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Original Article

Does the expression of BCL2 have prognostic significance in malignant peritoneal mesothelioma?

Krishna Pillai, Mohammad H Pourgholami, Terence C Chua, David L Morris

Department of Surgery, St. George Hospital, University of New South Wales, Kogarah, NSW 2217, Australia

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Abstract: Background Malignant peritoneal mesothelioma (MPM) is a rare neoplasm of the peritoneal membrane that is causally related to asbestos exposure. Survival after treatment is poor. Current therapy involving hyperthermic intraperitoneal chemotherapy has improved survival in selective patients. In the past, several prognostic factors have been identified in MPM patients and this has prompted the development of new therapies and patient management. Since BCL2, an antiapoptotic oncoprotein, is a favourable prognostic factor in breast cancer, we investigated to determine the significance of BCL2 in MPM. Materials and Methods Forty two archival patient tumour sections embedded in paraffin blocks were sectioned and subjected to immunohistochemistry to detect BCL2. The staining intensity and abundance was classified using standard procedures and classified into two groups (0-4 = low & 5-8 = high expression). The distribution of BCL2 groups was examined in the different clinicopathological categories to determine prognosis using Kaplan–Meier survival analysis. Results: Univariate analysis revealed that in almost all clinicopathological categories, high BCL2 expression predisposed patients to a favourable prognosis. Independent of BCL2 expression, univariate analysis also showed that male gender, sarcomatoid histology, high PCI and age at diagnosis ≥ 60 years were associated poor prognosis. Multivariate analysis indicated that for all tumours, males and females, high BCL2 expression was associated with good prognosis. Further, independent of BCL2, age ≥ 60 years is an unfavourable prognostic factor. Conclusion: Expression of BCL2 may serve to distinguish prognosis within the individual clinicopathological categories. BCL2 is also an independent variable in all tumours, males and females, with high expression being associated with good prognosis.

Keywords: BCL2, prognosis, peritoneal mesothelioma, survival

Introduction

BCL2 gene (B-cell lymphoma 2 gene, bcl-2) was first discovered in follicular B-cell lymphoma as a gene linked to immunoglobulin heavy chain locus at the breakpoint of t(14:18) translocation [1], the result being enhanced BCL2 protein transcription. Otherwise, in normal cells this gene is found on chromosome segment 18q21.3. The discovery that BCL2 inhibits cell death revolutionized the whole approach towards tumour genesis. Soon after it was recognized that BCL2 protein was also found in all other malignancies and can be independent of t(14:18) chromosomal translocation.

The mammalian BCL2 protein family consists of at least 30 related proteins, characterised by the presence of up to four relatively short sequence motifs (< 20 amino acid residue in length) called BCL2 homology (BH) domains [2-4]. On the whole, the BCL2 family is divisible into three different sub-classes based on structural and functional features. The details of which are fully discussed in recent reviews [5]. The anti-apoptotic member family includes BCL2, BCL-XL, BCL-W and MCL-1 proteins that possess all four conserved BH1-4 domains and a hydrophobic C-terminal part. BH1-3 domain forms a hydrophobic groove and N-terminal BH4 stabilizes the structure. BH4 domain is usually absent in apoptotic proteins and therefore is a key factor for anti-apoptotic activity. The BH4 domain of BCL2 consists of 26 amino acids and is permanently found in membranes compared to BCL-XL and BCL-W which appears after the initiation of cytotoxic signals [6].

