Expression of Silent Information Regulator-1 (Sirt1), CD163 and Tryptase; Implications for Immune Dys-regulation, Prognosis and Therapeutic Targeting in Classical Hodgkin Lymphoma

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Abstract

Background: Many studies have assessed the relation between cHL histology and its clinical outcome but the results are still controversial. The most recent issue was the discovery of the methods of interaction between the elements of benign reactive inflammatory cells and their effect on cHL metabolism. Silent information regulator-1 (Sirt1) is a class III HDACs family member, it was expressed in T-cell lymphoma and was considered a recently discovered therapeutic target, we tried to detect that the significance of Sirt1 expression in malignant Reed-Stemberg cells and surrounding inflammatory infiltrate in cHL. Cluster of Differentiation 163 (CD163) is a scavenger receptor cystein-rich (SRCR) family member which identifies monocytes and macrophages, Tryptase is the secretory granules that were serine proteinase derived and it has been used as a mast cell activation marker. The tumor-infiltrating macrophages and mast cells have been found to have different roles in malignancies of many organs but their role in cHL prognosis had not been sufficiently clarified.

Aim of the work: To assess the influence of Sirt1 expression in the malignant cells and the surrounding tumor microenvironment CD163 positive macrophages and tryptase positive mast cells on the pathological parameters and clinical outcome in cHL patients.

Methods: We evaluated clinicopathological and prognostic significance of Sirt1, CD163 and tryptase expressions in sections from fifty paraffin blocks of cHL using immunehistochemistry.

Results: The expression of Sirt1 in cHL was associated with advanced stage of the disease, presence of B symptoms, splenic affection, (p<0.001), bone marrow infiltration (p=0.002) and the presence of bulky mediastinal lymph node (p=0.004). The expression of CD163 and tryptase in cHL was associated with advanced stage of the disease and the presence of bulky mediastinal lymph node (p<0.001). High Sirt1, tryptase and CD163 expression was associated with poor response to therapy, high incidence of relapse after successful therapy and poor three-year overall survival rate (p<0.001).

Conclusion: High levels of expression of Sirt1, CD163 and tryptase were found to be markers of poor prognosis in cHL.

Keywords: Classical hodgkin lymphoma; Sirt1; CD163; Tryptase; Immunohistochemistry; Prognosis

Introduction

Classical Hodgkin lymphoma (cHL) forms ninety percent of all Hodgkin lymphomas and it is categorized into 4 subtypes; lymphocyte-rich (LR), nodular sclerosis (NS), mixed cellularity (MC) and lymphocyte-depletion (LD) subtype [1]. CHL is a treatable cancer [2], but still few patients are resistant to therapy or they may have lymphoma recurrence after successful therapy [3]. Novel researches had focused on recent therapeutic targets detection to improve the response to therapy and to decrease the incidence of lymphoma recurrence. CHL is composed of malignant giant cells i.e. Reed-Stemberg (RS) cells that are surrounded by reactive inflammatory infiltrate [2], e.g. lymphocytes, eosinophils, mast cells, macrophages which interact with RS cells via many cellular pathways and by production of cytokine by RS cells that affect the benign reactive inflammatory cells via autocrine and paracrine pathways [5,6]. Previous studies have focused on the cellular composition of cHL microenvironment to detect their clinicopathological and prognostic role, but conflicting results were detected. Silent information regulator-1 (Sirt1) is a class III HDACs family member that has a role in the gene silencing of yeast [7]. It was expressed in T-cell lymphoma and was considered a recent therapeutic target [8]. Our study attempted to acquire more understanding of the pathogenesis of cHL. As previously found that the RS cells and the surrounding reactive lymphocytes could express Sirt1 [9] we tried to detect the clinicopathological role of such expression in our patients. A more understanding of cHL microenvironment, may allow better management via restoration of the immune balance that could allow its better remission. Cluster of Differentiation 163 (CD163) is a protein that is encoded by the CD163 gene [10], it is a scavenger receptor cystein-rich (SRCR) family member [11,12], which has been found to mark macrophages [13]. Tryptase enzyme is detected in the secretory granules that were serine proteinase derived, were...
Evaluation of immunohistochemical expression of CD163 and Tryptase

For the CD163 staining, we counted 3 variable areas of the tumor in high power field x 400 magnifications and we scored the percentage of CD163 positivity in the cell membrane and cytoplasm of macrophages [26]. We have counted mast cells positive for tryptase in ten randomly selected high power fields x 200 magnifications [6]. We used a cutoff of twenty five percent for CD163 positive macrophage and tryptase positive mast cells above which was considered high expression and below which was low expression of both markers and that was applied for all patients.

