FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 42, No. 4, 2004
pp. 241-243

Angiogenesis measured by expression of CD34 antigen in lymph nodes of patients with non-Hodgkin's lymphoma

Grzegorz Mazur[1], Tomasz Wróbel[1], Piotr Dzięgiel[2], Michał Jeleń[3], Kazimierz Kuliczkowski[1] and Maciej Zabel[2]

[1]Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, [2]Department of Histology and Embryology and [3]Department of Pathology, Medical University, Wrocław, Poland

Abstract: Angiogenesis is important in development, maintenance and progression of haematological malignancies. Some clinical observations have indicated that in non-Hodgkin's lymphoma (nHL) tumour microvessel density (MVD) may correlate with tumour staging and outcome. The aim of the study was to examine relationship between MVD as a parameter of tumour angiogenesis measured by expression of CD34 and the grade of nHL histological malignancy as determined by REAL classification. 40 lymph node samples of patients with newly diagnosed nHL (17 women, 23 men; aged 48-70 yrs, median age 64 yrs; stage III and IV) and treated at the Department of Haematology, Wrocław Medical University in 1999-2002 were fixed in 10% buffered formalin and embedded in paraffin. In all the studied cases, sections were incubated with antibodies against CD34. The slides were stained with hematoxylin and eosin and evaluated histopathologically. Patients were divided into two groups according to histological malignancy: indolent nHL (19 patients) and aggressive nHL (21 patients). Mean MVD measured by expression of CD34 in aggressive and indolent nHL groups amounted to 19.45±11.24 vessels/0.375 mm^2 and 21.7±12.4 vessels/0.375 mm^2, respectively. Statistical analysis of microvessel staining demonstrated no correlation between tumour MVD and grade of histological malignancy in lymph nodes of nHL patients. Nevertheless, angiogenesis observed in nHL provides rationale for use of angiogenesis inhibitors in lymphoma therapy.

Key words: Angiogenesis - Non-Hodgkin's lymphoma - Microvessel density

Introduction

In recent years, the role of angiogenesis (neovascularisation) in neoplastic diseases, especially in growth of solid tumours, has been increasingly studied. There are some data that neovascularisation may be important in development, maintenance and progression of haematological malignancies. Some clinical observations have indicated that tumour microvessel density (MVD) measured by CD34, CD31 antigen or von Willebrand factor (vWF) expression is increased in lymphoproliferative disorders and correlate with tumour staging and outcome [1, 7, 8, 10, 11, 12, 18, 21]. Higher microvascular density and increased serum levels of proangiogenic factors such as vascular endothelial growth factor (VEGF) or basic fibroblasts growth factor (bFGF) have been reported in chronic lymphocytic leukemia, multiple myeloma and non-Hodgkin B-cell lymphomas (nHL) [10, 19]. nHL are heterogenous group of malignant neoplasms with different origin and clinical characteristics. In terms of clinical aggressiveness nHLs may be divided in two groups: agressive and indolent lymphomas.

The aim of the study was to examine the relationship between MVD as a parameter of tumour angiogenesis measured by expression of CD34 and the grade of nHL histological malignancy as determined by REAL classification.

Materials and methods

Angiogenesis was measured in 40 randomly selected adult patients with non-Hodgkin's lymphoma diagnosed and treated at the Department of Haematology, Wrocław Medical University in 1999-2002. The patients with nHL included 17 women and 23 men, aged 48-70 yrs (median 64 yrs). All patients had active disease at stage III and IV. Clinical data are shown in Table 1. Clinical staging was done according to the Ann Arbor classification system [9]. Histological classification was not reviewed in conjunction with the present study, but 19 lymphomas had been classified as indolent and 21 as aggressive nHL according to the REAL classification [6] by a pathologist with a profound experience in lymphoms. Samples of the

Correspondence: G. Mazur, Dept. Haematology, Blood Neoplasms and Bone Marrow Transplantation, Medical University, Pasteura 4, 50-367 Wrocław, Poland; e-mail: grzegmaz@hemat.am.wroc.pl

Table 1. Clinical data of patients with non-Hodgkin lymphoma (nHL) according to REAL classification

nHL classification	number of patients
AGRESSIVE nHL	21
Diffuse large B cell Follicular grade 3 Mantle cell	15 2 4
INDOLENT nHL	19
Small lymphocytic Lymphoplasmocytoid Marginal zone Follicular grade 1, 2	7 6 1 5

studied lymphomas were fixed in 10% buffered formalin and embedded in paraffin. The slides were stained with hematoxylin and eosin and evaluated histopathologically.

