Macrophage/histiocytic antigen CD68 expression in neoplastic and reactive lymph nodes

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Abstract

There are some reports, suggesting that infiltrating macrophages may promote tumour progression in non-Hodgkin's lymphoma (nHL). The aim of the study was an evaluation of macrophages, marked by antibody against CD68 in indolent and aggressive nHL. The study was performed in 65 patients: 50 with nHL and 15 with lymph nodes affected by reactive hyperplasia. Immunohistochemical analyses were performed on paraffin-embedded specimens with monoclonal anti-CD68 antibody. Scoring on the basis of the percentage of positive cells indicated CD68 expression in 34/50 (68%) of the patients with nHL and in only 5/15 (33%) of the patients with reactive hyperplasia. The expression of CD68 was statistically significantly higher in the aggressive nHL than in indolent nHL. An increased number of CD68 positive macrophages in clinically aggressive nHL may confirm their role in tumour progression.

Key words: macrophages, CD68, lymphomas.

Introduction

Non-Hodgkin's lymphomas (nHL) are a heterogeneous group of lymphoid malignancies with a different pattern of clinical behaviour and response to treatment. In terms of clinical aggressiveness nHL may be divided into two groups: aggressive and indolent lymphomas. The infiltration of neoplastic tumour by leucocytes was described in 1863 by Virchow [1]. A large part of those leucocytes was macrophages. Macrophages constitute a primary line of defence mechanisms. They are found in many solid tumours, where they are known as tumour associated macrophages (TAM) [2]. There are some clinical observations, indicating that macrophages are important in the processes of cancer progression (growth, invasion and metastasis), making a connection between inflammation and cancer [3, 4, 5]. A close relationship has been found between the number of macrophages and the inhibition of tumour growth, as well as tumour progression. Some authors suggest that infiltrating macrophages may promote tumour progression in nHL. Vacca et al. reported a correlation between the number of macrophages and tumour microvessel density in nHL. An increased number of macrophages may be recruited and activated locally by more malignant B-cells [6]. The aim of the study was an evaluation of macrophages, marked by antibody against CD68 in indolent and aggressive nHL.

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Material and methods

The study was performed on 50 patients with non-Hodgkin's lymphoma (NHL) and in 15 lymph nodes with reactive hyperplasia, diagnosed and histopathologically confirmed in at the Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation and the Department of Pathological Anatomy, Medical University in Wroclaw by a pathologist with a profound experience in lymphomas. Clinical staging was done, according to the Ann Arbor classification system. The histological diagnosis of enrolled patients with lymphomas was made, according to the WHO/REAL classification. Out of the 50 patients with lymphomas, 43 (87%) B-cell and 7 (13%) T-cell lymphomas were diagnosed. The median age of the population was 57 years, clinical stage III and IV. Aggressive lymphomas constituted group of 28 cases (54%) and indolent - 22 (46%) cases. Histological classification of B-cell lymphomas revealed the following: peripheral leukaemia/lymphoma - 12 (27%), fol-

Table 1. The expression of CD68 in lymph nodes: reactive; indolent and aggressive non-Hodgkin's lymphomas.

Grade of CD68 expression	**nHL 50 patients**	**Reactive hyperplasia 15 patients**	**p**
I and II	24	10	NS
III and IV	26	5	
Grade of CD68 expression	**Indolent nHL 22 patients**	**Aggressive nHL 28 patients**	**p**
I and II	15	9	0.011
III and IV	7	19	
Grade of CD68 expression	**Reactive hyperplasia 15 patients**	**Aggressive nHL 28 patients**	**p**
I and II	10	9	0.03
III and IV	5	19	

Figure 1. A. Peripheral B-cell leukaemia/lymphoma; 210x. D68 expression: grade II (5-25%)