Statistical Analysis

The statistics were evaluated by using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium). Continuous variables that were expressed as the mean ± SD & median (range), were checked for normality by using Shapiro-Wilk test. Mann Whitney U test was used to compare between two groups of non-normally distributed variables. Kruskal Wallis H test was used to compare between more than two groups of normally distributed variables. Stratification of OS and RFS was done according to all clinicopathological features and immunohistochemical markers. These time-to-death distributions were estimated using the method of Kaplan-Meier plot, and compared using two-sided exact log-rank test. All tests were two sided. A p-value <0.05 was considered significant.

Results

Patient data

The clinical data of our patients with cHL that were included in the current study were summarized (Table 1). We included 28 (56%) males and 22 (44%) females with age ranged from (20-80) years (Mean: 47.5 ± 20.7 years), 31 (62%) cases were having mixed cellularity subtype, 14 (28%) cases were of nodular sclerosis subtype and 5 (10%) cases were of lymphocyte rich subtype.

Sirt1 expression and its correlation with clinicopathological criteria of our patients

Sirt1 was overexpressed in the nuclei of both malignant cells and the background of tumor infiltrating lymphocytes in 29 cases out of 50 (58%) cases of cHL, and in the remaining 42% of cases there were no lymphocytes expressing Sirt1 at all. The expression of Sirt1 in was significantly positively correlated with advanced stage, presence of B symptoms, splenic affection, presence of extranodal spread, low serum albumin, low hemoglobin (p<0.001), presence of bone marrow infiltration (p=0.002) and the presence of bulky mediastinal lymph node involvement (p=0.004) (Table 1 and Figure 1A-D).

correlation clinicopathological features of our patients

High expression of CD163 in tumor infiltrating macrophages was detected in in 23 out of 50 (46%) cases of cHL.

High expression of tryptase in tumor infiltrating mast cells was detected in 22 out of 50 (44%) cases of cHL. The expression of CD163 and tryptase in cHL was significantly positively correlated with advanced stage, presence of B symptoms, splenic involvement, presence of extra-nodal spread, low albumin, low hemoglobin, bone marrow infiltration and the presence of bulky mediastinal lymph node (p<0.001) (Table 1 and Figures 2A-C, 3A-C).
### Table 1: Correlation between clinicopathological criteria, Sirt1, CD163 & Tryptase expression in our patients.

| Characteristics                      | All (N=50) | Sirt1 (N=29) | CD163 (N=23) | Tryptase (N=22) |
|--------------------------------------|------------|--------------|--------------|-----------------|
|                                      | Low (N=21) | High (N=29)  | Low (N=27)   | High (N=23)     |
|                                      |            |              |              |                 |
|                                     | p-value    | p-value      | p-value      | p-value         |
| Age (years)                          |            | 0.001        |              |                 |
| Mean ± SD                            | 47.5 ± 20.7| 35.9 ± 17.6  | 55.8 ± 18.8  |                 |
| Median (Range)                       | 50 (20-80) | 29 (20-72)   | 60 (20-80)   |                 |
| ≤ 45 years                           | 24 (48%)   | 8 (33.3%)    | 8 (30.8%)    | 15 (68.2%)      |
| >45 years                            | 26 (52%)   | 18 (80.8%)   | 12 (89.2%)   |                 |
| Sex                                  |            |              |              |                 |
| Male                                 | 28 (56%)   | 18 (64.3%)   | 14 (63.6%)   | 8 (36.4%)       |
| Female                               | 22 (44%)   | 10 (45.5%)   | 8 (36.4%)    |                 |
| B symptoms                           |            |              |              |                 |
| Absent                               | 35 (70%)   | <0.001       | 0.001        | <0.001          |
| Present                              | 15 (30%)   | 0 (0%)       | 0 (0%)       |                 |
| Bulky Med. LN                        |            |              |              |                 |
| Absent                               | 37 (74%)   | <0.001       | 0.004§       | <0.001          |
| Present                              | 13 (26%)   | 11 (84.6%)   | 7 (53.6%)    |                 |
| Number of nodal sites                |            |              |              |                 |
| One site                             | 7 (14%)    | 7 (100%)     | 0 (0%)       |                 |
| Two sites                            | 10 (20%)   | 0 (0%)       | 0 (0%)       |                 |
| Three sites                          | 3 (6%)     | 3 (100%)     | 0 (0%)       |                 |
| Four sites                           | 6 (12%)    | 5 (83.3%)    | 3 (50%)      |                 |
| Five sites                           | 6 (12%)    | 5 (83.3%)    | 3 (50%)      |                 |
| >Five sites                          | 18 (36%)   | 16 (88.9%)   | 1 (5.6%)     |                 |
| Spleen involvement                   |            |              |              |                 |
| Absent                               | 28 (56%)   | <0.001       | 0.004§       | <0.001          |
| Present                              | 22 (44%)   | 4 (18.2%)    | 8 (41.2%)    |                 |
| BM infiltration                      |            |              |              |                 |
| Absent                               | 36 (72%)   | 0.002§       | 0.001        | <0.001          |
| Present                              | 14 (28%)   | 1 (7.1%)     | 13 (92.9%)   |                 |
| Extraneodal lesions                  |            |              |              |                 |
| Absent                               | 14 (28%)   | <0.001       | 0.001§       | <0.001          |
| Present                              | 36 (72%)   | 27 (75%)     | 15 (41.7%)   |                 |
| Histopathological subtype            |            |              |              |                 |
| NS                                   | 14 (28%)   | 12 (85.7%)   | 2 (14.3%)    |                 |
| MC                                   | 31 (62%)   | 28 (88.5%)   | 3 (11.5%)    |                 |
| LR                                   | 5 (10%)    | 4 (80%)      | 1 (20%)      |                 |
| Stage                                |            | <0.001       | <0.001       | <0.001          |
| Stage I                              | 7 (14%)    | 7 (100%)     | 0 (0%)       |                 |
| Stage II                             | 13 (26%)   | 10 (76.9%)   | 3 (23.1%)    |                 |
| Stage III                            | 12 (24%)   | 10 (83.3%)   | 2 (16.7%)    |                 |
| Stage IV                             | 18 (36%)   | 16 (88.9%)   | 2 (11.1%)    |                 |
| Prognostic group                     |            | <0.001       | <0.001       | <0.001          |
| Unfavorable                          | 30 (60%)   | 26 (86.7%)   | 4 (13.3%)    |                 |
| Favorable                            | 20 (40%)   | 18 (90%)     | 2 (10%)      |                 |
| Risk group                           |            | <0.001       | <0.001       | <0.001          |
| Low favorable                        | 16 (32%)   | 16 (100%)    | 0 (0%)       |                 |
| Low unfavorable                      | 3 (6%)     | 3 (100%)     | 0 (0%)       |                 |
| Intermediate                         | 18 (36%)   | 13 (72.2%)   | 5 (27.8%)    |                 |
| High                                 | 13 (26%)   | 13 (100%)    | 0 (0%)       |                 |

