Prognostic Significance of Ki-67 Antigen Expression in Non-Hodgkin's Lymphomas

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Abstract. *Background: Non-Hodgkin's lymphomas (NHLs) are malignant tumours of the lymphoid system. Various risk factors have been described which are helpful in diagnosing, monitoring of the clinical course and predicting survival time of the patients. Proliferative activity of the tumour, measured by expression of Ki-67 antigen, is linked to the tumour proliferation rate and represents a recognised prognostic index in various tumours. In this study, the prognostic and predictive value of Ki-67 expression in NHL was evaluated. Patients and Methods: Expression of Ki-67 was examined using an immunohistochemical technique in archival paraffin-embedded sections taken from 56 previously untreated patients with diagnosed primary NHL. An attempt was made to test correlation between Ki-67 expression on one hand and clinical parameters of the patients and their survival on the other. Results: Expression of Ki-67 antigen was noted in 75% of the tumour cases. In the group manifesting higher Ki-67 indices, survival of the patients was significantly shorter (p=0.03). No significant correlation could be detected between Ki-67 antigen expression and clinical or pathological parameters of the patients. Conclusion: The results demonstrate that the most cases of NHL display the expression of Ki-67. Moreover, shortened survival was noted in patients with high expression of Ki-67.*

Despite continuous development of diagnostic and therapeutic methods, among all malignant tumours, NHLs occupy the sixth position in respect to their incidence and resulting mortality (1). NHLs form a heterogenous group of lymphoid tumours which vary in clinical pattern, dynamics of their course, response to treatment and in prognosis. Chemotherapy represents the principal means of treatment. Determination of an optimum therapeutic schedule is dependent on diagnosis of the histopathomorphological type of the tumour and on definition of prognostic indices, which modulate course of the disease and response to therapy. The prognostic parameters include variables related to individual patients (age, gender), those related to biological characteristics of the lymphoma (stage of advancement, mitotic activity, immunological markers and cytogenetic aberrations in lymphoma cells) and also variables dependent on the patient's capacity to adjust to the neoplastic disease (efficiency, anaemia, plasma albumin level) (2). The indices allow the evaluation of the relative severity of the clinical course in NHL but are frequently insufficient to effectively monitor the clinical course and to provide prognosis as to the duration of survival of the patients.

Studies in recent years stress the prognostic significance of the evaluation of tumour biology and, linked to it, processes of cell proliferation. The proliferative activity of the tumour is related to its growth rate, providing a recognised prognostic index, related to survival of patients with various types of tumours (3-7). The proliferative activity of a tumour can be examined using *e.g.* flow cytometry, autoradiography and evaluation of nucleolar organizer region. At present, immunohistochemical (IHC) methods represent an effective approach, detecting antigens typical for cells in the cell cycle (*e.g.* Ki-67, PCNA, Mcm-2) (8, 9).

Ki-67 antigen represents a non-histone nuclear antigen present only in proliferating cells. Its increased expression was noted in terminal stages of the S-phase in the cell cycle, in phases G_1-G_2 and in mitosis (10). No Ki-67 expression was noted at the G_0-phase. The antigen was found to be expressed both in normal and in neoplastic cells. In recent years, a number of reports have been published on the application of Ki-67 antigen in oncology (11). The results have demonstrated prognostic significance of the protein

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Key Words: Ki-67 antigen, non-Hodgkin's lymphoma, prognosis.

Table I. *Ki-67 and clinical characteristics of patients with non-Hodgkin's lymphoma.*