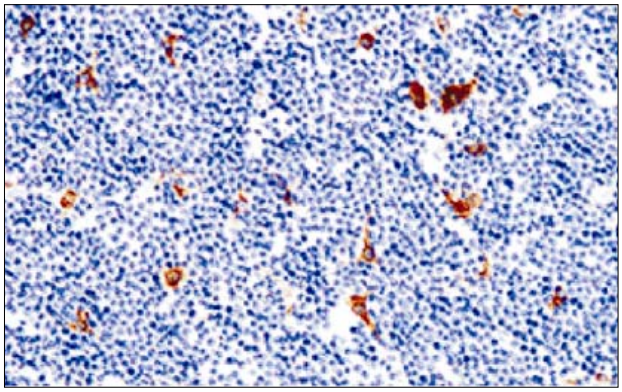
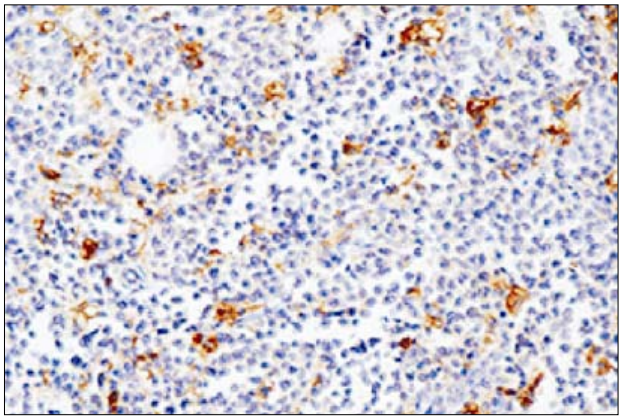


Figure 1. B. Immunoblastic B-cell lymphoma; 210x. CD68 expression: grade III (26-50%)



licular lymphoma - 7 (16%), mantle cell lymphoma - 7 (16%), immunoblastic/immunocytic - 4 (9%), other - 13 (32%). Immunohistochemical analyses were performed on paraffin-embedded specimens with monoclonal anti-human macrophage antigens - CD68 (cloneKP1) (DAKO, Denmark). The DAKO Fast Red Substrate System was used as a substrate and chromogen in immunocytochemical staining procedures, utilizing alkaline phosphatase. In each case, a control was included, in which, the specific antibody was omitted. CD68 expression was graded as the percentage of positive stained cells. Four grades of expression were established: grade I <5%; II - 5-25%; III - 26-50%; IV - >50% of positive cells. Statistical analysis was made using the Fisher test. Differences were considered statistically significant at $p<0.05$.

Results

CD68 expression in grade I (<5%) was only demonstrated in 18% (9, out of 50) of patients with lymphoma, examined by immunohistochemistry. In grade I, there were 16/50 nHL (31%) and no reactive lymph nodes. Grade II was found in 14/50 (28%) nHL patients and in 8/15 reactive lymph nodes (53%). Grade III was observed in 15/50 (30%) and 5/15 reactive (33%) observed. Grade IV was found in 13/50 (26%) nHL and 2/15 (13%) reactive lymph nodes noted. The results are presented in the (Fig. 1 a and b), Table 1 according to clinical aggressiveness. The expression of CD68 was statistically significantly higher in the aggressive nHL than that in indolent nHL and reactive lymph nodes.

Discussion

Lymph nodes, affected by lymphoma, are not only composed of malignant cells but also of many other cell types, including macrophages. TAMs have been considered to have an anti-tumour effect as a part of immune defence. Recently, it seems that they may have both protumour and antitumour activity. In tumours, macrophages are recruited and activated via several factors, secreted by tumour cells i.e. chemokines, VEGF. Their effect depends on tumour microenvironment and the tumour itself. TAMs can also produce various growth factors and cytokines, stimulating growth and angiogenesis, as well as facilitating the invasion by secreting cell membrane degrading enzymes [7, 8]. The results of various observations, using macrophage specific markers as a prognostic factor, are controversial. In papillary thyroid cancer, patients with tumours, containing TAMs exhibing neoplastic cell phagocytotic activities, had a better prognosis than patients without TAMs [9]. On the other hand, the presence of areas with high-density TAMs within "hot spots" was positively correlated with increased vascularity and metastasis and with reduced relapse-free and overall survival in breast cancer [10]. There is also a strong association between increased melanoma-specific mortality and the increasing number of CD68-positive macrophages [11]. The data, concerning the role of TAMs in nHL are very limited. Vacca et al. have demonstrated that macrophages promote angiogenesis and, in consequence, the progression of B-cell nHL [6]. In our study, we did not find any statistical differences in the CD68 expression between reactive lymph nodes and the whole nHL group. Nevertheless, the expression of CD68 was statistically significantly higher in the aggressive nHL than in

indolent nHL and reactive lymph nodes. This observation indicates a relationship between the number of TAMs in lymph nodes and the clinical aggressiveness of nHL.

Conclusions

An increased number of CD68 positive macrophages in clinically aggressive nHL may confirm their role in tumour progression.

Acknowledgements

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