Categorical variables were expressed as number (percentage); continuous variables were expressed as mean ± SD & median (range)

* Mann Whitney U test; § Chi-square test; ‡ Chi-square test for trend; p<0.05 is significant.

The 3-year recurrence free survival rate of our patients was 91.2% for all cases, 95.2% and 84.6% in patients with low and high Sirt1 expression respectively, 100% and 57.1% in patients with low and high tryptase and CD163 expression respectively (p<0.001).

The 3-year overall survival (OS) rate of our patients was 75.8% for all cases, 58.2% in patients with high Sirt1 expression (p=0.008), 46.6% in patients with high CD163 expression and 44.1% in patients with high tryptase expression (p<0.001).
Figure 1: Immunohistochemical staining of Sirt1 in classical Hodgkin lymphoma (cHL): (A & B) High expression in the nucleus of cHL cells x 400 (C & D); Low expression in the nucleus of cHL cells x 400.

Figure 2: Immunohistochemical expression of CD163 in macrophages that infiltrate classical Hodgkin lymphoma (A) High expression of CD163 in cHL x 100 (B & C) High expression of CD163 in cHL x 400, low expression of CD163 in cHL x 400.

Figure 3: Immunohistochemical expression of Tryptase in mast cells that infiltrate classical Hodgkin lymphoma (A) High expression of Tryptase in cHL x 400 (B & C) Low expression of Tryptase in cHL x 400, negative expression of Tryptase in cHL x 400.
**Table 2:** Correlation between clinicopathological criteria, Sirt1, CD163, Tryptase expression and response to treatment in our patients.

