | 0.17    |
| ≥ 10.5 g/dl             | 24%        |         | 21%         |         |
| WBCs category           |            |         |             |         |
| < 15 x 10^9/l           | 35%        | 0.8     | 30%         | 0.7     |
| ≥ 15 x 10^9/l           | 6%         |         | 7%          |         |
| Albumin level           |            |         |             |         |
| < 4 g/dl                | 16%        | 0.9     | 15%         | 0.03*   |
| ≥ 4 g/dl                | 24%        |         | 20%         |         |
| HCV virology            |            |         |             |         |
| Negative                | 35%        | 0.4     | 32%         | 0.9     |
| Positive                | 6%         |         | 5%          |         |
| B symptoms              |            |         |             |         |
| Absent                  | 23%        | 0.2     | 23%         | 0.04*   |
| Present                 | 18%        |         | 14%         |         |
| Bulky disease           |            |         |             |         |
| No                      | 40%        | 0.005*  | 36%         | 0.005*  |
| Yes                     | 1%         |         | 1%          |         |
| Histopathology subtype  |            |         |             |         |
| LR                      | 4%         | 0.5     | 5%          | 0.86    |
| MC                      | 21%        |         | 16%         |         |
| NS                      | 16%        |         | 16%         |         |
| Extra nodal disease     |            |         |             |         |
| No                      | 34%        | 0.07    | 29%         | 0.55    |
| Yes                     | 7%         |         | 8%          |         |
| Stage                   |            |         |             |         |
| Limited                 | 14%        | 0.02*   | 13%         | 0.02*   |
| Advanced                | 27%        |         | 24%         |         |
| IPS                     |            |         |             |         |
| < 3                     | 30%        | 0.04*   | 25%         | 0.02*   |
| ≥ 3                     | 11%        |         | 12%         |         |
| ORR                     |            |         |             |         |
| CR/PR                   | 38%        | 0.001*  | 34%         | 0.001*  |
| NO CR/PR                | 3%         |         | 3%          |         |

*HB* hemoglobin, *WBC* white blood cell count, *HCV* Hepatitis C virus, *MC* mixed cellularity, *NS* nodular sclerosis, *LR* lymphocyte rich, *IPS* International Prognostic score, *ORR* overall response rate, *CR* complete response, *PR* partial response
analysis for OS revealed that B symptoms \((p = 0.024)\), IPS \(\geq 3\) \((p = 0.023)\), and CD163 at cutoff > 25% (score 3) \((p = 0.003)\) statistically affected OS while, CD163 at cutoff > 5% (score 2) did not affect OS \((p\ value 0.44)\) (as illustrated in Table 4).

Relation of CD68 and CD163 to DFS
Kaplan Meier curve for the disease-free survival (DFS) revealed significant decrease in DFS with increasing both CD68 and CD163 expression (inverse relationship) (Fig. 3a, b).

The univariate analysis of DFS demonstrated that DFS was affected by bulky disease \((p = 0.05)\), CD68 score \((p = 0.001)\), and CD163 score \((p = 0.001)\) as illustrated in Table 3.

**Table 3** Univariate analysis of factors affecting OS and DFS

|                | OS Median | Log rank test | P value | DFS Median | Log rank test | P value |
|----------------|-----------|---------------|---------|------------|---------------|---------|
| B symptoms     |           |               |         |            |               |         |
| Absent         | 9.587     | 8.61          | 0.003*  | 5.822      | 0.459         | 0.49    |
| Present        | 7.216     |               |         | 5.626      |               |         |
| Bulky lesion   |           |               |         |            |               |         |
| No             | 8.726     | 7.66          | 0.006*  | 6.348      | 3.9           | 0.05*   |
| Yes            | 5.073     |               |         | 2.342      |               |         |
| IPS            |           |               |         |            |               |         |
| < 3            | 9.36      | 11.24         | 0.001*  | 6.18       | 0.14          | 0.7     |
| \(\geq 3\)    | 6.02      |               |         | 5.77       |               |         |
| CD68           |           |               |         |            |               |         |
| 1              | 9.198     | 4.28          | 0.1     | 7.084      | 13.07         | 0.001*  |
| 2              | 7.235     |               |         | 4.876      |               |         |
| 3              | 6.422     |               |         | 1.801      |               |         |
| CD 163         |           |               |         |            |               |         |
| 1              | 9.526     | 25.3          | < 0.001** | 7.216      | 27.7          | <0.001** |
| 2              | 7.849     |               |         | 5.059      |               |         |
| 3              | 4.768     |               |         | 1.305      |               |         |

