Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jobcr

Original Article

Clinicopathological correlation of Bcl-2 oncoprotein expression in oral precancer and cancer

Vandana Arya^{a,*}, Subash Singh^b, M. Jonathan Daniel^c^a Assistant Professor, Department of Oral Medicine & Radiology, KGMU, Lucknow, India^b Reader, Department of Pedodontics, BBD CODS, Lucknow, India^c Prof & Head, Department of Oral Medicine & Radiology, MGPGI, Puducherry, India

ARTICLE INFO

Article history:

Received 2 September 2015

Accepted 28 December 2015

Available online 19 January 2016

Keywords:

Oncoprotein

Bcl-2

Oral precancer

Oral cancer

Immunohistochemistry

ABSTRACT

Oral squamous cell carcinoma is the most common malignant tumor of the oral cavity. Normally the death of cell and the growth are active processes and depend not only on external factors but also on the expression of genes such as Bcl-2, which activate and inhibit apoptosis. The term Bcl-2 is an acronym for B-cell lymphoma/leukemia-2 genes. It has been reported that there is deregulation of Bcl-2 expression during progression from oral epithelial dysplasia to squamous cell carcinoma.

Expression of this oncoprotein can be detected by immunohistochemistry.

Aims and objectives: An attempt was made to evaluate Bcl-2 oncoprotein expression in patients with oral precancer and cancer.

Materials and methods: A selective prospective clinical and immunohistochemical study. Clinicopathological examination was correlated with immunohistochemical findings. The immunolocalization of Bcl-2 protein was performed using the labeled streptavidin biotin method. To visualize the reaction, 3,3-diaminobenzidine was used.

Results: Bcl-2 expression was positive in 11 [36.66%, low Bcl-2 expression 3 (10.00%), moderate Bcl-2 expression 7 (23.33%), and high Bcl-2 expression 1 (3.33%)] oral cancer cases and 14 [87.50%, low expression 8 (50%), moderate expression 6 (37.50%)] precancer cases.

Conclusion: On the basis of the results of our study, we conclude that positive Bcl-2 expression may be an indicator of poor prognosis in oral cancer and precancer.

© 2016 Craniofacial Research Foundation. Published by Elsevier B.V. All rights reserved.

1. Introduction

Oral squamous cell carcinoma is the most common malignant tumor of oral cavity. The malignancy is the result of disordered mechanisms that control cell growth and mitosis. Normally the death of cell and the growth are active processes and

depend not only on external factors but also on the expression of genes such as Bcl-2, which can activate and inhibit apoptosis.^{1–3}

Numerous studies have revealed a correlation between the expression of p53 and Bcl-2 proteins and the progression of neoplastic changes.⁴ It cannot be excluded that these proteins act as biomarkers of a threatening neoplastic transformation

* Corresponding author.

E-mail address: vasu22georgian@rediffmail.com (V. Arya).

<http://dx.doi.org/10.1016/j.jobcr.2015.12.011>

2212-4268/© 2016 Craniofacial Research Foundation. Published by Elsevier B.V. All rights reserved.

in precancerous states including leukoplakia. It has been reported that there is deregulation of Bcl-2 expression during progression from oral epithelial dysplasia to squamous cell carcinoma.⁵

The term Bcl-2 is an acronym for B-cell lymphoma/leukemia-2 genes and the gene is the primary regulator of apoptosis.^{6,7} Bcl-2 inhibits most kinds of programmed cell death and provides a selective survival advantage to various cell types.^{8,9}

It was first identified as the site of reciprocal translocation of chromosome 18 in follicular lymphoma and encodes a membrane associated protein that is present in the endoplasmic reticulum, nuclear and outer mitochondrial membrane.¹⁰ Normally, Bcl-2 proteins are found only in basal or proliferating cells.^{6,11} The structural abnormality (translocation t [14; 18]) of the Bcl-2 gene is confirmed to one neoplasm-follicular lymphoma; the deregulation mechanism in other neoplasms is largely unknown.⁶

Enhanced expression of Bcl-2 oncoprotein in various human neoplasms, suggests a role for this oncoprotein in the pathogenesis of neoplasia. Though for squamous cell carcinomas of head and neck, as a group, poor treatment outcome and shorter overall survival have been associated with Bcl-2 over expression in the tumor, irrespective of the treatment modality^{12,13} the biological significance of Bcl-2 expression for the development and progression of oral cancer is still to be evaluated.¹

Immunohistochemistry or IHC is the process of localizing proteins in cells of a tissue section exploiting the principle of antibodies binding specifically to antigens in biological tissues. Immunohistochemical staining is widely used in the diagnosis of abnormal cells and distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. This has made it a widely-used technique enabling researchers to examine protein expression within specific tissues.