| Characteristics | All (N=50) | Response | p-value | Response | p-value |
|-----------------|------------|----------|---------|----------|---------|
|                 | PD (N=6)   | SD (N=6) | PR (N=4) | CR (N=34) | NR (N=24) | OAR (N=38) |
| ESR 50 mm/h     | 34 (68%)   | 0 (0%)   | 2 (5.9%) | 32 (94.1%)| 0 (0%) | 34 (100%)   | <0.001§    | <0.001§ |
| Albumin <4 g/dl | 30 (60%)   | 6 (20%)  | 4 (13.3%)| 14 (46.7%)| 0 (0%) | 20 (100%)   | 0.001§     | 0.001§ |
| Hemoglobin <10 g/dl | 30 (60%) | 6 (20%)  | 4 (13.3%)| 14 (46.7%)| 0 (0%) | 20 (100%)   | 0.001§     | 0.001§ |
| WBCs <15 x 10^3/mm³ | 30 (60%) | 6 (20%)  | 4 (13.3%)| 14 (46.7%)| 0 (0%) | 20 (100%)   | 0.001§     | 0.001§ |
| Stage I         | 7 (14%)    | 0 (0%)   | 0 (0%)   | 7 (100%)  | 0 (0%) | 7 (100%)    | <0.001†    | 0.002‡ |
| Stage II        | 13 (26%)   | 0 (0%)   | 0 (0%)   | 13 (100%) | 0 (0%) | 13 (100%)   | 0 (0%)     | 0 (0%) |
| Stage III       | 12 (24%)   | 1 (8.3%) | 1 (8.3%) | 7 (58.3%) | 4 (33.3%)| 8 (66.7%)   | 8 (44.4%)  | 10 (55.6%)|
| Stage IV        | 18 (36%)   | 5 (27.8%)| 3 (16.7%)| 7 (39.9%) | 8 (44.4%)| 10 (55.6%)  | 0.001§     | 0.001§ |
| Prognostic group|            |          |         |          |        |            |
| Unfavorable     | 30 (60%)   | 6 (20%)  | 4 (13.3%)| 14 (46.7%)| 12 (40%)| 18 (60%)    | 0.001§     | 0.001§ |
| Favorable       | 20 (40%)   | 0 (0%)   | 0 (0%)   | 20 (100%)| 0 (0%) | 20 (100%)   | 0.001§     | 0.001§ |
| Risk group      |            |          |         |          |        |            |
| Low favorable   | 16 (32%)   | 0 (0%)   | 0 (0%)   | 16 (100%)| 0 (0%) | 16 (100%)   | <0.001†    | <0.001† |
| Low unfavorable | 3 (6%)     | 0 (0%)   | 0 (0%)   | 3 (100%) | 0 (0%) | 3 (100%)    | 0 (0%)     | 0 (0%) |
| Intermediate    | 18 (36%)   | 0 (0%)   | 0 (0%)   | 15 (83.3%)| 0 (0%) | 18 (100%)   | 0 (0%)     | 0 (0%) |
| High            | 13 (26%)   | 6 (46.2%)| 1 (7.7%) | 0 (0%)   | 12 (92.3%)| 1 (7.7%)    | 0.001§     | 0.001§ |
| Sirt1           |            |          |         |          |        |            |
| Low             | 21 (42%)   | 0 (0%)   | 0 (0%)   | 21 (100%)| 0 (0%) | 21 (100%)   | 0.001§     | 0.001§ |
| High            | 29 (58%)   | 6 (20.7%)| 4 (13.8%)| 13 (44.8%)| 12 (41.4%)| 17 (58.6%)  | 0.001§     | 0.001§ |
| CD163           |            |          |         |          |        |            |
| Low             | 27 (54%)   | 0 (0%)   | 0 (0%)   | 27 (100%)| 0 (0%) | 27 (100%)   | <0.001§    | <0.001 |
| High            | 23 (46%)   | 6 (20.7%)| 4 (13.8%)| 7 (30.4%) | 12 (52.2%)| 11 (47.8%)  | 0.001      | 0.001 |
| Tryptase        |            |          |         |          |        |            |
| Low             | 28 (56%)   | 0 (0%)   | 1 (3.6%) | 27 (96.4%)| 0 (0%) | 28 (100%)   | <0.001§    | <0.001§ |
| High            | 22 (44%)   | 6 (27.3%)| 3 (13.6%)| 7 (31.8%) | 12 (54.5%)| 10 (45.5%)  | 0.001§     | 0.001§ |

Categorical variables were expressed as number (percentage); continuous variables were expressed as mean ± SD & median (range).

* Mann Whitney U test; § Kraskall Wallis test for more than two groups; † Chi-square test; ‡ Chi-square test for trend; p<0.05 is significant.