Deparaffinised sections were boiled in citrate buffer to unmask antigenic determinants. In all studied cases sections were incubated with antibodies against CD34 (Class II, Clone QBEnd 10, 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 the control was included, in which the specific antibody was omitted. For the evaluation of microvessel density (MVD), slides were scanned in the light microscope at × 40 magnification and three areas of maximal MVD, so-called "hot spots" were identified. In each hot spot, microvessels (capillaries and small venules) were counted at × 400 magnification by two investigators (each field representing an area of 0.375 mm^2). For each slide, the mean number of microvessels from these three areas, including results obtained by two investigators, per 0.375 mm^2 was calculated. Statistical analysis was made using the Mann-Whitney U-test. Differences were considered statistically significant at $p<0.05$.

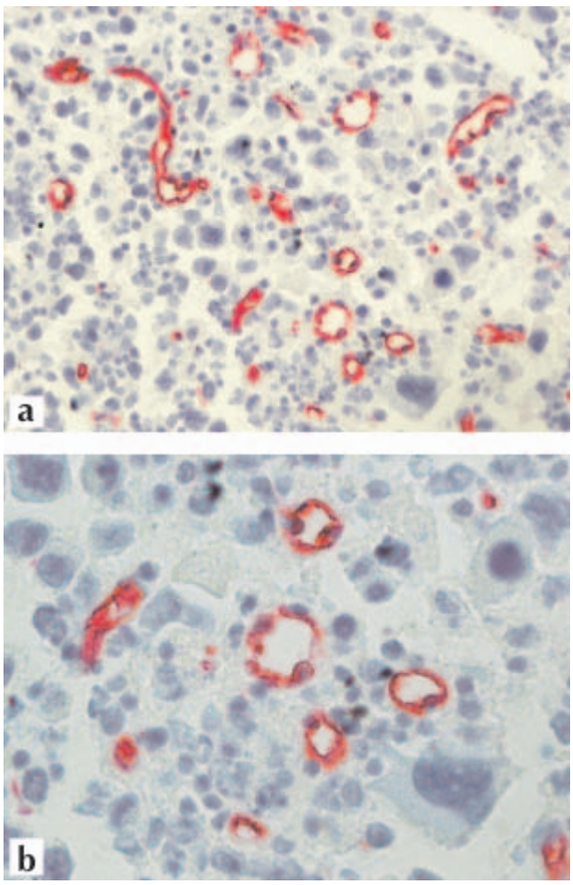

Fig. 1. Microvessels in lymph node of patient with nHL highlighted by antibodies against CD34: **a** - × 200; **b** - × 400.

Results and discussion

The immunocytochemical reactions demonstrated a variable intensity of staining in individual tumours. CD34 showed both the membrane and cytoplasmic localisation (Fig. 1). Mean MVD in aggressive and indolent nHL groups amounted to 19.45±11.24 vessels/0.375 mm^2 and 21.7±12.4 vessels/0.375 mm^2, respectively. Statistical analysis showed no significant differences between both nHL groups.

Angiogenesis plays a key role in tumour growth, invasiveness and progression. After tumours switch from the prevascular to the vascular and invasive phase, they stimulate growth of new capillary blood vessels to guarantee an adequate supply of oxygen and nutrients to their proliferating cells. CD34, a myeloid progenitor cell antigen also present in endothelial cells, is detectable in all types of endothelium [5]. The monoclonal antibody against CD34 reacts with endothelium of arteries and venules, and has been found to stain more intensely capillary endothelium [4]. It has been used for the diagnosis of vascular tumours and detection of small vessel proliferation representing angiogenesis [17]. In this study, we used data obtained from CD34 staining, since this procedure was highly sensitive for endothelium and produced the lowest background staining.

There are some clinical observations indicating that tumour MVD and expression of vascular endothelial growth factor (VEGF) correlate with tumour staging and outcome in solid cancers and lymphoid neoplasms. High pretreatment serum VEGF concentration is associated with poor outcome in nHL patients [16]. Ribatti *et al.* reported increased capillary proliferation in the lymph node biopsies of high grade nHL. MVD has been shown to correlate with the biological behaviour in nodal B-cell nHL and the frequency of nHL tissue microvessels increases simultaneously with pathological progression [13, 14, 19]. On the other hand, according to Ridell and Norrby [15] MVD is higher in the involved lymph nodes of patients with small lymphocytic lymphoma but the number of blood vessels does not correlate with the grade of tumour. There was no correlation between tumour MVD and response to chemotherapy in patients with diffuse large B-cell lymphoma [3]. Arias *et al.* [2] found a statistically significant difference in MVD

measured by immunostaining with anti-Factor VIII antibody between low and high grade nHL, when classified in either the Working Formulation (WF) or the Kiel Classification. However, the same authors found no differences between WF of high and intermediate lymphomas.

