Immunohistochemical markers for tumor associated macrophages and survival in advanced classical Hodgkin's lymphoma

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ABSTRACT

A subset of patients with advanced classical Hodgkin's lymphoma is refractory to standard therapies. Therefore, it is relevant to identify new biologically-based prognostic markers. Recently, tumor associated macrophages have been proposed as a factor that predicts survival, although contradictory results have also been reported.

Here we analyzed four macrophage markers (CD68, CD163, LYZ, and STAT1) using immunohistochemistry and automated quantification, in two independent series of advanced classical Hodgkin's lymphoma (n=266 and 103 patients, respectively).

Our results did not confirm that specific macrophage immunohistochemical markers could be used as surrogates for gene expression profiling studies. Survival analyses did not show correlation between CD163, LYZ or STAT1 and either failure-free or disease-specific survival. There was an association between CD68 and disease-specific survival, but it was not consistent in both series.

In conclusion, individual tumor associated macrophage markers cannot be used to predict outcome before technical standardization and prospective validation in independent series of patients with comparable stages and treatments.

Key words: Hodgkin's lymphoma, outcome, tumor associated macrophages.

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Introduction

Classical Hodgkin's Lymphoma (cHL) is characterized by a minority of neoplastic Hodgkin's and Reed-Sternberg (HRS) cells associated with a heterogeneous background of non-neoplastic bystanders, mostly T cells, but also macrophages, eosinophils, basophils and plasma cells. Hodgkin's and Reed-Sternberg cells secrete numerous cytokines, including granulocyte-macrophage colony-stimulating factor that are likely responsible for the assembly of inflammatory cells in involved lymph nodes.[1] Recently, there has been increasing interest in the reactive components of the microenvironment as potential markers predictive of prognosis in cHL patients, and a number of studies have shown that bystander cells, such as T-cell subsets,[2] might be of prognostic importance.

Tumor associated macrophages (TAM) have been associated with adverse outcome in patients with different types of cancer,[3] including non-Hodgkin's lymphomas, and particularly follicular lymphoma (FL).[4,5] In cHL, the role of TAM was first suggested some decades ago.[6,7] More recently, these observations have been reinforced by gene-expression profiling (GEP) studies.[8,9] Most recently, Steidl *et al.*[10] described a gene signature that is over-expressed among patients who do not respond to therapy, containing genes described in TAM, including metallopeptidases such as MMP11, gene signatures of adipocytes, angiogenic and HRS cells, and underexpression of genes of germinal center B cells. These investigators selected CD68 as a surrogate marker for macrophages, and demonstrated in an independent cohort of patients by immunohistochemical (IHC) analysis that less than 5% of CD68$^+$ TAM correlated with longer progression-free survival (PFS) after primary treatment and lower relapse rates after autologous transplantation. The authors also showed that a very low percentage of TAM could identify a subset of patients with low

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Members of the Spanish Hodgkin Lymphoma Study Group are listed in the Appendix.

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stage disease who had a survival rate of 100%. These results suggest that semi-quantitative assessment of CD68+ TAMs can be used to identify patients at high risk of disease relapse or progression, as well as patients with early-stage disease with low risk for relapse who are currently over-treated.[11] However, this approach has a major drawback as the number of low-risk patients in the Steidl *et al.* study was low because very few cases of cHL had less than 5% of TAM. Therefore, about 72% of cases in the original report fall into the high-risk group[10] and might be selected for alternative treatments.