While current methods of tumor classification are still based on the traditional concepts of histogenesis and differentiation, we need to re-evaluate these concepts in the light of pertinent immunomarker studies.

With this background an attempt was made to evaluate Bcl-2 oncoprotein expression in patients with oral precancer and cancer.

2. Aims and objectives

- To evaluate Bcl-2 oncoprotein expression in patients with oral precancer and cancer.
- To assess possible correlation between Bcl-2 oncoprotein expression and clinicopathological features of oral precancer and cancer.
- To validate its role as a prognostic marker in oral precancer and cancer.

3. Materials and methods

A selective prospective cross sectional clinical and immunohistochemical study was done in the Department of Oral Medicine and Radiology.

The patients 46 [30 oral Squamous cell carcinoma and 16 precancer] clinically diagnosed and histopathologically proven to have oral squamous cell carcinoma/precancer (leukoplakia) were examined and evaluated for the expression of the Bcl-2 oncoprotein by immunohistochemical staining.

The study was approved by the research and ethical committee of the institute. Informed consent was obtained from all patients, who participated in the study.

4. Inclusion criteria

Individuals with clinical evidence and histopathologically proven oral precancer (dysplasia) and cancer were included in the study.

5. Exclusion criteria

- Individuals with known systemic disease and other skin disease.
- Individuals who had undergone previous radiotherapy, surgery or chemotherapy.

6. Methodology

6.1. Clinical examination

Thorough clinical examination was done according to a specially devised Proforma. For squamous cell carcinoma patients, TNM staging and Cancer stage grouping were done according to American Joint Committee on Cancer (AJCC) 2003.

6.2. Histopathological examination

Incisional/excisional biopsy was done for histopathologic confirmation of dysplasia and/or neoplasia. Tissue blocks were collected immediately after tumor/lesion removal, fixed in 10% buffered formaldehyde solution and embedded in paraffin at 56 °C. The tissue sections were stained with HE, and histopathological examination was done by an oral pathologist.

Histopathologically, the tissue sections of OSCC/epithelial dysplasia were graded into three classes:

OSCC

- Well differentiated OSCC
- Moderately differentiated OSCC
- Poorly differentiated OSCC

Epithelial dysplasia

- Mild dysplasia
- Moderate dysplasia
- Severe dysplasia

6.3. Immunohistochemical procedure for Bcl-2 expression

The slides were stained immunohistochemically with the antihuman Bcl-2 antibody (DAKO/Bcl-2, No M0887). The

immunolocalization of Bcl-2 protein was performed using the labeled streptavidin biotin method. To visualize the reaction, 3,3-diaminobenzidine was used.

Tissue blocks of the selected cases were sectioned (4 μ m) by automated microtome and fixed on silane coated glass slides at 65 °C and subjected to immunohistochemical staining.

Bcl-2 expression was evaluated semiquantitatively (at least 100 neoplastic/dysplastic cells per sample) using a light microscope (40 $\times$ magnification) and graded as follows:

Grade 0	0–10%	Negative expression
Grade 1	10–30%	Low expression
Grade 2	30–60%	Moderate expression
Grade 3	>60%	High expression

The correlations between cancer/precancer Bcl-2 expression and patient age, gender, tumor site, tumor staging, tumor/lesion morphology and histological grading, were subjected to statistical analysis.

6.4. Data analysis

- Paired t-test was performed to evaluate Intra-Rater and Inter-Rater Reliability for Bcl-2 expression in % and to compare the Bcl-2 expression (in %) between Cancer and Pre-Cancer Groups.
- Kappa coefficient was used for Intra-Rater Reliability for Bcl-2 expression in grade values.
- Chi-square test was performed to (A) compare the Bcl-2 expression in grade between Cancer and Pre-Cancer Groups, (B) compare Bcl-2 expression with negative and positive values between Cancer and Pre-Cancer and (C) calculate correlation between Bcl-2 expression and clinicopathologic features of oral cancer and precancer.