OS overall survival, DFS disease-free survival, IPS International Prognostic score

Multivariate Cox regression analysis was applied to study factors affecting DFS including both of CD68 and CD133 at cutoff > 5% (score 2) and at cutoff > 25% (score 3). This multivariate analysis revealed that neither bulky disease, nor CD68 or CD163 (at either cutoff values) statistically affected DFS as illustrated in Table 4.

**Discussion**
Macrophages are heterogeneous cells which are pivotal in remodeling of the tumor immune microenvironment. Relation between TAM and outcome of several malignancies attracted many researchers [13].

CD68 is recognized as a pan-macrophage biomarker and also expressed by other cells as monocytes, some
subtypes of CD34-positive hematopoietic stem cells (mye-
loid cells), fibroblasts, dendritic cells, neutrophils, langer-
hans cells, basophils, and mast cells [14]. It is therefore
non-specific for the monocyte/macrophage lineage.

On the other hand, expression of CD163 is largely re-
stricted to a subdivision of macrophage known as M2
macrophages. These M2 macrophages are involved in
anti-inflammatory function. They may be related to cell
proliferation and migration. They may also play an im-
portant role in new blood vessel formation by regulating
the angiogenic switch via secretion of vascular endo-
thelial growth factor (VEGF) and hypoxia-inducing factors
(HIF). M2 macrophages, therefore, may induce tumor
growth and metastasis unlike M1 macrophages that kill
tumor cells [13, 15, 16].

Therefore, unlike CD68, CD163 staining pattern is re-
ported to show a cleaner background with less non-
specific staining of RS cells and other inflammatory cells.
Thus, CD163 expression was easier to evaluate than
CD68 expression.

This work spots light on the possible role of cHL
microenvironment in disease biological behavior using
IHC assessment of CD68 and CD163 expression in
TAM of 100 cases diagnosed as cHL and study its rela-
tion to different clinico-pathological parameters, re-
response to treatment, and overall survival.

In this study, we noticed that CD68 and CD163 scores
 correlated statistically to tumor stage ($p$ value = 0.02 for
both), bulky disease ($p$ value = 0.005 for both) and IPS
($p$ value = 0.04 and 0.02 respectively). Additionally,
CD163 score was also related to gender ($p$ value = 0.02),
serum albumin ($p$ value = 0.03), B symptoms ($p$ value = 0.04). This agrees with Guo et al. who revealed that
CD68 or CD163 positive TAMs were associated with ad-
vanced tumor stage, presence of bulky disease, higher
IPS, and presence of B symptoms after meta-analysis of
22 studies [9].

On the other hand, Azambuja et al. found no associ-
ation between various clinico-pathological characteristics
and the expression of either CD68 or CD163 [10]. This

| Table 4 Multivariate analysis of factors affecting OS and DFS |
|---------------------------------------------------------------------------------------------------------------|
|                        | OS                      | DFS                        |
|                        | HR  | 95.0% CI     | P          | HR  | 95.0% CI     | P          |
| B symptoms             | 4.55| 1.21–16.96   | 0.02*      | Bulky disease | 1.22| 0.24-6.06    | 0.8        |
| Bulky disease          | 0.66| 0.2–2.18     | 0.5        | CD68 | 0.88         |            |
| IPS $\geq$ 3           | 3.55| 1.95–10.521  | 0.02*      | CD68 (score II) | 0.83| 0.12–5.8     | 0.85       |
| CD163 (score II)       | 0.003*|            |            | CD68 (score III) | 0.36| 0.007–19.6   | 0.62       |
| CD163 (score III)      | 0.39| 2.19–40.22   | 0.003*     | CD163 (score II) | 0.36| 0.3–13.1     | 0.47       |

OS overall survival, DFS disease-free survival, HR hazard ratio, CI confidence interval, IPS International Prognostic score.