BCL2 and its anti-apoptotic orthologous seems to inhibit apoptosis by maintaining mitochondrial membrane integrity through its hydropho-

Table 1. Clinicopathological features of 42 patients screened for BCL2 expression using immunohistochemistry

Variables	All patients	*p*	IHC (0-4)	*p*	IHC (5-8)	*p*
Total	42		28 (66%)		14 (34%)	
Age (median)	57		53		42	
Age (range)	23-69		23-66		30-69	
Gender-M	20 (48%)	0.062	14 (34%)	0.062	6 (14%)	0.028
Gender-F	22 (52%)		14 (34%)		8 (18%)	
Epithelial	35 (83%)		23 (55%)		12 (28%)	
Epithelial-M	15 (36%)	0.050	11 (26%)	0.078	4 (10%)	0.213
Epithelial F	20 (48%)		12 (28%)		8 (19%)	
Sarcomatoid	7 (17%)		5 (12%)		2 (5%)	
Sarcomatoid-M	4 (9%)		2 (4.5%)		-2 (4.5%)	
Sarcomatoid-F	3 (7%)		3 (7%)		-	
PCI < 20	20 (48%)	0.038	13 (31%)	0.031	7 (17%)	0.321
PCI > 20	22 (52%)		15 (35%)		7 (17%)	
AAD < 60y	31 (74%)	0.029	21 (50%)	0.022	10 (24%)	0.049
AAD > 60y	11 (26%)		7 (17%)		4 (9%)	

M = Male; F = Female; PCI = Peritoneal Cancer Index, AAD = Age at diagnosis; χ^2 = Student T test or Mann Whitney U test when sample size is small. *P* values below 0.05 are considered significant. IHC = immunohistochemical score.

bic carboxyl terminal domain that is linked to outer membrane. BCL2 also prevents BAX/BAK oligomerization that would otherwise lead to release of numerous apoptotic molecules from the mitochondrion. BCL2 may also regulate several initiator caspases like caspase-2 that act upstream or independently of cytochrome c. BCL2 also blocks directly the release of cytochrome c and hence prevent APAF-1 and caspase-9 activation. Over expression of BCL2 not only is associated with poor prognosis in nasopharangeal cancer [7] but also is related to chemoresistance in a number of cancers [8, 9].

Although BCL2 is renowned for its antiapoptotic property, the protein has also an anti-proliferative effect that influences cell cycle entry [10-14]. Indeed, the anti apoptotic role of BCL2 has been well characterised but its function in cell cycle control has received less attention. The latter property is well supported by cell line studies which show that BCL2 expression delays G1 progression and G1-S transition by prolonging GO and is capable of growth inhibitory effects similar to those of p53 [10, 13]. BCL2 is a highly favourable prognostic factor in breast cancer [15, 16]. More recently, it has been combined with mitotic index as a method of modifying tumour grade [17]. Further, the development of Ki67/BCL2 index is highly prognostic in ER-positive breast cancer [18]. Hence, the anti-proliferative role of BCL2 seems to be useful in predicting survival outcome in cancer patients.

Malignant peritoneal mesothelioma (MPM) is a rare disease of the mesothelial lining of the abdominal cavity, the known causative agent is asbestos [19], although Simian Virus 40 has been implicated as a co-carcinogen [20]. The latency period between exposure and disease expression is in the range of 10-30 years. MPM symptoms are non-specific and hence patients are diagnosed late in the course of the disease with poor outcome [21]. The mean survival period is in the region of a year [22], although, with the introduction of cytoreductive surgery and hyperthermic intraoperative and perioperative chemotherapy, survival has improved in selective patients [23]. Therefore, the identification of prognostic factors may help to demarcate those who are seriously ill and hence improve patient management. Currently, a number of prognostic factors have been identified, the majority are clinical factors such as peritoneal cancer index, gender, age at diagnosis, tumor node status and cytoreductive score [24-27]. We have recently identified estrogen receptor-β, MUC1 and Ki67 as independent prognostic variables that affect prognosis (under review). Since we found that high expression of MUC1 and Ki67 are poor prognostic factors in MPM,

we were interested in determining how the presence of BCL2 may affect the survival pattern in the 42 patients that we have examined earlier. The prognostic significance of BCL2 in malignant mesothelioma (MM) has been rarely investigated, although a study in 54 pleural malignant mesothelioma found no significance with survival and neither any correlation with the other anti-apoptotic markers such as BCL xl and MCL-1 [28]. In another study on 35 malignant mesothelioma patients, BCL2 has been reported to be infrequently expressed and has been associated inversely with apoptotic index [29]. Our study will be the first to determine the prognostic significance of BCL2 in MPM.