7. Results

The study group comprised of 30 Oral Cancer patients, 16 Oral Precancer patients. Age of the study group patients ranged from 28 to 80 years with a mean of 61 years. Bcl-2 expression was evaluated by two observers, observer A and observer B.

Fig. 1 – Bcl-2 expression in oral cancer: photomicrograph low power (40 $\times$).

Bcl-2 expression was positive in 11 [36.66%, low, 3 (10.00%), moderate 7 (23.33%), and high 1 (3.33%)] oral cancer cases and 14 [87.50%, low expression 8 (50%), moderate expression 6 (37.50%)] precancer cases. There was a statistically significant difference in the Bcl-2 expression between oral cancer and precancer with p value of <0.01 (Table 1, Fig. 1).

Other findings are shown in the following tables (Tables 2 and 3).

Intra-Rater Reliability & Inter-Rater Reliability for Bcl-2 expression in percentage was significant.

8. Discussion

In India, oral cancer is the leading cancer among men and within the first five cancers of women, with an incidence rate of 12% of all cancers in men and 8% of all cancers among women.¹⁴

Clinical and pathological parameters alone are not sufficient to determine the biological characteristics of the tumor. The identification of molecular markers provides an insight into the potential behavior or aggressiveness of tumors. There are many important regulatory molecules

Table 1 – Bcl-2 expression (percentage and graded values) in oral cancer and precancer.

Groups	Bcl-2 expression				Chi-square	p-Level
	Negative	Positive				
		Grades				
		Low	Moderate	High		
Oral cancer	19 (63.33)	3 (10.00)	7 (23.33)	1 (3.33)	14.16	<0.01
Precancer	2 (12.50)	8 (50.00)	6 (37.50)	0 (0.00)		

Figures in brackets are in percentage; $p < 0.01$ – significant at 1% level.

Table 2 – Correlation between Bcl-2 expression and clinicopathologic features in oral cancer.

Features		Negative		Positive		Chi-square	p-Value
		N	%	N	%		
Gender	Male	14	63.6	8	36.4	0.003	NS
	Female	5	62.5	3	37.5		
Age	≤60 years	12	70.6	5	29.4	0.889	NS
	>60 years	7	53.9	6	46.2		
Lesion site	Buccal Mucosa	9	81.8	2	18.2	6.27	NS
	Tongue	5	62.5	3	37.5		
	Alveolus	2	33.3	4	66.7		
	Floor of mouth	1	33.3	2	66.7		
Lesion morphology	Palate	2	100.0	0	0.0	5.44 ^a	<0.05
	Proliferative	4	36.4	7	63.6		
	Ulcerative	15	79.0	4	21.1		
Staging	I	0	0.0	2	100.0	5.84	NS
	II	3	100.0	0	0.0		
	III	8	72.7	3	27.3		
	IV	8	57.1	6	42.9		
Histopathology	WDSCC	17	85.0	3	15.0	12.87 ^a	<0.01
	Poor	0	0.0	3	100.0		
	Moderate	2	28.6	5	71.4		

^a NS: not significant.**Table 3 – Correlation between Bcl-2 expression and clinicopathologic features in oral precancer.**

Features		Negative		Positive		Chi-square	p-Value
		N	%	N	%		
Gender	Male	2	14.3	12	85.7	0.327	NS
	Female	0	0.0	2	100.0		
Age	≤60 years	2	14.3	12	85.7	0.327	NS
	>60 years	0	0.0	2	100.0		
Lesion site	Buccal Mucosa	2	12.5	14	87.5	Not applicable	NS
	Tongue	0	0.0	0	0.0		
	Alveolus	0	0.0	0	0.0		
	Floor of mouth	0	0.0	0	0.0		
	Palate	0	0.0	0	0.0		
	Homogenous	0	0.0	8	100.0		
Lesion morphology	Speckled	2	25.0	6	75.0	2.286	NS
	Moderate	1	11.1	8	88.9		
Histopathology	Mild	1	14.3	6	85.7	0.036	NS