Discussion

In our study we found that Sirt1 was overexpressed in the nuclei of both Reed-Sternberg (RS) cells and the background of tumor infiltrating lymphocytes in 58% cases of cHL and in the remaining 42% of cases there were no lymphocytes expressing Sirt1 at all. Sirt1 expression was associated with poor clinicopathological parameters and poor prognosis, and our results were the same like Quesada et al., and Frazzi et al. [27,28]. Our results were explained that one of the Sirt1 de-acetylation targets is FoxP3 that is a transcription factor which is essential for T regulatory lymphocytes (Tregs) differentiation and function. So, Sirt1 de-acetylation of FoxP3 could result in FoxP3 destabilization and increased its degradation that decreased Tregs activity, those tregs stimulate the immunity against cancer and had onco-suppressive role, so, inhibition of Sirt1 increases the suppressive action of Tregs cells [29,30]. Moreover, Sirt1 inhibition in cHL allows RS cells to promote CD4+ naïve T cells differentiation toward Tregs as RS could promote differentiation of CD4+ naïve T cells toward both Tregs and cytotoxic T-cells that are both increase their onco-suppressive role [31]. All these results are in line with ours that Sirt-1 over expression in cHL is a marker of poor prognosis and its inhibition was a novel therapeutic target to improve patient outcome and also histone deacetylase inhibitors (such as vorinostat [SAHA]) could increase the efficacy of the present therapeutic agents by down inhibition was a novel therapeutic target to improve patient outcome and also histone deacetylase inhibitors (such as vorinostat [SAHA]) could increase the efficacy of the present therapeutic agents by down regulating Sirt1 and decreasing Sirt1 deacetylase activity [32,33], which could decrease the inhibitory effects on Tregs and restore the immune balance in cHL microenvironment. We found that cHL patients with Sirt1 over expression had higher incidence of recurrence after successful therapy which suggest the possibility that immune dys-regulation played an essential role in the cHL pathogenesis and could decrease Tregs resulting in imbalance between them and cytotoxic T-cells. This was in agreeing with immune dysregulation.
Table 3: Correlation between clinicopathological criteria, Sirt1, CD163, Tryptase expression and relapse of our patients.

| Characteristics | All (N=34) | Relapse | p-value | Median RFS | Relapse Free Survival (RFS) | HR (95%CI) | p-value |
|-----------------|------------|---------|---------|------------|---------------------------|------------|---------|
|                 | No (N=20)  | No (N=14) |         |            |                           |            |         |
| Initial site    |            |          |         |            |                           |            |         |
| Cervical        | 14 (41.2%) | 9 (64.3%) | 5 (35.7%) | 0.474§     | NR 100% 91.2% 56.5%     |            | 0.242† |
| SC              | 3 (8.8%)   | 1 (33.3%) | 2 (66.7%) |            | 45 month 100% 100% 33.3% |            |         |
| Axilla          | 8 (23.5%)  | 4 (50%)   | 4 (50%)   | NR 100% 100% 50% |           |            |         |
| Mediastinum     | 8 (23.5%)  | 6 (75%)   | 2 (25%)   | NR 100% 100% 75% |           |            |         |
| Inguinal        | 1 (2.9%)   | 0 (0%)    | 1 (100%)  | NR 100% 100% 0%   |           |            |         |
| Bulky LN        |            |          |         |            |                           |            |         |
| Absent          | 23 (67.6%) | 18 (78.3%)| 5 (21.7%) | 0.002§     | NR 100% 100% 75.3% 6.931| <0.001† |         |
| Present         | 11 (32.4%) | 2 (18.2%) | 9 (81.8%) | 42 month 100% 72.7% 18.2% (1.888-25.304) |         |         |
| Bulky Med. LN   |            |          |         |            |                           |            |         |
| Absent          | 32 (94.1%) | 19 (59.4%)| 13 (40.6%)| 1.000§     | NR 100% 90.6% 56.8% 1.399 | 0.737† |         |