Similar results about the predictive value of TAM in cHL have recently been reported by other groups.[12,13] However, the consistency of IHC markers in different series has not been clearly confirmed.[14,15] Differences in the composition of the case series, technical variability, and different cut offs for expression of TAM associated markers could, in part, explain these diverse results. Also, roles for Epstein-Barr virus (EBV) and age as confounding factors have been claimed, since both variables influence the host immune response and TAM composition.[12,16]

We recently used GEP to identify specific genes associated with treatment failure in cHL patients[8,17] including gene signatures associated with reactive cells in the microenvironment. This gene signature was related to outcome in a selected series of advanced stage cHL patients using a different approach: RT-PCR analyses and integration of gene expression levels into a logistical regression model.[18] In this study, high expression of the *LYZ* or *STAT1* genes was found to correlate with prolonged FFS and better outcome[18] in the final model. To better understand the discrepancies between different series, we used IHC to investigate four different potential TAM markers in two independent series of patients.

Design and Methods

Using IHC, we analyzed four different macrophage markers, including CD68 , CD163, LYZ and STAT1, in two different series of patients: a Spanish cohort of 266 patients from the Spanish Hodgkin Lymphoma Study Group and an independent series of 103 cHL patients from the MD Anderson Cancer Center, Houston, Texas (Table 1). Specific approval of the IRB Committees from the participant institutions was obtained for this study. Markers were selected according to recent reports on cHL.

Tissue microarrays (TMAs) were constructed with duplicate or triplicate cores from selected areas of formalin-fixed, paraffin-embedded tumor samples. IHC analyses were performed using TMA sections as described earlier.[12] The sections were stained with anti-CD68 (clones KP1 and PGM1, Dako, Glostrup, Denmark), anti-CD163 (clone 10D6, Novocastra Laboratories, Newcastle Upon Tyne, UK), anti-STAT1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-LYZ (Dako) following routine procedures. Quantification of positivity was analyzed by an automated scan, Chroma Vision Systems ACIS III (Dako), as previously described,[12] analyzing the whole area of the tissue cores included in the TMAs. Additionally, CD68 (PGM1 clone) expression was manually scored by one of the authors (JFG) and the results were compared with the automated system.

All immunostaining and slide analyses were centralized and performed in a single institution (MDACC, Spain).

Correlation of the results of different IHC markers and outcome was estimated using Kaplan-Meier analyses and the log rank test. For survival analyses, we used four different IHC cut-off points, based on previous reports: median of IHC expression, 4th quartile, 5% and 25%.[8,10,12,14]

We performed quantitative RT-PCR analyses for the LYZ and STAT1 genes, as previously described,[18] using a group of 209 cases randomly selected on the basis of the availability of sufficient tissue for mRNA extraction, and extracting RNA from the whole tissue sections.

Clinical end points were failure-free survival (FFS) and disease-specific survival (DSS) as described.[8,18] Spearman's test was used to test correlations between quantitative variables. $P<0.05$ was considered significant.

Results and Discussion

Results are summarized in Table 2 and in the *Online Supplementary Appendix*. Briefly, we did not find any significant associations between CD68, CD163, LYZ or STAT1 and FFS in either case series. We also analyzed the CD163/CD68 ratio as a putative surrogate for M2-type activation of macrophages[19] and found no significant associations.

In DSS analyses, there was a significant association between higher CD68 levels and shorter DSS, independent of the cut off used, in the Spanish series. However, this result was not significant in the Houston series. Furthermore, we could not find a clear relationship between CD68 levels and treatment response as indicated by FFS. When the PGM1 clone was used, only one of the different cut-off points showed significant association between CD68 IHC levels and DSS (Table 2).

Technical factors may explain some of the discrepancies between the results reported here and those in the literature, since different authors have used different cut offs and quantification techniques (automated scanning of slides *vs.* visual counting of positive cells). Based on the results in this study, a significant correlation can be found when CD68 IHC was manually scored and compared

Table 1. Patients' clinical characteristics.