*NS: not significant.

involved in the control of cellular apoptosis, and development of cancer.¹⁵ One critically important group of apoptosis-regulatory proteins is the Bcl-2 family, which is comprised of at least 15 proteins with either anti-apoptotic (Bcl-2-X_L) or pro-apoptotic effects (Bax, Bak). Bcl-2 protein product seems to be one of the most promising members of molecular markers to evaluate cancer malignant behavior (Yao et al.).¹⁶

Bcl-2 over-expression has been observed in multiple tumor types, including cancers of the lung, colon, and breast (Pezzella et al., Silvestrini et al., Sinicrope et al.).^{17–19} Increased expression of either Bcl-2 or Bcl-X_L has been detected in oral dysplastic lesions and oral squamous cell carcinoma, as well as in carcinogen-induced papillomas in a mouse model (Popović et al.).²⁰ The expression of Bcl-2 has been correlated with prognosis of the tumor. In oral cancer, however, a clear relationship between Bcl-2 over expression and poor prognosis is yet to be established.

The purpose of the present study was to assess and correlate clinicopathological features with the Bcl-2 expression in oral cancer and precancer and to determine whether its expression has any prognostic significance (Fig. 2).

In normal epithelium Bcl-2 expression is very infrequent and seen in the basal layer only. It is mainly localized in the cytoplasm. In our study, in dysplasia cases Bcl-2 expression was found in basal as well as parabasal layer as grainy and yellowish brown staining in the cytoplasm. In oral cancer cases the staining was present in the central and peripheral area of the tumor. Patielli et al.¹¹ also observed the same pattern of Bcl-2 immunostaining in dysplastic and oral cancer cases.

In our study, positive Bcl-2 expression was present in 36.66% (11/30) oral cancer cases. Zhang et al.²¹ observed Bcl-2 expression in 47.27% of cases. Singh et al.⁵ reported Bcl-2 expression in 25% of oral cancers, whereas Ravi et al.²² in 87% of the cases. The discrepancies might be attributed to geographic distribution, genetic discrepancies as well as

Fig. 2 – Bcl-2 expression in oral precancer (leukoplakia): photomicrograph low power.

different etiological factors involved in oral cavity carcinogenesis. In our cases, the main etiologic factors recorded were tobacco and areca nut chewing.

In our study, positive Bcl-2 expression was found in 87.50% (14/16) precancer (dysplasia) cases whereas Fernando et al.²³ reported in 16.67% precancer cases. Piattelli et al.¹¹ found inverse relationship between Bcl-2 expression and premalignant lesions. The differences in Bcl-2 expression, from the various studies may reflect inherent difference in upstream genetic events between the different population groups, and also the environmental difference. It has been reported that genetic events and environmental factors are responsible for the inherited capacity to metabolize carcinogens or pro-carcinogens, or impaired ability to repair the consequent DNA damage.⁶

Stefania et al.²⁴ recorded a mean age of 62.04 years in their patients which is higher than the mean age of 61 years in our patients. Among our cases, cancer and precancer were predominant in male patients 78.2%. In both the studies, the mean age was above 60 years. Male predominance may be due to the fact that habit of tobacco and areca nut chewing is more predominant in males.

Similar to Sulowska et al.⁸ in our study also there was no correlation between positive Bcl-2 expression, and gender as well as age and lesion site of oral cancer and precancer cases. In our study group there was an unequal distribution of sample, most of the oral cancer and precancer were of buccal mucosa.

Similar to Zhang et al.,²¹ we did not find any correlation of positive Bcl-2 expression with, tumor size and nodal metastasis.

The histologic significance of Bcl-2 over-expression in the neoplasm has varied widely in different reports. Yao et al.¹⁶ reported significant correlation between Bcl-2 over expression and histologic grading in tongue squamous cell carcinoma. In our study the positive Bcl-2 expression was. There was statistically significant correlation between histopathologic differentiation of cancer and positive Bcl-2 expression (100%, 3/3) in poorly differentiated cancer and 15.0%, 3/20 in well differentiated oral cancer.