Material and methods

Patients

This study was conducted with the approval of ethics committee of St. George Hospital (SESIAH), Kogarah, NSW, Australia. The study participants included 20 males and 22 females, with a median age of 57 years (range 23-69), that were diagnosed with MPM in St George Hospital, 1999- Dec 2012. All participants were uniformly treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (cisplatin and doxorubicin). Of the 42 patients, 40 (95%) had an optimal cytoreduction (CCR 0-1) whilst 2 patients (5%) had incomplete cytoreduction (CCR-2). The clinical features of these patients are summarized in **Table 1**.

Immunohistochemistry

Tumour specimens of PMP, retrieved from patients were formalin preserved and paraffin embedded. 3 μm sections were cut and mounted on glass slides. The slides were deparaffinised, rehydrated and then subjected to antigen retrieval in citrate buffer at pH. 6.0. The sections were subsequently incubated in 3% aqueous hydrogen peroxide for 15 minutes to quench endogenous peroxide activity and with protein block serum-Free (Dako, Carpentaria, CA, USA), for 20 minutes at room temperature to suppress non-specific binding of subsequent reagents. The reaction was followed with incubation of the primary antibody, Clone 124 (Dako, Carpentaria, CA, USA), dilutions 1:100, for 40 minutes. Slides were then washed for 5 minutes with phosphate buffer saline (PBS) before applying biotin labelled secondary antibody (goat anti–rabbit, Dako, 1:500) for 15 minutes, then washed again and incubated for 15 minutes with avidin–biotin–HRP complex as directed by Dako. Slides were then washed with PBS; and finally immunoprecipitation was visualized by treating with aminobenzidene tertahydrochloride, Dako) for 30 minutes and counter staining with haematoxylin.

Immunohistochemical evaluation and controls

Three blinded and independent evaluations of the stained slides were carried out, without any knowledge of patient characteristics or the expression of other IHC markers. Discrepancies in evaluation were resolved by re-examination of the slides. Immunoreactivity was categorised as previously described [30, 31] and compared to positive control (Malignant peritoneal mesothelioma). Brown stains were classified as positive. Negative controls were patient samples without primary antibody.

BCL2 expression was assessed according to intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; 3 = strong staining) and proportion of staining cells were assigned as 0 = < 1%; 1 = > 1–10%, 2 = > 10–20%; 3 = > 20–30%; 4 = > 30%-40% and 5 = > 40%. The scores were added to give the final expression of BCL2. A score of 4 and below was classified as low expression.

Statistical analysis

X^2 test was performed for comparison of two groups and Mann-Witney U test was performed as appropriate. Overall survival probabilities were calculated using the Kaplan-Meier method and compared using log-rank test. A Cox proportion hazard model was used for determining factors related to overall survival. These analyses yielded a hazard ratio, their 95% confidence intervals and *P* values. Only *P* values < 0.05 were considered significant.

Results

Expression of BCL2

The immunohistochemical staining to detect BCL2 expression in 42 patients showed that there was a wide variability in expression of the BCL2 protein (**Figure 1**). Categorizing the stain-

