Molecular and Immunological Analysis of the Effect of Tumor Associated Macrophages on Prognosis of Hodgkin's Lymphoma in Saudi Patients

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Introduction

Hodgkin's lymphoma (HL) is a malignant lymphoma that most commonly affects younger patients. HL is characterized by a minority of neoplastic cells: Hodgkin's Reed-Sternberg Cells (HRS). HRS are surrounded by the tumor microenvironment (TME), with frontline tumor associated macrophages (TAMs). The high number of TAMs constitutes a major part of the cellular milieu and was identified as the major determinant of the clinical outcome of HL. Over expression of CD68 and CD163 antigens by TAMs is considered a significant predictive biomarker for risk stratification of HL. This is thought to be caused by genetic polymorphism (SNPs) at the promoter or the coding region of their genes. Moreover, common DNA polymorphisms of related DNA repair genes in Saudi patients and their link to disease progression and/or survival can be used as prognostic biomarkers and may help researchers identify a curative therapy for HL.

The classical HL (CHL) comprises the majority of all HL cases. A review of records at John Hopkins Aramco Healthcare Center-Dhahran-KSA showed that CHL represents more than 85% of HL cases. Although HL is a relatively curable disease, a considerable percentage of cases (20-30 %) fail to respond to therapy and an equal percentage are over treated with current protocols. Early identification of those cases has become an important objective for clinical research. Even though the epidemiological behavior of HL is continuously updated in various parts of the world, studies from the Kingdom of Saudi Arabia are limited. Recent studies confirmed a molecular/gene signature associated with macrophage infiltration in CHL.

Objectives: The aim of this study was to investigate the significance of expression of CD68 and CD163 antigens on TAMs and the presence of their related SNPs on the prognosis of CHL in Saudi patients. The overall objective is to establish a gene expression profile of HL specific biomarkers to predict the outcome and survival of HL patients in Saudi Arabia.

Keywords: Hodgkin's lymphoma, Tumor associated macrophages, Immunohistochemistry, CD68, CD163, Single nucleotide polymorphism, DNA repair genes, Predictive biomarkers.

Methods

This is a retrospective case-control study. The protein expression of CD68 and CD163 on TAMs was studied using Immunohistochemistry Staining Technique (IHC). A prognosis index was calculated for both proteins to assess the risk stratification of HL. We intended to introduce a new methodology for calculating a "Prognosis Index" of CD163 and of CD68 as well. That was calculated by dividing the average number of CD163 positive TAMs surrounding a cluster of HRS by the average number of CD30 HRS positive cells. The equation is as follows:

$$\text{Prognosis Index of CD163} = \frac{\text{Average Number of CD163 Positive TAMs}}{\text{Average Number of CD30 Positive cells}}$$

The same was done for calculating the CD68 prognosis index. Although CD30 IHC may stain activated B-lymphocytes in addition to HRS cells, the fact that we used a CD30 positive denominator for both CD68 and CD163 index calculation should not affect the value of the index. The CD30 positive reactive B-lymphocytes were counted from the normal lymph node tissues selecting the inter-follicular areas (not inside the follicles nor in the sinusoidal spaces) and selecting the scattered non-clustered cells in that area.

Molecular genotyping of selected SNPs located in the promoters and the protein-coding region of those two antigens plus selected DNA repair genes and a transcription regulator gene SNP was performed for 100 CHL cases and normal controls using Real Time PCR.

Table 1: Comparison of CD68 and CD163 indices in CHL patients versus the control group.

	HL PATIENTS	CONTROLS	T-TEST
	n= 100	n=20	P value
CD68 INDEX (MEAN)	12.3	2.8	< 0.001
CD163 INDEX (MEAN)	13.5	2.8	< 0.001

Table 2: Summary of DR and OS in studied CHL patients at JHAH.

	DR				OS	
	0-6 months	6 months-5 years	5-10 years	> 10 years	< 5 years	> 5 years
NO. OF CHL PATIENTS	3/100	10/100	2/100	1/100	9/100	2/100

Results

Data revealed that both CD68 and CD163 created indices are highly correlated to each other. The expression levels of these two proteins were significantly correlated with the disease relapse (DR) and overall survival (OS) with a p value of < 0.001 for both. In addition, the CD163 index threshold of (15.0) was significantly correlated with the relapse rate (p=0.022). None of the CD68 gene SNPs were correlated with DR or with the OS. At the same time, all the studied CD163 SNPs were not correlated with DR and OS of CHL patients except for rs75608120, which is located in the promotor region. This SNP is showing a significant correlation with the relapse rate of CHL (p=0.032) but not with OS. The analysis of 5 DNA repair genes SNPs showed that the XPG gene has a statistical significance correlation with CHL patient survival (p=0.036). This finding suggests that the XPG gene can be used as a molecular predictive biomarker for HL clinical prognosis.

Figure 1: Estimated survival curve based on CD163 Index (time was calculated in years)

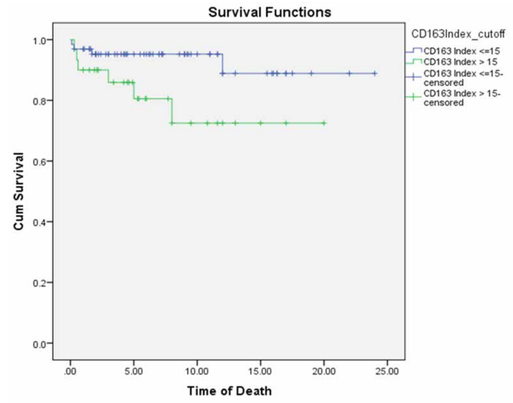
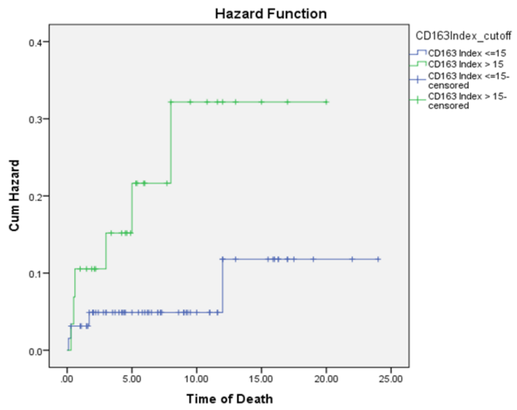


Figure 2: Estimated Hazard curve based on CD163 Index (time was calculated in years).



Conclusions

Through this study we gained valuable insights on the molecular genetics and immunological contribution of CD68 and CD163 on HL in Saudi Arabian patients. We have confirmed that CD68 and CD163 over expression on TAMs is significantly associated with early relapse and reduced survival post therapy, with more specificity of CD163 antigen. Notwithstanding, we have also defined, for the first time, a specific genetic pattern that is clearly associated with the clinical outcome of HL. A specific SNP in XPG DNA repair gene and a SNP located in the CD163 promotor sequence of its gene were both significantly correlated with HL relapse risk and poor overall survival rate. Accordingly, they are defined to be useful predictive immunological and genetic biomarkers for CHL prognosis in Saudi patients.

Moreover, our findings pave the way to improve the clinical management of CHL patients in the future using molecular analyses for gene expression and immunohistochemistry. Our findings may help to achieve better therapeutic protocols.

Pharmacogenetics, specifically monoclonal antibodies, could be used to block TAMs receptors for CD68 and CD163 in conjunction with the current chemotherapy or immunotherapy used to treat HL.

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