Feature	Spain N = 266 (%)	Houston N = 103 (%)	*P*
Age (years)			
<45	188 (70. 7%)	75 (72. 8%)	0.684
≥45	78 (29. 3%)	28 (27. 2%)	
Gender			
Male	148 (55.6%)	58 (56.3%)	0.907
Female	118 (44.4%)	45 (43.7%)	
IPS			
0-2	177 (66.5%)	83 (80.6%)	0.008
≥3	89 (33.5%)	20 (19.4%)	
Ann Arbor stage			
<IV	212 (79.7%)	83 (80.6%)	0.849
≥IV	54 (20.3%)	20 (19.4%)	
Treatment response			
CR	190 (71.4%)	74 (71.8%)	0.937
Failure	76 (28.6%)	29 (28.2%)	
cHL subtype			
Nodular sclerosis	141 (58.5%)	74 (72.5%)	
Mixed cellularity	82 (34.0%)	22 (21.6%)	0.129
Lymphocyte rich	12 (5.0%)	4 (3.9 %)	
Lymphocyte depletion	6 (2.5%)	2 (2.0%)	

with the automated system (Spearman's coefficient 0.432; $P<0.001$) (*Online Supplementary Figure S1*).

Importantly, previous studies used the KP1 antibody to identify cells that express CD68. The KP1 antibody is known to also react with myeloid and fibroblastic subpopulations in addition to TAM, in contrast with a more specific clone such as PGM1.[20] In agreement, overall values for CD68 (KP1) expression were higher than those of CD68 (PGM1) (Figure 1). When the two different anti-CD68 clones were compared, we observed a weak but significant correlation (Spearman's coefficient 0.226; $P<0.000$).

Many studies of TAM populations in various cancers have been published, but few have dealt with the role of activation of macrophage phenotypes, such as M1 and M2, in tumor tissue. All tumor types are invaded by macrophages and it was thought that this represented a host immunological response to tumor. However, M2-type macrophages represent part of the innate immune response, can be associated with cell proliferation and migration, and have been shown to mediate blood vessel formation by regulating the angiogenic switch through secretion of VEGF and hypoxia inducing factors.[3,21] M2 macrophages are known to highly express CD163 which may be a useful marker for identifying specific activation states of macrophages. Recent analyses of angioimmunoblastic T-cell lymphoma suggest that the ratio of $CD163^+$ to $CD68^+$ cells reflects the proportion of macrophages polarized toward the M2 phenotype, and this ratio correlates with worse prognosis.[19] We, therefore, assessed the CD163/CD68 ratio in cHL cases. There was no significant association between the CD163/CD68 ratio and either FFS or DSS.

Other investigators have advocated the predictive value of TAM in cHL[12,13] using either CD68 or CD163, the latter an IHC marker more selective for M2-alternatively activated macrophages.[3] However, the cut offs used differ substantially. Kamper *et al.*[12] identified the fraction of patients with the highest quartile of IHC expression, representing approximately a quarter of patients with the highest risk of relapse. In the report by Jakovic *et al.*[13] a cut off of 25% $CD68^+$ cells was associated with shorter OS, but not with event-free survival. In addition, the number of macrophages in the reactive background of cHL tumors seems to be related to specific cell populations, such as regulatory and cytotoxic T cells. Tzankov *et al.*[22] recently showed a combined microenvironment score using specific markers PD1, Granzyme B, and FOXP3. In this study, a cut off of 82% $CD68^+$ cells was associated with OS, but not with disease-specific survival or relapse-free survival. The functional consequences of these complex interactions between tumor and the background cells require further investigation.

Also, the consistency of TAM detection using IHC markers in different series has not been clearly confirmed. A recent report by Azambuja *et al.*[14] did not find any statistically significant association between CD68 or CD163 IHC expression and outcome in cHL patients. Similar results have recently been reported by Agostinelli *et al.*[15] Higher levels of CD68 and CD163 expression, however, were correlated with the presence of EBV-positive HRS tumor cells in the two series, confirming that the presence of EBV represents a relevant factor in the immune response and the polarization status of macrophages. The roles of EBV and age as confounding variables must be further investigated.

Table 2. Survival analyses for the different inmunohistochemical markers included in the study. Numbers correspond to the different *P* values (log rank test).