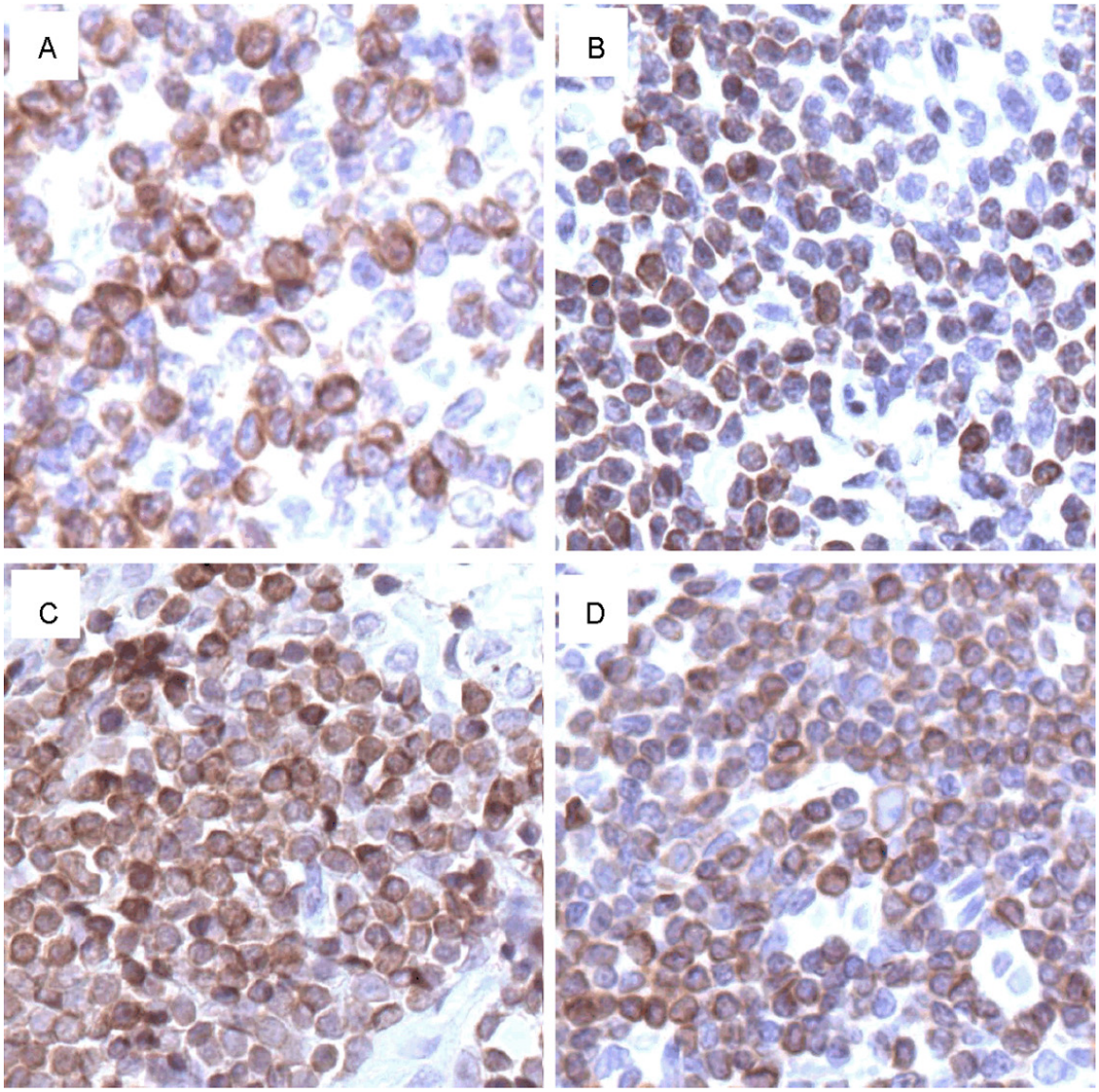

Figure 1. A shows the staining pattern in control slide with expression of BCL2 (brown stain). B, C, and D are staining pattern in three MPM patients. Patient B has higher intensity of staining, whilst patient C and D have a lower intensity of staining. A higher abundance of staining is shown in patience C, compared to patient B and D. For immunohistochemical staining, glass tissue mounted slides were deparaffinised in xylene and ethanol, microwave irradiated and treated with mouse monoclonal anti-BCL2 followed by rabbit ant-mouse secondary antibody (En Vision Method). Magnification is 20 x 0.45 (detail of method referenced earlier).

ing intensity and abundance of BCL2 expression into 2 groups, low with a score ranging from 0-4 (group 1) and high with a score from 5-8 (group 2), we found that the majority 28 (66%) of patients belonged to group 1 whilst the remaining 14 (34%) patients in group 2. The ratio of patients in group 1 : group 2 is 2:1 showing a preferential expression of low level of BCL2.