![3a: Kaplan Meier curve for disease-free survival. It revealed statistically significant decrease in DFS with increasing CD68 expression (inverse relationship).](image-url)  

![3b: Kaplan Meier curve for disease-free survival. It revealed statistically significant decrease in DFS with increasing CD163 expression (inverse relationship).](image-url)
discrepancy between our results and that of Azambuja et al. could be attributed to different methods of IHC interpretation, in addition to different number of cases in each study.

Also, our results elicited that higher scores of CD68 and CD163 were associated with lower ORR (p value = 0.001); this copes with many previous studies that found TAMs enhance the resistance to curative chemotherapy and radiotherapy in many malignancies [13, 17, 18].

For impact of TAM on prognosis, as regard DFS, we found higher CD68 and CD163 scores were associated with lower DFS (p value = 0.001 and < 0.001 respectively) using univariate analysis but neither CD68 nor CD163 statistically affected DFS after applying multivariate Cox regression analysis that identified the presence of B symptoms (p value = 0.02), IPS ≥ 3 (p value = 0.02), and high CD163 score (> 25%) (P value = 0.003) as variables statistically associated with lower OS but not CD68 score. This gives advantage for CD163 over CD68 in prediction of poor overall survival. Our results agree with Klein et al. who identified CD163 score ≥ 25% and IPS ≥ 4 as predictors of poor survival after assessment of CD68 and CD163 expression by five hematopathologists [19]. Also, Guo et al. noted that high expression of CD163 and CD68 in TAM was related to poor prognosis [9]. On the contrary, Azambuja et al. showed no association between CD68 and CD163 in TAM and patients’ outcome and prognosis.

Discrepancies among studies could be attributed in part to the different number of cases in each study and in other part to the different assessment of low scores (5 to 25%) of CD68 due to the non-specific reactivity of RS cells as well as the reactive inflammatory cells in the background.

Conclusion
Increased expression of CD68 and CD163 in TAM were associated with adverse clinico-pathological features including bulky lesion, advanced tumor stage, high IPS ≥ 3 and lower response rate; however, CD163 is an outstanding predictor for OS in cHL rather than CD68. Thus, new therapeutic strategies altering cHL microenvironment may be helpful in patients with conventional therapy failure.

Abbreviations

ABVD: Adriamycin, bleomycin, vinblastine, and dacarbazine; cHL: Classic Hodgkin Lymphoma; CR: Complete remission; DFS: Disease-free survival; EDTA: Ethylenediaminetetraacetic acid; HBs Ag: Hepatitis B surface antigen; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; HIER: Heat-induced epitope retrieval; HIF: Hypoxia inducing factor; HRS: Hodgkin Reed-Stemmbel cells; IRT: Involved-field radiotherapy; IHC: Immunohistochemistry; IPS: International Prognostic Score; IRB: Institutional review board; ORR: Overall response rate; OS: Overall survival; PBS: Phosphate-buffered saline; PD: Progressive disease; PET/CT: Positron emission tomography/computerized tomography scan; PR: Partial response; SD: Stable disease; SPSS: Statistical Package for Social Sciences; TAM: Tumor-associated macrophages; VEGF: Vascular endothelial growth factor; WHO: World Health Organization

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Authors’ contributions
MYA interpreted histopathologic and immunohistochemical stained slides, captured figures provided in this study; also, she contributed in the study design. MWF and SE equally participated in the design of the study, collecting clinical, radiological, histopathological data, and statistical analysis. Also the three authors participated in writing and editing of final version of manuscript. The authors read and approved the final manuscript.

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Availability of data and materials
All the clinical, radiological, and pathological data used in this manuscript is available on Mansoura University medical system ( Ibn Sina Hospital management system): http://srv137.mans.edu.eg/mus/newSystem/ IHC results for CD68 and CD163 are available from Associate Professor of Pathology Dr. Mona Y.Y. Abd Allah on reasonable request.

Ethics approval and consent to participate
The current study was reviewed and approved by the Institutional review board (IRB) of Faculty of Medicine, Mansoura University (Code number: R/ 18.02.27).