	CD68 (KP1 clone)				CD163			
	Spain		Houston		Spain		Houston	
Cut-off point	FFS	DSS	FFS	DSS	FFS	DSS	FFS	DSS
Median	0.333	0.000*	0.400	0.661	0.058	0.320	0.242	0.169
Q4 (75%)	0.100	0.000*	0.563	0.517	0.037	0.218	0.437	0.350
5%	0.288	0.047*	0.898	0.866	0.318	0.074	0.933	0.654
25%	0.146	0.000*	0.501	0.768	0.064	0.211	0.124	0.076

	STAT1				LYZ			
	Spain		Houston		Spain		Houston	
Cut-off point	FFS	DSS	FFS	DSS	FFS	DSS	FFS	DSS
Median	0.280	0.840	0.622	0.065	0.437	0.028*	0.621	0.908
Q4 (75%)	0.800	0.892	0.639	0.005*	0.565	0.071	0.178	0.573
5%	0.492	0.957	0.893	0.986	0.402	0.242	0.107	0.382
25%	0.178	0.520	0.938	0.078	0.397	0.020*	0.465	0.931

	CD68 (PGM1 clone)			
	Spain		Houston	
Cut-off point	FFS	DSS	FFS	DSS
Median	0.850	0.068	0.156	0.529
Q4 (75%)	0.686	0.026*	0.300	0.779
5%	0.457	0.052	0.397	0.262
25%	0.884	0.131	0.423	0.639

FFS: failure free survival; DSS: disease specific survival.
**means a P value < 0.05*

In this study, we also compared protein expression levels measured by IHC and gene expression levels measured by RT-PCR for the two genes included in the previous model,[18] *LYZ* and *STAT1*. There were no significant correlations between the variables (Spearman's test, *data not shown*). These results indicate that different laboratory techniques can give inconsistent results. Results obtained using GEP with RNA extracted from frozen tissues, RT-PCR analyses using RNA extracted from formalin-fixed, paraffin-embedded tissue, or IHC analyses on tissue sections, represent different procedures that are not comparable. Additionally, microarray-based GEP and RT-PCR techniques assess RNA extracted from whole tissue and do not analyze specific cell types.

The results we present to some extent confirm previous observations concerning the role of the microenvironment in follicular lymphoma,[4] where the impact of the tumor microenvironment on prognosis in follicular lymphoma has been shown to be dependent on specific treatment protocols[23,24] illustrating the limitations in the comparison of biomarkers between different series of patients and different treatment strategies.

CD68 has been used as a standard marker for TAM. However, it is not clear whether the expression of other markers, such as STAT1, LYZ and CD163, are directly related to the absolute number of macrophages, or identify specific cell subpopulations, or specific activation stages. Also, comparison between different series of patients with different inclusion criteria (advanced *vs*. all stages of cHL), diverse technical approaches, or different biomarker selection could explain contradictory observations. We can conclude that technical standardization and