Examining gender distribution within the two groups of BCL2 expression, out of 20 (48%) males, 14 (34%) were in group 1 and 6 (14%) fell into group 2. In the females out of total of 22 (52%) patients, 14 (34%) were in group 1 whilst the remaining 8 (18%) belonged to group 2 (**Table 1**). The ratio of group 1 : group 2 in males is 2.4:1, whilst in females it is 1.8:1, hence showing a higher predisposition in the males towards having low expression of BCL2 that is indicative of poor prognosis.

Amongst 42 patients, 35 (84%) had epithelial tumour histology, out of which 23 (53%)

Table 2. Univariate analysis and prognosis in different clinicopathological gategories of malignant peritoneal mesothelioma (n=42)

	UNIVARIATE ANALYSIS		
VARIABLES	HR	(CI 95 %)	P
ALL TUMORS			
(Low vs High BCL2 Expression)	6.0	2.23-16.44	0.004
GENDER			
Male vs Female	2.0	0.64-7.10	0.007
Male (Low vs High BCL2 Expression)	4.3	1.15-15.90	0.030
Female (Low vs High BCL2 Expression)	7.0	1.63-30.25	0.001
HISTOLOGY			
Sarcomatoid vs Epithelial	2.0	0.64-7.10	0.001
Epithelial (Low vs High BCL2 Expression)	5.5	1.57-19.62	0.008
Epithelial Male (Low vs High BCL2 Expression)	2.8	6.46-17.06	0.257 NS
Epithelial Female (Low vs High BCL2 Expression)	6.5	1.07-39.47	0.042
PERITONEAL CANCER INDEX (PCI)			
PCI ≥ 20 vs PCI < 20	1.1	0.3-3.80	0.013
PCI < 20 (Low vs High BCL2 Expression)	4.1	1.0-16.4	0.043
PCI ≥ 20 (Low vs High BCL2 Expression)	4.2	0.56-31.6	0.011
AGE AT DIAGNOSIS (AAD)			
AAD ≥ 60 vs < 60 years	3.1	1.0-9.10	0.001
AAD < 60 (Low vs High BCL2 Expression)	5.9	1.72-20.47	0.005
AAD ≥ 60 (Low vs High BCL2 Expression)	3.9	0.52-30.18	0.182 NS

Low BCL2 = BCL2 Immunohistochemical score (IHC) = 0-4; High BCL2 = BCL2 IHC core of 5-8; NS = Not significant; CI = 95% Confidence interval; *P* values < 0.05 are considered significant.

belonged to group 1, the remaining 12 (28%) in group 2, a ratio of 2:1 suggesting that low BCL2 expression is more common in epithelial tumours. Examining gender distribution in the epithelial category, 15 (36%) were males, out of which 11 (26%) belonged to group 1, remaining 4 (10%) in group 2. Of the 20 (48%) females with epithelial histology, 12 (28%) fell into group 1, remaining 8 (19%) into group 2. Hence, there was a higher percentage of tumours with low expression of BCL2 (group 1) in both the sexes. Examining the ratio of percentage expression of BCL2 in group 1 : group 2, amongst the sexes, the males had a ratio of 2.6:1, whilst for the females it was 1.5:1, again in favour of the females.