| Present         | 2 (5.9%)   | 1 (50%)   | 1 (50%)   | NR 100% 100% 50% (0.132-14.785) |         |         |
| Number of nodal sites |       |        |         |            |                           |            |         |
| One site        |            |          |         |            |                           |            |         |
| Two sites       | 10 (29.4%) | 9 (90%)   | 1 (10%)   | NR 100% 100% 85.7% |           |         |
| Three sites     | 3 (8.8%)   | 0 (0%)    | 3 (100%)  | 45 month 100% 100% 0%   |           |         |
| Four sites      | 4 (11.8%)  | 2 (50%)   | 2 (50%)   | 52 month 100% 100% 37.5% |         | <0.001† |
| Five sites      | 3 (8.8%)   | 1 (33.3%) | 2 (66.7%) | 45 month 100% 100% 33.3% |         |         |
| >Five sites     | 7 (20.6%)  | 1 (14.3%) | 6 (85.7%) | 40 month 100% 57.1% 14.3% |         |         |
| BM infiltration |            |          |         |            |                           |            |         |
| Absent          | 33 (38.2%) | 19 (57.6%)| 14 (42.4%)| 1.000§     | NR 100% 90.9% 55.1% 0.000 | 0.438† |         |
| Present         | 1 (2.9%)   | 1 (100%)  | 0 (0%)    | NR 100% 100% 100% |           |         |
| Extranodal lesions |        |        |         |            |                           |            |         |
| Absent          | 13 (38.2%) | 12 (92.3%)| 1 (7.7%)  | 0.002§     | NR 100% 100% 87.5% 11.043| 0.002† |         |
| Present         | 21 (61.8%) | 8 (38.1%) | 13 (61.9%)| 45 month 100% 85.7% 37.5% (3.860-31.595) |         |         |
| Histopathological subtype |       |        |         |            |                           |            |         |
| NS              | 14 (41.2%) | 12 (85.7%)| 2 (14.3%) | 0.005§     | NR 100% 100% 86.7% | 0.028† |         |
| MC              | 16 (47.1%) | 9 (50%)   | 7 (50%)   | NR 100% 100% 100% |           |         |
| LR              | 4 (11.8%)  | 0 (0%)    | 4 (100%)  | 52 month 100% 100% 0% |           |         |
| Stage           |            |          |         |            |                           |            |         |
| Stage I         | 7 (20.6%)  | 7 (100%)  | 0 (0%)    | NR 100% 100% 100% |           |         |
| Stage II        | 13 (38.2) | 9 (69.2%) | 4 (30.8%) | 45 month 100% 100% 65.9% |         | <0.001† |
| Stage III       | 7 (20.6%)  | 3 (42.9%) | 4 (57.1%) | 52 month 100% 100% 38.1% |         |         |
| Stage IV        | 7 (20.6%)  | 1 (14.3%) | 6 (85.7%) | 40 month 100% 57.1% 14.3% |         |         |
| Prognostic group |        |        |         |            |                           |            |         |
| Unfavorable     | 14 (41.2%) | 4 (28.6%) | 10 (71.4%)| 0.003§     | 45 month 100% 78.6% 26.8% 0.181 | <0.001† |         |
| Favorable       | 20 (58.8%) | 16 (80%)  | 4 (20%)   | NR 100% 100% 77.9% (0.058-0.561) |         |         |
| Sirt1           |            |          |         |            |                           |            |         |
| Low             | 21 (61.8%) | 18 (85.7%)| 3 (14.3%) | <0.001§    | NR 100% 95.2% 82.7% 9.590 | <0.001† |         |
| High            | 13 (38.2%) | 2 (15.4%) | 11 (84.6%)| 45 month 100% 84.6% ----- (2.973-30.935) |         |         |
| CD163           |            |          |         |            |                           |            |         |
| Low             | 27 (79.4%) | 20 (74.1%)| 7 (25.9%) | 0.011§     | NR 100% 100% 71.2% 11.545 | <0.001† |         |
| High            | 7 (20.6%)  | 0 (0%)    | 7 (100%)  | 40 month 100% 57.1% 11.545 |         |         |
| Tryptase        |            |          |         |            |                           |            |         |
| Low             | 27 (79.4%) | 20 (74.1%)| 7 (25.9%) | 0.011§     | NR 100% 100% 71.2% 11.545 | <0.001† |         |
| High            | 7 (20.5%)  | 0 (0%)    | 7 (100%)  | 40 month 100% 57.1% 11.545 |         |         |