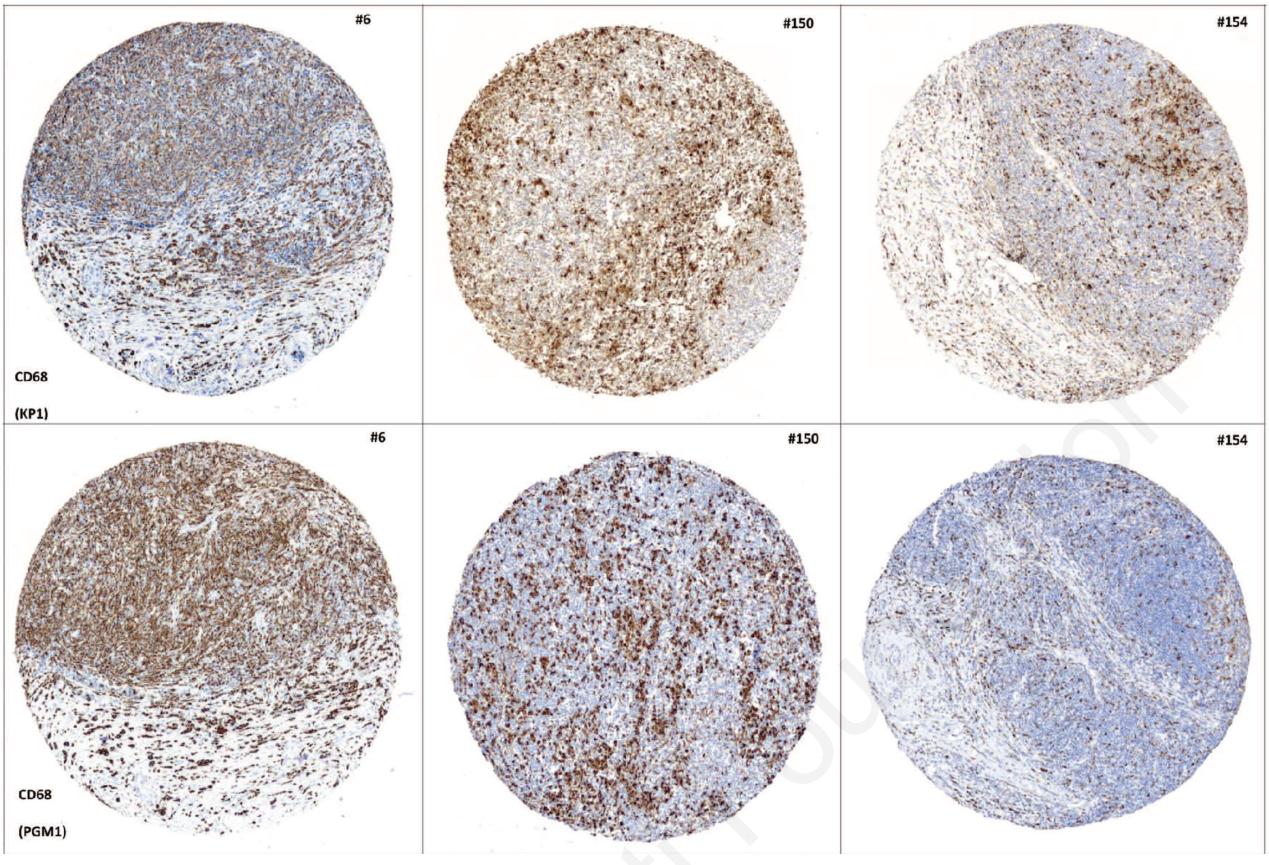

Figure 1. Immunohistochemical analyses for CD68 in representative cases. KP1 (top) and PGM1 (bottom) scores were, respectively: case 6, 85 *vs.* 74%; case 150, 48 *vs.* 32%; case 114, 10 *vs.* 5%.

prospective validation in independent series of cHL patients, with comparable clinical stages and similar treatment, are needed before IHC analysis of CD68, or any other individual or combination of markers for TAMs, can be used to predict outcome of cHL patients.