In sarcomatoid tumours, out of a total of 7 (17%), 5 (12%) were in group 1, the remaining 2 (5%) in group 2. Hence sarcomatoid tumours mainly expressed low level of BCL2. Examining the gender distribution, there were 4 (9%) of males and 3 (7%) of females in this category. In the males, 2 (4.5%) fell into group 1, and equal number in group 2. In the females, all the 3 (7%) patients belonged to group 1 with low expression of BCL2.

In the peritoneal cancer index (PCI) category, out of 42 patients, 20 (48%) belonged to PCI < 20 category, the remaining 22 (52%) fell into category of PCI > 20. In the category of PCI < 20, 13 (31%) patients fell into group 1 with low expression of BCL2, the remaining 7 (17%) belonged to group 2. In the PCI category > 20, out of 22 (52%) patients, 15 (35%) had low expression of BCL2 (group 1) and the remaining 7 (17%) fell into group 2. Hence, almost double the number of patients in both the PCI category belonged to group 1 with low expression of BCL2. The distribution of tumours within the two groups of BCL2 was quite similar in both the categories of PCI. Hence, the BCL2 expression did not show any difference within the two categories of PCI.

In the category, Age at diagnosis (AAD), out of 42 tumours, 31 (74%) belonged to AAD < 60

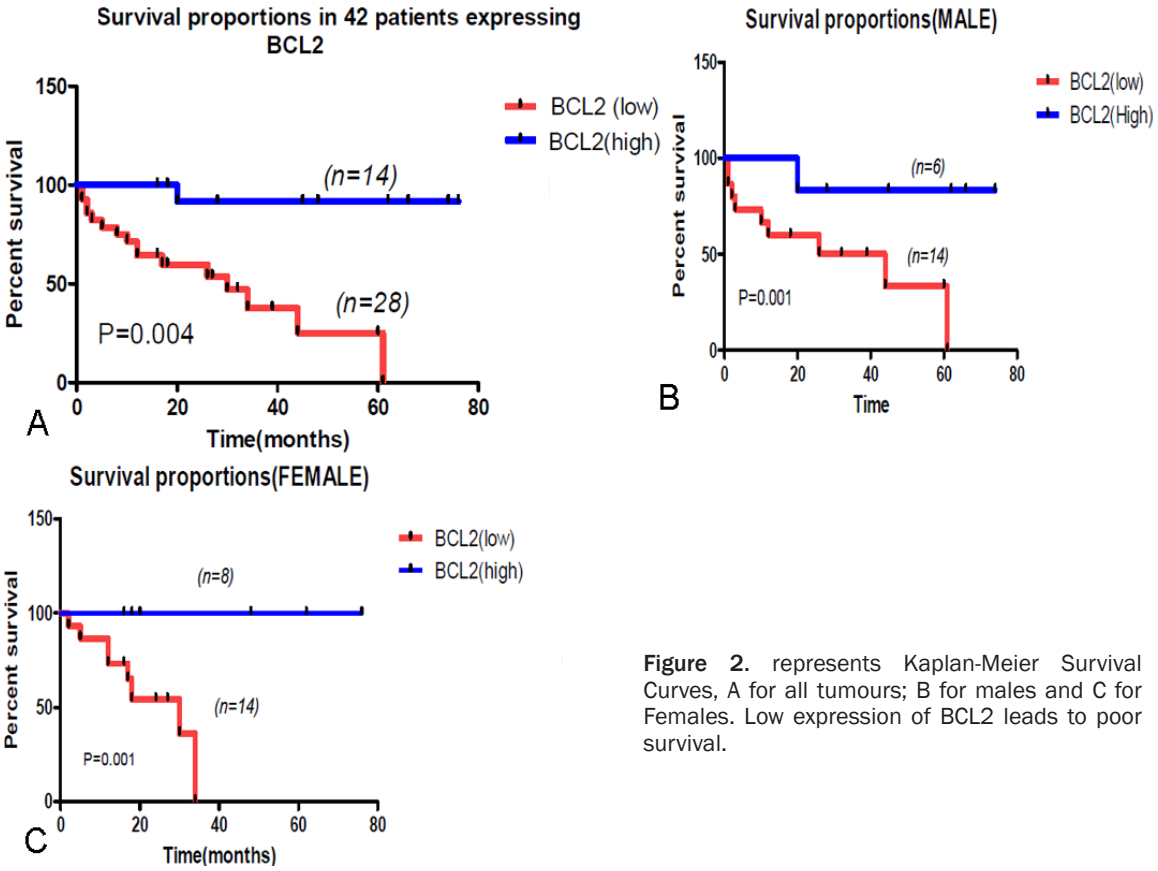

Figure 2. represents Kaplan-Meier Survival Curves, A for all tumours; B for males and C for Females. Low expression of BCL2 leads to poor survival.