Our data demonstrate that there is no correlation between tumour MVD revealed by CD34 staining and grade of histological malignancy in lymph nodes of nHL patients classified according to REAL classification. It must be emphasised that the degree of angiogenesis may vary between patients, over time, and in relation to the disease status of the site biopsied [20]. Nevertheless, angiogenesis observed in nHL provides rationale for the use of angiogenesis inhibitors in lymphoma therapy.

Acknowledgements: Supported by grant no. 4 PO5B 009 18 from the National Committee for Scientific Research (KBN).

References

[1] Aguayo A, Kantarjian H, Manshouri T, Gidel C, Estey E, Thomas D, Koller C, Estrov Z, O'Brien S, Keating M, Freireich E, Albitar M (2000) Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood 96: 2240-2245
[2] Arias V, Soares FA (2000) Vascular density (tumor angiogenesis) in non-Hodgkin's lymphomas and florid follicular hyperplasia: a morphometric study. Leuk Lymphoma 40: 157-166
[3] Bairey O, Zimra Y, Kaganovsky E, Shaklai M, Okon E, Rabizadeh E (2000) Microvessel density in chemosensitive and chemoresistant diffuse large B-cell lymphomas. Med Oncol 17: 314-318
[4] Fina L, Molgaard H, Robertson D, Bradley NJ, Monaghan P, Delia D, Sutherland DR, Baker MA, Greaves MF (1990) Expression of the CD34 gene in vascular endothelial cells. Blood 75: 2417-2420
[5] Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1: 27-31
[6] Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC (1994) A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 84: 1361-1392
[7] Hussong JW, Rodgers GM, Shami PJ (2000) Evidence of increased angiogenesis in patients with acute myeloid leukemia. Blood 95: 309-313
[8] Juczewska M, Chyczewska E, Naumnik W, Chyczewski L, Niklinska W, Rogalewska A, Kowalczuk O, Niklinski J (2001) Endothelial cells and angiogenesis intensity in lung cancer. Folia Histochem Cytobiol 39: 253-258
[9] Lister TA, Crowdther D (1990) Staging for Hodgkin's disease. Semin Oncol 17: 696-703
[10] Mangi MH, Newland AC (2000) Angiogenesis and angiogenic mediators in haematological malignancies. Br J Haematol 111: 43-51
[11] Miettinen M, Lindenmayer A, Chaubal A (1994) Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willibrand factor. Mod Path 7: 82-85
[12] Perez-Atayde AR, Sallan SE, Tedrow U (1997) Spectrum of tumor angiogenesis in bone marrow of children with acute lymphoblastic leukemia. Am J Pathol 150: 815-821
[13] Ribatti D, Vacca A, Marzullo A, Nico B, Ria R, Roncali L, Dammacco F (2000) Angiogenesis and mast cell density with tryptase activity increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. Int J Cancer 15: 171-175
[14] Ribatti D, Vacca A, Nico B, Fanelli L, Roncali L, Dammacco F (1996) Angiogenesis spectrum in the stroma of B cell NHL. An immunohistochemical and ultrastructure study. Eur J Haematol 56: 45-53
[15] Ridell B, Norrby K (2001) Intratumoral microvascular density in malignant lymphomas of B-cell origin. APMIS 109: 66-72
[16] Salven P, Teerenhovi L, Joensuu H (1997) A high pretreatment serum vascular endothelial growth factor concentration is associated with poor outcome in non-Hodgkin's lymphoma. Blood 90: 3167-3172
[17] Schaerer L, Schmid MH, Mueller B, Dummer RG, Burg G, Kempf W (2000) Angiogenesis in cutaneous lymphoproliferative disorders: microvessel density discriminates between cutaneous B-cell lymphomas and B-cell pseudolymphomas. Am J Dermatopathol 22: 140-143
[18] Usnarska-Zubkiewicz L, Mazur G, Wróbel T, Poręba M, Kuliczkowski K (2003) Expression of serum vascular endothelial growth factor correlates with clinical outcome in multiple myeloma (in Polish). Pol Arch Med Wewn 110: 719-724
[19] Vacca A, Ribatti D, Roncalli L, Dammacco F (1995) Angiogenesis in B cell lymphoproliferative diseases. Biological and clinical studies. Leuk Lymphoma 20: 27-38
[20] Wadleigh M, Niedzwiecki D, Davis P, Payne N, Sen F, Mann KP, Proia A, Hollis D, Rizzieri DA (2001) Increased microvessel density in non-Hodgkin's lymphoma (NHL) is localized to the site of disease changes in accordance with disease response. Blood 98: 236b
[21] Wróbel T, Mazur G, Surowiak P, Dzięgiel P, Zubkiewicz L, Jeleń M (2003) Increased expression of vascular endothelial growth factor (VEGF) in bone marrow of multiple myeloma (MM) patients. Eur J Int Med 14: 98-100

Accepted June 14, 2004