Appendix's

The following centers and investigators participate in the Spanish Hodgkin Lymphoma Study Group and contribute to the studies with tumor samples and clinical data: R. Ramos, J. Rodríguez, F. Mestre (Hospital Son Dureta, Palma de Mallorca); P. Domínguez, C. Jara (Fundación Hospital Alcorcón, Madrid); M. J. Mestre, R. Quibén, M. Méndez, L. Borbolla (Hospital de Móstoles, Madrid); M. A. Martínez, C. Grande (Hospital 12 de Octubre, Madrid); M. García-Cosío, C. Montalbán, J. García-Laraña (Hospital Ramón y Cajal, Madrid); M. Canales, J. Alves (Hospital La Paz, Madrid); C. Bellas, M. Provencio (Hospital Puerta de Hierro, Madrid); A. Castaño, P. Sánchez-Godoy (Hospital Severo Ochoa, Leganés, Madrid); C. Martín, R. Martínez (Hospital Clínico Universitario San Carlos, Madrid); J. Menárguez, P. Sabín, E. Flores (Hospital Gregorio Marañón, Madrid); J. González-Carrero, C. Poderós (Hospital Xeral-Cies, Vigo); A. Salar, S. Serrano (Hospital del Mar, Barcelona); T. Álvaro, L. Font (Hospital Verge de la Cinta, Tortosa); V. Romagosa, A. Fernández de Sevilla (Hospital Duran i Reynalds, Institut Catala d'Oncologia, Barcelona); M. Mollejo, M. A. Cruz (Hospital Virgen de la Salud, Toledo); A. Cánovas, C. Camarero (Hospital de Cruces, Baracaldo). H. Álvarez-Arguelles, M. Llanos (Hospital Universitario Canarias, Tenerife); R. Arranz, A. Acevedo (Hospital La Princesa, Madrid); R. García-Sanz, T.Flores (Hospital Universitario de Salamanca); C. Morante (Hospital Cabueñes, Gijón); A. Marín, E. Ríos (Hospital Virgen del Rocío, Seville); F. Mazorra, E. Conde (Hospital Marqués de Valdecilla, Santander); M. F. Fresno, C. Rayón, C. Nicolás (Hospital Central de Asturias, Oviedo); C.Santonja, Jose L. López (Fundación Jiménez Díaz, Madrid); T. Flores, R. García-Sanz (Hospital Universitario de Salamanca, Salamanca); J. Guma (Hospital Sant Joan, Reus); P. Gonzalvo (Hospital Comarcal de Jarrio, Coaña); G. Fernández (Hospital Alvarez Buylla, Mieres); J. Forteza, M. Fraga, J. L. Bello (F Med Santiago de Compostela); A. Bas (Hospital Universitario Virgen de la Arrixaca, Murcia); J. R. Méndez (Hospital Valle de Nalón, Asturias); J.F. Tomás, M. Estevez (MD Anderson España, Madrid); C.Ruiz-Marcellán , A.López (Hospital Vall d'Hebron) and J. F. García, M. M. Morente and M. A. Piris (CNIO, Madrid).