years. In this category 21 (50%) fell into group 1 (low BCL2 expression) whilst the remaining 11 (24%) belonged to group 2 (High BCL2 expression). In the category of AAD ≥ 60 years, 7 (17%) belonged to group 1, the remaining 4 (9%) were in group 2. Hence, there was twice the percentage of tumours in group 1 compared to group 2, for both the classes of AAD, indicating that there was no preferential distribution of BCL2 within the two classes of AAD.

Clinical outcome

The clinical outcome in this study was measured by survival in patients as monitored from the date of diagnosis and treatment to death or survival within the last 11 years. Using Kaplan-Meier analysis, with log-Rank and Cox-proportional Hazard ratio, we were able to determine how the expression of BCL2 affected prognosis in the two patient groups (low BCL2 expression vs High BCL2 expression). Both univariate and multivariate analysis were conducted to determine survival.

Univariate analysis

In almost all clinicopathological variables examined, high BCL2 expression was correlated with good prognosis (**Table 2**) and they are as follows: All tumours (*p = 0.004*), Males *(p = 0.030)*, Females *(p = 0.001)*, Epithelial tumours *(p = 0.008),* Epithelial Female *(p = 0.042)*, PCI < 20 *(p = 0.043)*, PCI ≥ 20 *(p = 0.011)* and AAD < 60 years *(p = 0.005)*. For the other variables that were tested independent of BCL2 expression, Male *(p = 0.007),* Sarcomatoid histology *(p = 0.001)*, PCI ≥ 20 *(p = 0.013)* and AAD (≥ 60 years) (*p = 0.001),* were poor prognostic factors (**Figures 2** and **3**).

Multivariate analysis and overall survival

Only variables that tested to be significant in univariate analysis were entered into multivariate analysis. Of the variables that were tested high BCL2 expression were correlated with good prognosis in the following categories: All tumors, HR = 6.4 (95% CI, 2.32–17.61), *p* =