Characteristic	No.	Ki-67 expression [n (%)]		*P*-value
		Low (score 0-2) n=29	High (score 3) n=24	
All cases	53 (100)			
Age (years, mean±SD)	56.36±13.19			
≤60	28	13(27)	15(31)	0.527[b]
>60	21	12(24)	9(18)	
Gender				
Female	22	11(22)	11(22)	0.512[a]
Male	27	14(29)	13(27)	
Ann Arbor stage				
I/II	7	2(5)	5(12)	0.366[a]
III/IV	36	20(46)	16(37)	
Performance status				
≥2	14	7(30)	7(30)	0.382[a]
<2	9	4(17)	5(22)	
Tumour grade				
Indolent	22	15(31)	7(15)	0.072[a]
Aggressive	25	10(21)	15(31)	
Very aggressive	1	0	1(2)	
B symptoms				
Yes	28	12(29)	16(38)	0.507[a]
No	14	9(21)	5(12)	
IPI score				
Low	22	12(28)	10(24)	0.759[a]
Medium/low	6	3(7)	3(7)	
Medium/high	7	3(7)	4(10)	
High	7	2(5)	5(12)	
Extranodal involvement				
≤1	22	18(40)	4(9)	0.948[a]
>1	23	20(44)	3(7)	
BM/CNS involvement				
Yes	16	9(20)	7(16)	0.463[a]
No	29	13(29)	16(35)	
LDH level (before Ch-th)				
≤Normal	15	8(35)	7(30)	0.210[b]
>Normal	8	4(17)	4(17)	
LDH level (after Ch-th)				
≤Normal	13	6(24)	7(28)	0.420[b]
>Normal	12	5(20)	7(28)	
β_2– Microglobulin* level				
≤Normal	3	1(11)	2(22)	0.656[b]
>Normal	6	3(33)	3(33)	
Anaemia				
Yes	16	5(11)	11(25)	0.104[a]
No	28	17(39)	11(25)	
Infection with HCV				
Yes	2	1(2)	1(2)	0.698[a]
No	46	24(50)	22(46)	
Infection with *H. pylori*				
Yes	2	1(2)	1(2)	0.678[a]
No	46	24(50)	22(46)	
Clinical response				
CR	10	3(10)	7(23)	0.409[a]
PR	15	10(33)	5(17)	
SD	4	2(7)	2(7)	
PD	1	0	1(3)	
Relapse				
Yes	7	5(11)	2(5)	0.765[a]
No	37	16(36)	21(48)	
Progression				
Yes	26	11(25)	15(34)	0.525[a]
No	18	10(23)	8(18)	
Death				
Yes	22	9(20)	13(30)	0.591[a]
No	22	12(27)	10(23)	

CR: Complete response, PR: partial response, SD: stable disease, PD: progressive disease; normal level of LDH: 200-480 U/L, of β_2–Microglobulin: 0.7-1.8 mg/l; [a]ANOVA rank test of Kruskal-Wallis; [b]rank correlation according to Spearman.

expression in various human malignant tumours, *e.g.* in multiple myeloma, soft tissue tumours, prostate tumours and in mammary cancer (12, 13).

In this study we attempted to correlate expression of Ki-67 antigen with clinical and pathological variables in primary NHL and to define the prognostic value of the antigen in the prediction of survival time and relapse-free time in NHL patients.

Patients and Methods

Specimens. The IHC studies were conducted on retrospective samples of tissues sent for routine diagnostic studies to the Chair and Department of Pathological Anatomy in Wrocław, in 1994-2003, isolated from patients treated at the Chair and Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, University School of Medicine in Wroclaw, Poland. A group of 56 earlier untreated patients with NHL qualified to participate in the studies (Table I); this group included 52 cases of lymph node tumours and four cases of extranodular tumours. In 49 cases, B-cell tumours and in 5 cases T-cell tumours were diagnosed. In the patients, tumour clinical advancement was defined according to the Ann Arbor classification (14). Twenty-six cases (14 women and 12 men) were found to represent a low degree of malignancy, while 27 cases (9 women and 18 men) carried an aggressive (in one case highly aggressive) lymphoma. All the patients were subjected to chemotherapy and/or radiotherapy and were then monitored using periodic control examinations. During the period of observation (102 months), 7 patients (14%) developed a relapse, 31 patients (62%) manifested progression and 25 patients (50%) died. The