Categorical variables were expressed as number (percentage); continuous variables were expressed as mean ± SD & median (range). * Mann Whitney U test; § Chi-square test; † Chi-square test for trend; ‡ Log rank test; NR denote not reached yet; HR Hazards Ratio; 95%CI: 95% confidence interval; p<0.05 is significant.
theory that played a role in cHL pathogenesis. Proofs of theory of the role of inhibition of Sirt1 pathway and Treg stimulation to restore immune balance in cHL are recently proved [34,35]. In our study we demonstrate that, in patients with cHL, the recurrence of the disease after successful therapy can be correlated with Sirt-1 expression so it carries prognostic significances, so Histone deacetylation inhibitors can increase the efficacy of pre-existing drugs by inhibiting Sirt1 gene mRNA/Sirt1 protein function, which subsequently improve prognosis and decrease remission in cHL [29,30,32-34]. Targeting Sirt1 gene mRNA/Sirt1 protein function, which subsequently improve

| Characteristics | All (N=50) | Spleen involvement | Histopathological subtype | Stage | Prognostic group | Risk group | Sirt1 | CD163 | Tryptase |
|-----------------|-----------|--------------------|--------------------------|-------|-----------------|-----------|-------|-------|---------|
| All patients    | 50 (100%) | NR                 | NR                       | NR    | NR              | NR        | NR    | NR    | NR      |
| Absent          | 28 (56%)  | 24 (85.7%)         | 3 (21.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Present         | 22 (44%)  | 10 (45.5%)         | 12 (54.5%)               | NR    | NR              | NR        | NR    | NR    | NR      |
| NS              | 14 (28%)  | 11 (78.6%)         | 3 (21.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| MC              | 31 (62%)  | 19 (61.3%)         | 12 (38.7%)               | NR    | NR              | NR        | NR    | NR    | NR      |
| LR              | 5 (10%)   | 4 (80%)            | 1 (20%)                  | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage I         | 7 (14%)   | 6 (85.7%)          | 1 (14.3%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage II        | 13 (26%)  | 11 (84.6%)         | 2 (15.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage III       | 12 (24%)  | 7 (58.3%)          | 5 (41.7%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage IV        | 18 (36%)  | 10 (55.6%)         | 8 (44.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Unfavorable     | 30 (60%)  | 17 (56.7%)         | 13 (43.3%)               | NR    | NR              | NR        | NR    | NR    | NR      |
| Favorable       | 20 (40%)  | 17 (85%)           | 3 (15%)                  | NR    | NR              | NR        | NR    | NR    | NR      |
| Low favorable   | 16 (32%)  | 15 (93.8%)         | 1 (6.3%)                 | NR    | NR              | NR        | NR    | NR    | NR      |
| Low unfavorable | 3 (6%)    | 1 (33.3%)          | 2 (66.7%)                | 50 month | 100% | 100% | 0% | 0% | 0% |
| Intermediate    | 18 (38%)  | 18 (100%)          | 0 (0%)                   | NR    | NR              | NR        | NR    | NR    | NR      |
| High            | 13 (26%)  | 0 (0%)             | 13 (100%)                | 25 month | 84.6% | 7.6% | 0% | 0% | 0% |
| Low             | 21 (42%)  | 20 (95.2%)         | 1 (4.8%)                 | NR    | NR              | NR        | NR    | NR    | NR      |
| High            | 29 (58%)  | 14 (48.3%)         | 15 (51.7%)               | 48 month | 93.1% | 58.2% | ---- | (6.399-45.617) | <0.001† |
| CD163 Low       | 27 (54%)  | 24 (88.9%)         | 3 (11.1%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| CD163 High      | 23 (46%)  | 10 (43.5%)         | 13 (56.5%)               | 36 month | 91.3% | 46.6% | ---- | (3.227-26.407) | <0.001† |
| Tryptase Low    | 28 (56%)  | 25 (89.3%)         | 3 (10.7%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Tryptase High   | 22 (44%)  | 9 (40.9%)          | 13 (59.1%)               | 36 month | 90.9% | 44.1% | ---- | (3.576-30.873) | <0.001† |

Categorical variables were expressed as number (percentage); continuous variables were expressed as mean ± SD & median.

Table 4: Correlation between clinicopathological criteria, Sirt1, CD163, Tryptase expression and survival of our patients.

| Characteristics | All (N=50) | Spleen involvement | Histopathological subtype | Stage | Prognostic group | Risk group | Sirt1 | CD163 | Tryptase |
|-----------------|-----------|--------------------|--------------------------|-------|-----------------|-----------|-------|-------|---------|
| All patients    | 50 (100%) | NR                 | NR                       | NR    | NR              | NR        | NR    | NR    | NR      |
| Absent          | 28 (56%)  | 24 (85.7%)         | 3 (21.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Present         | 22 (44%)  | 10 (45.5%)         | 12 (54.5%)               | NR    | NR              | NR        | NR    | NR    | NR      |
| NS              | 14 (28%)  | 11 (78.6%)         | 3 (21.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| MC              | 31 (62%)  | 19 (61.3%)         | 12 (38.7%)               | NR    | NR              | NR        | NR    | NR    | NR      |
| LR              | 5 (10%)   | 4 (80%)            | 1 (20%)                  | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage I         | 7 (14%)   | 6 (85.7%)          | 1 (14.3%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage II        | 13 (26%)  | 11 (84.6%)         | 2 (15.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage III       | 12 (24%)  | 7 (58.3%)          | 5 (41.7%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Stage IV        | 18 (36%)  | 10 (55.6%)         | 8 (44.4%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Unfavorable     | 30 (60%)  | 17 (56.7%)         | 13 (43.3%)               | NR    | NR              | NR        | NR    | NR    | NR      |
| Favorable       | 20 (40%)  | 17 (85%)           | 3 (15%)                  | NR    | NR              | NR        | NR    | NR    | NR      |
| Low favorable   | 16 (32%)  | 15 (93.8%)         | 1 (6.3%)                 | NR    | NR              | NR        | NR    | NR    | NR      |
| Low unfavorable | 3 (6%)    | 1 (33.3%)          | 2 (66.7%)                | 50 month | 100% | 100% | 0% | 0% | 0% |
| Intermediate    | 18 (38%)  | 18 (100%)          | 0 (0%)                   | NR    | NR              | NR        | NR    | NR    | NR      |
| High            | 13 (26%)  | 0 (0%)             | 13 (100%)                | 25 month | 84.6% | 7.6% | 0% | 0% | 0% |
| Low             | 21 (42%)  | 20 (95.2%)         | 1 (4.8%)                 | NR    | NR              | NR        | NR    | NR    | NR      |
| High            | 29 (58%)  | 14 (48.3%)         | 15 (51.7%)               | 48 month | 93.1% | 58.2% | ---- | (6.399-45.617) | <0.001† |
| CD163 Low       | 27 (54%)  | 24 (88.9%)         | 3 (11.1%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| CD163 High      | 23 (46%)  | 10 (43.5%)         | 13 (56.5%)               | 36 month | 91.3% | 46.6% | ---- | (3.227-26.407) | <0.001† |
| Tryptase Low    | 28 (56%)  | 25 (89.3%)         | 3 (10.7%)                | NR    | NR              | NR        | NR    | NR    | NR      |
| Tryptase High   | 22 (44%)  | 9 (40.9%)          | 13 (59.1%)               | 36 month | 90.9% | 44.1% | ---- | (3.576-30.873) | <0.001† |