Ravi et al.²² reported that lesions expressing p53 and Bcl-2 had a high probability of having histology of dysplasia or worse. In our study, in oral precancer cases, the positive Bcl-2 expression was more in moderate dysplasia (88.9%, 8/9) and less in mild dysplasia (85.7%, 5/7), though it was not statistically significant.

For squamous cell carcinoma of head and neck as a group, poor treatment outcome and shorter overall survival have been associated with Bcl-2 over-expression in the tumor, irrespective of the treatment modality (Friedman et al.).¹² The type of neoplasm and anatomic site play a significant role in the prognostic significance of Bcl-2 over-expression. Piris²⁵ assessed that positive expression of Bcl-2 was correlated with poor prognosis in patients with B-cell lymphomas. In our study a statistically significant correlation between positive Bcl-2 expression and histopathologic differentiation of tumor and lesion morphology, in oral cancer was observed. Based on lesion morphology the Bcl-2 expression was significantly more (63.6%, 7/11) in proliferative cancer. Our study showed positive Bcl-2 expression in 100% (3/3) cases of poorly differentiated oral cancer. In precancer cases also positive Bcl-2 expression was high in moderate dysplasia 88.9% (8/9). Overall, there was an upregulation of Bcl-2 expression, proportional to degree of differentiation or dysplasia suggesting that positive Bcl-2 expression may be a valuable factor in establishing unfavorable histopathological features of oral cancer and precancer and thus indicating poor prognosis.

Overall our study showed no correlation between positive Bcl-2 expression and various clinicopathologic features in oral cancer and precancer, except histopathologic grading and lesion morphology in oral cancer and only histopathologic grading in precancer, as Zhang et al.²¹ also reported in their study.

The results of our study may be considered to be valid as there was substantial to good agreement between inter-rater and intra-rater for the graded values of positive Bcl-2 expression.

Genetic complexity dictates that expression of single gene has to be considered in the context of other related genes and gene products. In the case of Bcl-2, the related genes are Bax Bcl-xl, Bcl-xs and Bad. Staibano et al.²⁴ demonstrated correlation of high expression of PCNA, Bax, Bcl-1 and Bcl-2 with a worse prognosis. Before ascribing importance to Bcl-2 expression in oral cancer and precancer, it is necessary to sort out the interrelationship of related genes.

Low sample size, unequal distribution of sample, and use of only one molecular marker may be considered as limitations of the present study. As the study mainly included the patients from a limited geographic area Puducherry, Southern India, further multicentric studies from all parts of the world may be required to establish a more precise correlation between clinicopathologic features and positive Bcl-2 expression in oral cancer and precancer.

9. Conclusion

In our study we found that positive Bcl-2 expression was associated with a more aggressive histopathologic behavior of tumor cells, thus indicating unfavorable clinical outcome or

poor prognosis. There was no correlation between positive Bcl-2 expression and various clinicopathological features of oral cancer and precancer.

Based on the results of our study, we conclude that positive Bcl-2 expression may be an indicator of poor prognosis in oral cancer and precancer, and in future, multicentric studies are needed to establish a definite and more precise correlation between positive Bcl-2 expression and clinicopathologic features of oral cancer and precancer.

Conflicts of interest

The authors have none to declare.

Acknowledgement

We would like to thank Dr. Surendra Verma, Prof & Head, Department of General Pathology, JIPMER, Puducherry for his able guidance and support.