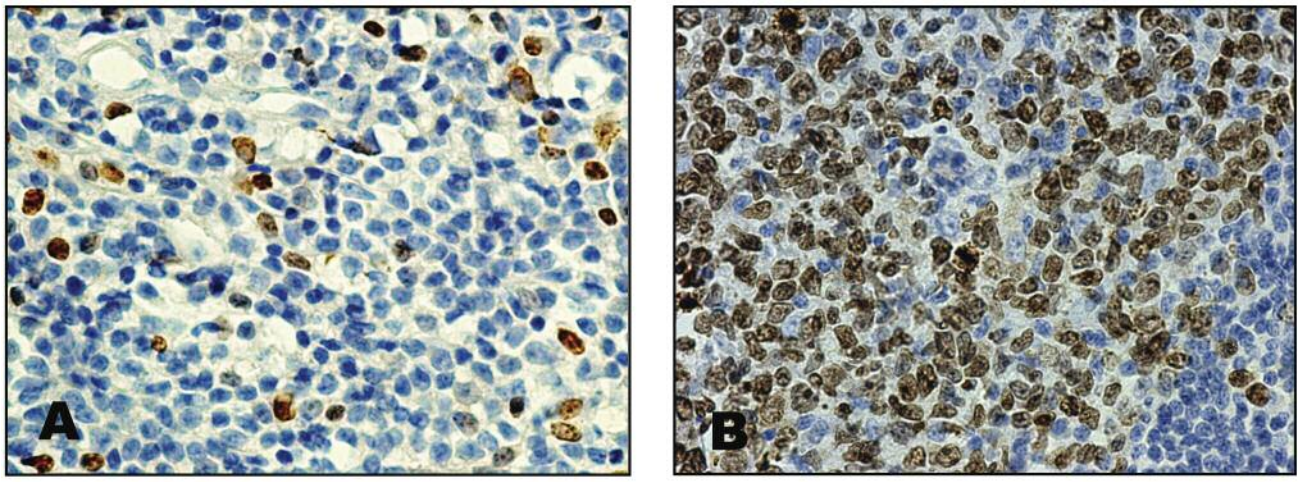

Figure 1. *Immunohistochemical localization of Ki-67 expression in non-Hodgkin's lymphoma. A: Faint nuclear reaction; B: Strong nuclear reaction. Magnification x400.*

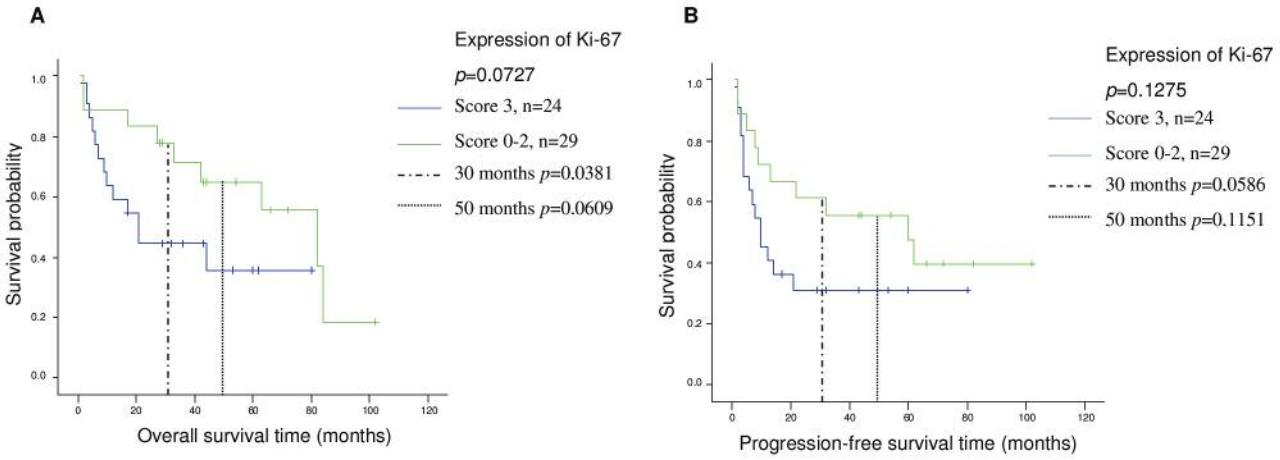

Figure 2. *Survival curves according to Kaplan-Meier in the studied group of non-Hodgkin's lymphomas with lower or higher expression of Ki-67: (A) expression of Ki-67 and survival time, (B) expression of Ki-67 and progression-free time.*

mean progression-free survival amounted to 32 months and the mean total survival time was 40 months. In line with the International Prognostic Index (IPI) (15), patient's age, advancement of the tumour, serum lactic dehydrogenase (LDH) level, efficiency and number of extranodular sites involved by the disease were taken into account to divide the patients into four risk groups: low, moderately low, moderately high and high risk.