Categorical variables were expressed as number (percentage); continuous variables were expressed as mean ± SD & median.

Table 4: Correlation between clinicopathological criteria, Sirt1, CD163, Tryptase expression and survival of our patients.
Figure 4: Kaplan Meir plot of Relapse Free Survival (Left panel) and Overall Survival (Right panel) of Hodgkin's lymphoma patients: (A) & (D) stratified by SIRT-1 expression; (B) & (E) stratified by CD163 expression; (C) & (F) stratified by Tryptase expression.
Similar to our results Barros et al. proved that progression-free survival rate (PFS) was lower in cases with high numbers of CD163+ macrophages [46]. But, Gupta et al. presented that there was no association between survival rates in macrophages evaluated with either CD163 or CD68 [47]. These conflicting data could be due to different number of patients used, variable follow up period, different clones of antibodies used or different method of evaluation.

There are many questions regarding the exact mechanisms of how RS cells can attract the macrophages, how macrophages affect RS cell growth and how RS cells could acquire immune isolation by such interaction. Recently, there are many discovered molecules that had been incriminated in macrophage chemotaxis and signaling in cHL pathobiology, e.g. CSF1R, MIF, fractalkine and CD74 [48], some of these molecules could be promising targets for discovering novel drug therapies that target the macrophages directly or by causing disturbances in the signaling pathways between RS cells and the surrounding tumor microenvironment.

We found that high number of mast cell infiltration in cHL as evidenced by tryptase overexpression was correlated to advanced stage and the presence of B-symptoms that was similar to Molin et al. who found that high mast cell infiltration had found to be related to advanced disease and poor relapse-free survival in adults [49]. Andersen et al. detect the relation between high mast cell count and poor outcome of patients similar to our results but that was only restricted to the mixed cellularity subtype [20]. In our results higher incidence of disease remission after successive therapy was higher in patients with higher mast cells count that was explained by RS cells stimulation by mast cells through CD30-CD30L interaction [49].

**Conclusion**

We evaluated the clinicopathological and prognostic roles of the microenvironment in cHL and found a significant relations between Sirt1 expression which was related to Tregs differentiation and oncopsressive role, macrophages as evidenced by CD163 expression, mast cells as evidenced by tryptase expression, poor clinicopathological parameters, advanced stage and poor patient prognosis, which may help to detect novel therapeutic targets to improve cHL outcome mainly in advanced and relapsed patients. Because of the conflicting results between our study and different previous studies we recommend that in advanced and relapsed patients. Because of the conflicting results between our study and different previous studies we recommend that in advanced and relapsed patients. But, Gupta et al. presented that there was no association between survival rates in macrophages evaluated with either CD163 or CD68 [47]. These conflicting data could be due to different number of patients used, variable follow up period, different clones of antibodies used or different method of evaluation.

There are many questions regarding the exact mechanisms of how RS cells can attract the macrophages, how macrophages affect RS cell growth and how RS cells could acquire immune isolation by such interaction. Recently, there are many discovered molecules that had been incriminated in macrophage chemotaxis and signaling in cHL pathobiology, e.g. CSF1R, MIF, fractalkine and CD74 [48], some of these molecules could be promising targets for discovering novel drug therapies that target the macrophages directly or by causing disturbances in the signaling pathways between RS cells and the surrounding tumor microenvironment.

We found that high number of mast cell infiltration in cHL as evidenced by tryptase overexpression was correlated to advanced stage and the presence of B-symptoms that was similar to Molin et al. who found that high mast cell infiltration had found to be related to advanced disease and poor relapse-free survival in adults [49]. Andersen et al. detect the relation between high mast cell count and poor outcome of patients similar to our results but that was only restricted to the mixed cellularity subtype [20]. In our results higher incidence of disease remission after successive therapy was higher in patients with higher mast cells count that was explained by RS cells stimulation by mast cells through CD30-CD30L interaction [49].

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