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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References
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017; 67(1):7–30
2. Eichenauer DA, Akeran BMP, André M, Federico M, Hutchings M, Illidge T, et al. Hodgkin lymphoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018; Oct 1;29(Suppl 4):iv19–29. https://doi.org/10.1093/annonc/mdy080.
3. Ibrahim AS, Khaled HM, Mikhail NNH, Baraka H, Kamel H. Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol. 2014;2014:437971. https://doi.org/10.1155/2014/437971. Epub 2014 Sep 21.
4. Montanari F, Diefenbach CS. Hodgkin lymphoma: targeting the tumor microenvironment as a therapeutic strategy. Clin Adv Hematol Oncol. 2015; 13(8):518–24.
5. Hollander P, Kamper P, Smedby KE, Enblad G, Ludvigsen M, Mortensen J, et al. High proportions of PD-1+ and PD-L1+ leukocytes in classical Hodgkin lymphoma microenvironment are associated with inferior outcome. Blood Adv. 2017;1(18):1427–39.
6. Hollander P, Rostgaard K, Smedby KE, Molin D, Loskog A, de Nully BP, et al. An anergic immune signature in the tumor microenvironment of classical Hodgkin lymphoma is associated with inferior outcome. Eur J Haematol. 2018;100(1):88–97.
7. Derenzini E, Younes A. Predicting treatment outcome in classical Hodgkin lymphoma: genomic advances. Genome Med. 2011;3(4):26.
8. DeVita VT Jr, Costa J. Toward a personalized treatment of Hodgkin's disease. N Engl J Med. 2010 Mar 11;362(10):942–3. https://doi.org/10.1056/NEJMe0912481.
9. Guo B, Cen H, Tan X, Ke Q. Meta-analysis of the prognostic and clinical value of tumor-associated macrophages in adult classical Hodgkin lymphoma. BMC Med. 2016 Oct 17;14(1):159.
10. Azambuja D, Natkunam Y, Biasoli I, Lossos IS, Anderson MW, Morais JC, et al. Lack of association of tumor-associated macrophages with clinical outcome in patients with classical Hodgkin’s lymphoma. Ann Oncol. 2012;23(3):736–42.
11. Hasenclever D, Dkhil N, Armitage JO, Assouline D, Björkholm M, Busanpolino E, et al. A prognostic score for advanced Hodgkin’s disease. International Prognostic Factors Project on Advanced Hodgkin’s Disease. N Engl J Med. 1998;339(21):1506–14. https://doi.org/10.1056/NEJM199811193392104.
12. Harris JA, Jain S, Ren Q, Zarineh A, Liu C, Ibrahim S. CD163 versus CD68 in tumor associated macrophages of classical Hodgkin lymphoma. Diagn Pathol. 2012 Jan 30;7:12. https://doi.org/10.1186/1746-1596-7-12.
13. Guo Q, Jin Z, Yuan Y, Liu R, Xu T, Wei H, et al. New mechanisms of tumor-associated macrophages on promoting tumor progression: recent research advances and potential targets for tumor immunotherapy. J Immunol Res. 2016;2016:920912 Epub 2016 Nov 16.
14. Rehg JE, Bush D, Ward JM. The utility of immunohistochemistry for the identification of hematopoietic and lymphoid cells in normal tissues and interpretation of proliferative and inflammatory lesions of mice and rats. Toxicol Pathol. 2012;40(2):345–74.
15. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140(6):883–99.
16. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. J Hematol Oncol. 2017;10(1):58.
17. Wilton WH. Treatment strategies for aggressive lymphomas: what works? Hematology Am Soc Hematol Educ Program. 2013(1):584–90.
18. Wechselsebaum RR, Liang H, Deng L, Fu Y-X. Radiotherapy and immunotherapy: a beneficial liaison? Nat Rev Clin Oncol. 2017;14(6):365-379. doi: https://doi.org/10.1038/nrclinonc.2016.211. Epub 2017 Jan 17.
19. Klein JL, Nguyen TT, Bien-Willner GA, Chen L, Foil KV, Bartlett NL, et al. CD163 immunohistochemistry is superior to CD68 in predicting outcome in classical Hodgkin lymphoma. Am J Clin Pathol. 2014;141(3):381–7.

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