Immunohistochemistry. Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. In each case, hematoxylin and eosin-stained preparations were subjected to histopathological evaluation by two pathologists. Formalin-fixed, paraffin-embedded tissue was freshly cut (4 μm). The paraffin sections were mounted on Superfrost slides (Menzel Gläser, Germany), dewaxed with xylene and gradually rehydrated. The IHC reactions were performed on paraffin sections, using antibodies and reagents originated from Dako (Copenhagen, Denmark). Activity of endogenous peroxidase was blocked by 30 min exposure to 1% H_2O_2. All the studied sections were boiled in Target Retrieval Solution for 20 min at 500W in a microwave oven. IHC reactions were performed using monoclonal mouse antibody against Ki-67 (clone MIB1 diluted 1:50). The antibody was diluted in antibody

Table II. *Evaluation scale for appraising frequency of cells with expression of Ki-67.*

Percentage of cells with colour reaction	Score
<5%	0
5-15%	1
16-30%	2
>30%	3

diluent with the background reducing component. Tested sections were incubated with antibody against Ki-67 overnight at a temperature of 4˚C. The investigated antigen was visualized using biotinylated antibodies, streptavidin-biotinylated peroxidase complex (LSAB+ kit) and diaminobenzidine (DAB). All the sections were counterstained with Meyer's hematoxylin (20 second). Every test was accompanied by a negative control in which specific anti-Ki-67 antibody was substituted by PBS (Phosphate Buffered Saline).

Evaluation of the intensity of IHC reaction. The intensity of Ki-67 antigen expression was conducted using a scale which took into account the percentage of cells manifesting nuclear colour reactions: less than 5% - 0 points, 5-15% - 1 point, 16-30% - 2 points and more than 30% - 3 points (Table II). Percentage of positive cells was evaluated scoring the brownish-labeled cell nuclei detected after screening of all cell nuclei under x400 magnification (Olympus BX 41 light microscope with the visual mode AnalySis 3.2 software for computer-assisted image analysis). The intensity of the IHC reactions was independently evaluated in coded preparations by two pathologists; in doubtful cases a re-evaluation was performed using a double-headed microscope and staining was discussed until a consensus was achieved. The examining pathologists knew no clinical details related to the respective patient.

Statistical analysis. The results were subjected to statistical analysis using Statistica 97 PL software (Statsoft, Poland). The relationship between the expression of Ki-67 antigen and age of patients and serum protein levels (LDH, β-microglobulin) was examined using Spearman's rank correlation. The relationship between intensity of Ki-67 expression and clinical and pathological data was examined using ANOVA rank test of Kruskal-Wallis. Analysis of the relationship between protein expression and overall survival time and progression-free time took advantage of Kaplan-Meier's analysis using SPSS, version 10.0 software (SPSS Inc., Chicago, IL, USA). Differences were accepted as significant at $p<0.05$.

Results

Expression of Ki-67 antigen in NHL. IHC reactions were performed in the group of 56 cases of NHL. In evaluation of protein expression, 3 cases were rejected due to significant damage of the studied material.

Positive Ki-67 reaction was demonstrated in 40 cases (75%). It developed in the cell nuclei (Figure 1) and the mean expression of the protein on the evaluation scale of 0 to 3 was 2.04±1.15 SD.

Relationship between Ki-67 antigen expression and clinicopathological data. In our studies a hypothetical relationship was tested between Ki-67 expression and clinicopathological variables in the patients. All the clinicopathological variables qualified for the analysis are listed in Table I. No significant relationships were demonstrated between Ki-67 expression and the listed variables ($p>0.05$; Table I).

Expression of Ki-67 antigen and overall survival and relapse-free survival. Survival time was diagnosed as the time between diagnosis and a patient's death, while progression-free survival was defined as the time period between diagnosis and progression or relapse. The survival time and the progression-free survival time proved to be comparable between groups differing in intensity of Ki-67 antigen expression. Using the three-degree scale for evaluation of the frequency of immunopositive cells, two groups were distinguished: a group with the highest degree of intensity in Ki-67 antigen expression (valued at 3) and a group with a lower degree of Ki-67 antigen expression (valued at 0 to 2). No relationship was demonstrated between the expression of the studied antigen and overall survival or progression-free survival of NHL patients over the entire period of observation (102 months) ($p>0.05$; Figure 2A, B). Considering the fact that the studied group of patients were subjected to intense chemotherapy which could significantly affect the biology of the tumour cells, the relationship between Ki-67 expression and survival were examined in short-term follow-up. The analysis was conducted with the observations considered up to 30 and 50 months. The Kaplan-Meier analysis demonstrated that the overall survival time was significantly shorter in the group with higher expression of Ki-67 in the course of 30 months observation ($p=0.0381$; Figure 2A). No effect of Ki-67 expression could be demonstrated on survival over 50 months observation ($p>0.05$; Figure 2A) or on progression-free survival time over either 30 months or 50 months observation ($p>0.05$; Figure 2B).