REFERENCES

- Kuropkat C, Venkatesan TK, Caldarelli DD, et al. Abnormalities of molecular regulators of proliferation and apoptosis in carcinoma of the oral cavity and oropharynx. *Auris Nasus Larynx*. 2002;29:165–174.
- Williams GT, Smith CA. Molecular regulation of apoptosis: genetic controls on cell death. *Cell*. 1993;74:777–779.
- Wyllie AH. The biology of cell death in tumors. *Anticancer Res*. 1985;5(1):131–136.
- Birchall MA, Schock E, Harmon BV, Gobe G. Apoptosis, mitosis, PCNA and Bcl2 in normal, leukoplakic and malignant epithelia of the human oral cavity: prospective, in-vivo study. *Oral Oncol*. 1997;33:419–425.
- Singh BB, Chandler Jr FW, Whitaker SB, Forbes Nelson AE. Immunohistochemical evaluation of Bcl-2 oncoprotein in oral dysplasia and carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol*. 1998;85(June (6)):692–698.
- Batsakis JG. The molecular biology of oral cancer. In: Shah JP, Johnson WJ, Batsakis H, eds. *Oral Cancer* 1st ed. United Kingdom: Martin Dunitz, an imprint of the Taylor & Francis Group; 2003:165–182.
- Hockenbery DM. Bcl-2 in cancer, development and apoptosis. *J Cell Sci Suppl*. 1994;18:51–55.
- Sulkowska M, Famulski W, Sulkowski S, et al. Correlation between Bcl-2 protein expression and some clinicopathological features of oral squamous cell carcinoma. *Pol J Pathol*. 2003;54:49–52.
- Oltvai ZN, Millman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog Bax, that accelerates programmed cell death. *Cell*. 1993;74(August (4)):609–619.
- Wang Y, Szekely L, Okan I, Klein G, Wiman KG. Wild-type p53-triggered apoptosis is inhibited by bcl-2 in a v-myc-induced T-cell lymphoma line. *Oncogene*. 1993;8:3427–3431.
- Piattelli A, Rubini C, Fioroni M, Iezzi G, Santinelli A. Prevalence of p53, bcl-2, and Ki-67 immunoreactivity and of apoptosis in normal oral epithelium and in premalignant and malignant lesions of the oral cavity. *J Oral Maxillofac Surg*. 2002;60:532–540.
- Friedman M, Grey P, Venkatesan TK, et al. Prognostic significance of Bcl-2 expression in localized squamous cell carcinoma of head and neck. *Ann Otol Rhinol Laryngol*. 1997;106:445–450.
- Ravi D, Ramdas K, Mathew BS, Nalinakumari KR, Nair MK, Pillai MR. De novo programmed cell death in oral cancer. *Histopathology*. 1999;34:241–249.
- Epstein J, Wanderwaal I. Oral cancer. In: Greenberg MS, ed. *Burket's Oral Medicine* 11th ed. New Delhi: CBS Publishers; 2008:153–193.
- Kroemer G. The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nat Med*. 1997;3:614–620.
- Yao L, Iwai M, Frusta I. Correlations of Bcl-2 and p53 expression with the clinicopathological features in tongue squamous cell carcinomas. *J Oral Oncol*. 1999;35:56–62.
- Pezzella F, Turley H, Kuzu I, et al. Bcl-2 protein in non-small cell lung carcinoma. *N Engl J Med*. 1993;329:690–694.
- Silvestrini R, Veneroni S, Daidone MG, et al. The Bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph node-negative breast cancer patients. *J Natl Cancer Inst*. 1994;86(April (7)):499–504.
- Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ, Levin B. Bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. *Cancer Res*. 1995;55(January (2)):237–241.
- Popovic B, Jekic B, Novakovic I, et al. Bcl-2 expression in oral squamous cell carcinoma. *Ann N Y Acad Sci*. 2007;1095(January):19–25.
- Zhang M, Zhang P, Zhang C, et al. Prognostic significance of Bcl-2 and Bax protein expression in the patients with oral squamous cell carcinoma. *J Oral Pathol Med*. 2009;38:307–313.
- Ravi D, Nalinakumari KR, Rajaram RS, Nair MK, Pillai MR. Expression of programmed cell death regulatory p53 and bcl-2 proteins in oral lesions. *Cancer Lett*. 1996;105:139–146.
- Sousa FA, Paradella TC, Carvalho YR, Rosa LE. Immunohistochemical expression of PCNA, p53, bax and bcl-2 in oral lichen planus and epithelial dysplasia. *J Oral Sci*. 2009;51(March (1)):117–121.
- Staibano S, Migonogva MD, Lo Muzio L, et al. Over expression of cyclin-D1, bcl-2, and bax proteins, proliferating cell nuclear antigen (PCNA), and DNA-ploidy in squamous cell carcinoma of the oral cavity. *Hum Pathol*. 1998;29(November (11)):1189–1194.
- Piris MA, Pezzella F, Martinez Montero JC, et al. P53 and Bcl-2 expression in high grade B-cell lymphomas: correlation with survival time. *Br J Cancer*. 1994;69(February (2)):337–341.