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References

1. Aldinucci D, Lorenzon D, Cattaruzza L, Pinto A, Gloghini A, Carbone A, et al. Expression of CCR5 receptors on Reed-Sternberg cells and Hodgkin lymphoma cell lines: involvement of CCL5/Rantes in tumor cell growth and microenvironmental interactions. Int J Cancer. 2008;122(4):769-76.
2. Alvaro T, Lejeune M, Salvadó MT, Bosch R, García JF, Jaén J, et al. Outcome in Hodgkin's lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T cells. Clin Cancer Res. 2005;11(4):1467-73.
3. Mantovani A, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev. 2006;25 (3):315-22.
4. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004;351(21): 2159-69.
5. Farinha P, Masoudi H, Skinnider BF, Shumansky K, Spinelli JJ, Gill K, et al. Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). Blood. 2005;106(6):2169-74.
6. Ree HJ, Kadin ME. Macrophage-histiocytes in Hodgkin's disease. The relation of peanut-agglutinin-binding macrophage-histiocytes to clinicopathologic presentation and course of disease. Cancer. 1985;56(2): 333-8.
7. Coppleson LW, Rappaport H, Strum SB, Rose J. Analysis of the Rye classification of Hodgkin's disease. The prognostic significance of cellular composition. J Natl Cancer Inst. 1973;51(2):379-90.
8. Sanchez-Aguilera A, Montalban C, de la Cueva P, Sanchez-Verde L, Morente MM, Garcia-Cosio M, et al. Tumor microenvironment and mitotic checkpoint are key factors in the outcome of classic Hodgkin lymphoma. Blood. 2006;108(2):662-8.
9. Devilard E, Bertucci F, Trempat P, Bouabdallah R, Loriod B, Giaconia A, et al. Gene expression profiling defines molecular subtypes of classical Hodgkin's disease. Oncogene.2002;21(19):3095-102.
10. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010; 362(10):875-85.
11. Carde P, Koscielny S, Franklin J, Axdorph U, Raemaekers J, Diehl V, et al. Early response to chemotherapy: a surrogate for final outcome of Hodgkin's disease patients that should influence initial treatment length and intensity? Ann Oncol. 2002;13 (Suppl 1):86-91.
12. Kamper P, Bendix K, Hamilton-Dutoit S, Honore B, Nyengaard JR, d'Amore F. Tumor-infiltrating macrophages correlate with adverse prognosis and Epstein-Barr virus status in classical Hodgkin's lymphoma. Haematologica. 2011;96(2):269-76.
13. Jakovic LR, Mihaljevic BS, Perunicic Jovanovic MD, Bogdanovic AD, Andjelic BM, Bumbasirevic VZ. The prognostic relevance of tumor associated macrophages in advanced stage classical Hodgkin lymphoma. Leuk Lymphoma. 2011;52(10): 1913-9
14. 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. 2011; doi: 10.1093/annonc/mdr157.
15. Agostinelli C, Gallamini A, Rigacci L, Vitolo U, Stracqualursi L, Piccaluga P, et al. The prognostic value of biologic markers in classical Hodgkin lymphoma (cHL) patients. Ann Oncol. 2011;22:153-4.
16. Chetaille B, Bertucci F, Finetti P, Esterni B, Stamatoullas A, Picquenot JM, et al. Molecular profiling of classical Hodgkin lymphoma tissues uncovers variations in the tumor microenvironment and correlations with EBV infection and outcome. Blood.2009;113(12):2765-3775.
17. Sanchez-Espiridion B, Sanchez-Aguilera A, Montalban C, Martin C, Martinez R, Gonzalez-Carrero J, et al. A TaqMan low-density array to predict outcome in advanced Hodgkin's lymphoma using paraffin-embedded samples. Clin Cancer Res. 2009;15(4):1367-75.
18. Sanchez-Espiridion B, Montalban C, Lopez A, Menarguez J, Sabin P, Ruiz-Marcellan C, et al. A molecular risk score based on 4 functional pathways for advanced classical Hodgkin lymphoma. Blood. 2010;116(8): e12-7.
19. Niino D, Komohara Y, Murayama T, Aoki R, Kimura Y, Hashikawa K, et al. Ratio of M2 macrophage expression is closely associated with poor prognosis for Angioimmunoblastic T-cell lymphoma (AITL). Pathol Int. 2010;60(4):278-83.
20. Kunisch E, Fuhrmann R, Roth A, Winter R, Lungershausen W, Kinne RW. Macrophage specificity of three anti-CD68 monoclonal antibodies (KP1, EBM11, and PGM1) widely used for immunohistochemistry and flow cytometry. Ann Rheum Dis. 2004;63(7):774-84.
21. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res. 2006;66(2):605-12.
22. Tzankov A, Matter MS, Dirnhofer S. Refined prognostic role of CD68-positive tumor macrophages in the context of the cellular micromilieu of classical Hodgkin lymphoma. Pathobiology. 2010;77(6):301-8.
23. Canioni D, Salles G, Mounier N, Brousse N, Keuppens M, Morchhauser F, et al. High numbers of tumor-associated macrophages have an adverse prognostic value that can be circumvented by rituximab in patients with follicular lymphoma enrolled onto the GELA-GOELAMS FL-2000 trial. J Clin Oncol. 2008;26(3):440-6.
24. Taskinen M, Karjalainen-Lindsberg ML, Nyman H, Eerola LM, Leppa S. A high tumor-associated macrophage content predicts favorable outcome in follicular lymphoma patients treated with rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone. Clin Cancer Res. 2007;13(19): 5784-9.