Discussion

The study aimed at the examination of the prognostic and predictive significance of Ki-67 expression in malignant non-Hodgkin's lymphomas. Ki-67 undergoes expression exclusively in proliferating cells. Synthesized at the beginning of the cell proliferation process, it is indispensable for cell division and it is effectively degraded at the end of the proliferative cycle. Nevertheless, the specific function of the protein remains unknown. IHC reaction with anti-Ki-67 allows proliferating cells to be distinguished from cells in the G_0-phase of the cell cycle. Demonstration of Ki-67 expression in tumour cells followed by calculation of the proportion of immunopositive cells allows the determination of the proliferative activity of the tumour. The Ki-67 index permits the growth fraction in the tumour to be determined and for years has focused attention of investigators. Among the numerous reports, papers which document the prognostic value of the protein expression in various human malignant tumours constitute a significant share, although the results are not always unequivocal (3-6, 9, 16).

In our experiments, we demonstrated a high level of Ki-67 expression in the NHL cases studied. However, no significant relationship was shown between Ki-67 expression on one hand and clinical or pathological variables on the other. Abbreviation of the observation to 30 and 50 months permitted lesions accompanying the long-term therapy to be eliminated. Thirty months observation showed that survival in the group with lower expression of Ki-67 was extended as compared to the group with more pronounced Ki-67 expression. Similar results have been obtained by Tiemann *et al*. (17), who have shown that in patients with diagnosed mantle zone lymphoma, high proliferation index has correlated with shorter survival time. Martin *et al*. (18), examining follicular lymphomas, also found that a lower proliferative index was associated with longer survival time even though no prognostic value as related to relapse-free survival time was found.

Both the original studies from the early 1990 and later ones have indicated a relationship between expression levels of Ki-67 on one hand and the histological type of the tumour, which affects the degree of malignancy, and survival time in NHL on the other (3-6, 19-23). In line with other clinical parameters, the Ki-67 antigen has been recognised as a marker useful in establishing biological prognosis of NHL development. However, in studies on primary central nervous system lymphomas (PCNSL) determination of tumour proliferative activity seems to carry just marginal significance. In the study of Aho *et al*. (16), the Ki-67 index failed to correlate with tumour histological type and duration of survival. Studies of Roser *et al*. (24) have confirmed that most PCNSL manifest less advantageous response to treatment independently of their proliferative activity and degree of malignancy. The apoptotic index has shown an inverse correlation with the proliferative index, corresponding to a more aggressive clinical course of the disease. Other studies on, for example malignant tumours of soft tissues, mammary gland carcinoma, lung cancer, brain tumours and melanoma, have shown that a high Ki-67 index has been associated with large size of the tumour, a high degree of malignancy, an aggressive course of the disease, worse response to therapy and shorter survival (3, 25-28). Similar results have been obtained by Scholzen and Gerdes (12), who confirmed the prognostic value of Ki-67 expression in predicting survival in multiple myeloma, soft tissue sarcoma and mammary gland carcinoma. On the other hand, no relationship between Ki-67 proliferative marker expression and the clinical course of a disease, a lack of its prognostic value, as compared to other prognostic markers, were demonstrated in studies on astrocytoma and primary ovarian cancer (29, 30). In turn, in cancer of the uterine cervix and prostate cancer, expression of Ki-67 has been found to be less important for predicting the course of the disease (11, 13). Similarly, Nylander *et al*. (31) negated the significance of the Ki-67 index for prognosis in squamous cancer of the head and neck, while Spafford *et al*. (32) reproduced the result in patients with laryngeal cancer. On the other hand, Welkoborsky *et al*. (33) found that in laryngeal cancer, the Ki-67 index carries a prognostic value as to the duration of the tumour-free time and expression of PCNA antigen allows prediction of overall survival time of the patients.

In conclusion, expression of Ki-67 was demonstrated here in most cases of NHL. No relationship was established between the expression of Ki-67 antigen and clinical or pathological variables in the NHL group. At 30 months observation, a lower expression of Ki-67 antigen was linked to longer survival time. The evaluation of proliferative activity using the Ki-67 index allows a group of NHL patients with a predicted survival time to be distinguished.

Acknowledgements

The study was supported by the grant No. 2 PO5B 058 28 from the Committee of Scientific Investigations, Poland.

References

1 Skarin AT and Dorfman DM: Non-Hodgkin's lymphomas: current classification and management. CA-Cancer J Clinicians *47*: 351-372, 1997.

2 Shipp MA, Harrington DP, Anderson JR and Armitage JO: A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med *329*: 987, 1993.

3 Gerdes J: Ki-67 and other proliferation markers useful for immunohistologocal diagnostic and prognostic evaluations in human malignancies. Semin Cancer Biol *1*: 199-206, 1990.

4 Hall PA, Richards MA, Gregory WM, d'Ardenne AJ, Lister TA and Stansfeld AG: The prognostic value of Ki-67 immunostaining in non-Hodgkin's lymphoma. J Pathol *154*: 223-235, 1998.

5 Velders GA, Kluin-Nelemans JC, De Boer CJ, Hermans J, Noodijk EM, Schuuring E, Kramer Mh, Van Deijk WA, Rahder JB, Kluin PM and Van Krieken JH: Mantle-cell lymphoma: a population-based clinical study. J Clin Oncol *14(4)*: 1269-1274, 1996.

6 Kologeraki A, Tzardi M, Panagiotides I, Koutsoubi K, Bolioti S, Rontogianni D, Stefanaki K, Zois E, Karidi E, Darivianaki K, Delides G and Kanavaros P: MIB-1 (Ki-67) expression in non-Hodgkin's lymphomas. Anticancer Res *17(1A)*: 487-491, 1997.

7 Pastuszewski W, Dziegiel P, Krecicki T, Podhorska-Okolow M, Ciesielska U, Gorzynska E and Zabel M: Prognostic significance of metallothionein, p53 protein and Ki-67 antigen expression in larygeal cancer. Anticancer Res *27*: 335-342, 2007.

8 Gryczynski M and Pastuszewska W: Selected aspects of apoptosis and cell proliferation in laryngeal carcinoma. Otolaryngologia *1*: 151-160, 2002 (in Polish).

9 Szelachowska J, Dziegiel P, Jelen-Krzeszewska J, Jelen M, Matkowski R, Pomiecko A, Spytkowska B, Jagas M, Gisterek I and Kornafel J: Mcm-2 protein expression predicts prognostic better than Ki-67 antigen in oral squamocellular carcinoma. Anticancer Res *26*: 2473-2478, 2006.

10 Dzięgiel P and Zabel M: Role of immunohistochemical expression of Ki-67 in adenocarcinoma of large intestine. *In*: Immunohistochemistry and *In Situ* Hybridization of Human Carcinomas. Hayat MA (eds.). London, Elsevier Academic Press, pp. 127-134, 2006.
11 Brown DC and Gatter KC: Ki-67 protein: the immaculate deception? Histopathology *40*: 2-11, 2002.
12 Scholzen T and Gerdes J: The Ki-67 protein: from the known and the unknown. J Cell Physiol *182*: 311-332, 2000.
13 Dziegiel P, Salwa-Zurawska W, Zurawski J, Wojnar A and Zabel M: Prognostic significance of augmented metallothionein (MT) expression corellated with Ki-67 antigen expression in selected soft tissue sarcomas. Histol Histopathol *20*: 83-89, 2005.
14 Hennessy BT, Hanrahan EO and Daly PA: Non-Hodgkin Lymphoma: an update. Lancet Onkol *5*: 341-353, 2004.
15 Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred C, Clarc GM, Ruby SG, O'Malley F, Simpson JF, Connolly JL, Hayes DF, Edge SB, Lichter A and Schnitt SJ: Prognostic factors in breast cancer. Arch Pathol Lab Med *124*: 7, 2000.
16 Aho R, Haapasalo H, Alanen K, Haltia M and Kalimo H: Proliferative activity and DNA index do not significantly predict survival in primary central nervous system lymphoma. J Neuropathol Exp Neurol *54(6)*: 826-832, 1995.
17 Tiemann M, Schrader C, Klapper W, Dreyling MH, Campo E, Norton A, Berger F, Kluin P, Ott G, Pileri S, Pedrinis E, Feller AC, Merz H, Janssen D, Hansmann ML, Krieken H, Moller P, Stein H, Unterhalt M, Hiddemann W and Parwaresch R: Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL Network. Br J Haematol *131*: 29-38, 2005.
18 Martin AR, Weisenburger DD, Chan WC, Ruby EI, Anderson JR, Vose JM, Bierman PJ, Bast MA, Daley DT and Armitage JO: Prognostic value of cellular proliferation and histologic grade in follicular lymphoma. Blood *85*: 3671-3678, 1995.
19 Gerdes J, Schwab U, Lemke H and Stein H: Production of mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer *31*: 13-20, 1983.
20 Gerdes J, Dallenbach F, Lennert K, Lemke H and Stein H: Growth fractions in malignant non-Hodgkin's lymphomas (NHL) as determined *in situ* with the monoclonal antibody Ki-67. Hematol Oncol *2*: 365-371, 1984.
21 Itami M, Takenouchi T, Tamatru J, Harigaya K and Mikata A: Expression of functional molecules in non-Hodgkin's lymphoma. Correlation with bone morrow involvement and serum LDH value. Acta Pathol Jpn *41(4)*: 277-285, 1991.
22 Tominaga k, Yamaguchi Y, Nozawa Y, Abe M and Wakasa H: Proliferation in non-Hodgkin's lymphomas as determined by immunohistochemical double staining for Ki-67. Hematol Oncol *10(3-4)*: 163-169, 1992.
23 Xu W, Sheng R, Zheng Z and Xu T: The expression and clinical significance of proliferative antigen Ki-67 and apoptosis-antagonizing antigen Bcl-2 in non-Hodgkin's lymphoma Zhonghua Nei Ke Za Zhi *40(7)*: 452-455, 2001 (in Chinese).
24 Roser F, Saini M, Meliss R, Ostertag H, Samii M and Bellinzona M: Apoptosis, vascularity, and proliferation in primary central nervous system lymphomas (PCNSL): a histopathological study. Surg Neurol *62(5)*: 393-399, 2004.
25 Brown DC and Gatter KC: Monoclonal antibody Ki-67: its use in histopathology. Histopathology *17*: 489-503, 1990.
26 Pence J, Kerns B and Dodge R: Prognostic significance of the proliferation index in surgically resected non-small cell lung cancer. Arch Surg *128*: 1382-1390, 1993.
27 Pugsley JM, Schmidt RA and Vesselle H: The Ki-67 index and survival in non-small cell lung cancer: a review and relevance to positron emission tomography. Cancer *8*: 222-233, 2002.
28 Haerslev T, Jacobson GK and Zedeler K: Correlation of growth fraction by Ki-67 and proliferating cell nuclear antigen (PCNA) immunohistochemistry with histopathological parameters and prognosis in primary breast carcinomas. Breast Cancer Res Treat *37*: 101-113, 1996.
29 Nakamura M, Konishi M, Tsunoda S, Nakase H, Tsuzuki T, Aoki H, Sakitani H, Inui T and Sakaki T: Analysis of prognostic and survival factors related to treatment of low-grade astrocytomas in adults. Oncology *58*: 108-116, 2000.
30 Reitmaier M, Rudlowski C, Biesterfeld S, Rath W and Schroder W: Comparative studies on the biological significance of the marker for proliferation Ki-67-antigen and PCNA in primary ovarian carcinoma. Zentralbl Gynakol *122*: 361-7, 2000 (in German).
31 Nylander K, Stenling R, Gustafsson R, Zackrisson B and Roos G: p53 Expression and cell proliferation in squamous cell carcinoma of the head and neck. Cancer *75*: 87-93, 1995.
32 Spafford MF, Koeppe J, Pan Z, Archer PG, Meyers AD and Franklin WA: Correlation of tumour markers p53, bcl-2, CD34, CD44H, CD44v6 and Ki-67 with survival and metastasis in laryngeal squamous carcinoma. Arch Otolaryngol Head Neck Surg *122*: 644-648, 1996.
33 Welkoborsky HJ, Hinni M, Dienes HP and Mann WJ: Predicting recurrence and survival in patients with laryngeal cancer by means of DNA cytometry, tumour front grading, and proliferation markers. Ann Otol Rhinol Laryngol *104*: 503-510, 1995.

Received October 16, 2007
Revised January 11, 2008
Accepted February 5, 2008