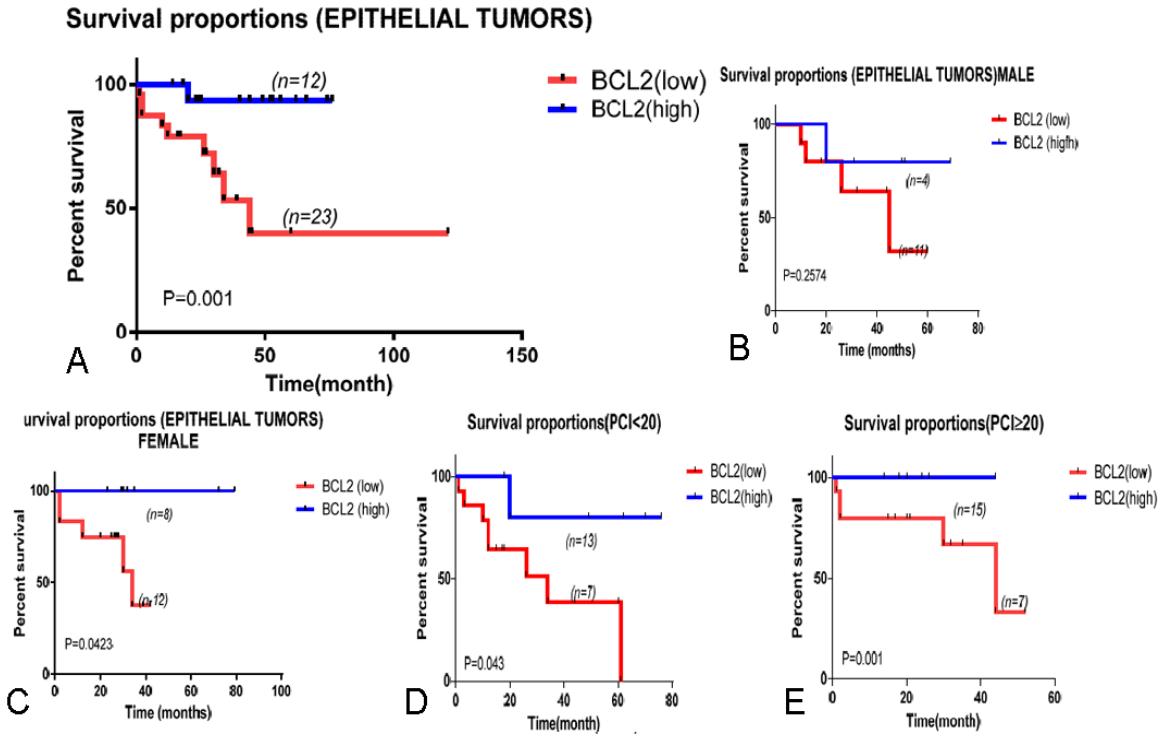

Figure 3. represent Kaplan-Meier Survival Curves, A for all epithelial tumors; B & C for males and females; D & E for age at diagnosis < 60 and ≥ 60 years. Low BCL2 expression leads to poor survival.

0.038; Male, HR = 4.7 (CI, 1.41–16.2), *p = 0.038*; Female, HR = 7.3 (CI, 1.72–38.6), *p = 0.048).* Amongst the variables tested independent of BCL2 expression, only age at diagnosis ≥ 60 years was an independent variable with poor prognosis, HR = 3.3 (CI, 1.4-16.4), *P = 0.049*. (**Table 3**).

Discussion

BCL2 protein is a member of the BCL family that is known to regulate apoptosis in both normal and cancer cells. It is the balance between pro-apoptotic and anti-apoptotic BCL family of proteins that decides the survival of the cells [3, 9]. Further, the tumuroigenic potential of BCL2 has also been demonstrated in animal models [32]. Over expression has been found in a variety of tumours [33, 34] and in oral squamous cell carcinoma where BCL2 acts as an oncogene [35]. BCL2 enhances carcinogenesis by preventing cell death, rather than increasing the rate of cell division [36]. It has been proposed that BCL2 expression level determines response to chemotherapy and radiotherapy [8, 9]. Hence BCL2 has been extensively studied in many cancers as a prognostic marker. Recently, it has been shown that BCL2 expression is a prognostic marker in triple negative breast cancer [37].

In the current study, we have examined the expression status of BCL2 in 42 patients in order to determine the correlation between the presence of this oncogene and survival. Our study indicates that high expression of BCL2 in tumours is related to survival advantage, and this may be related to its ability to control tumour growth by its anti-proliferative action [10, 13].

Further, our investigation also shows that the majority of the patients (66%) displayed low expression of BCL2 which was related to poor prognosis by Kaplan-Meier analysis. On examining the gender distribution of low against high expression of BCL2 for both the males and the females, we found the ratio to be 2.4:1 and 1.7:1, respectively, suggesting that the males were more inclined to express a low level of BCL2 which is an indicator of poor prognosis. This difference may have a bearing on the gender difference observed in malignant peritoneal mesothelioma as reported in some studies [26, 27].

Table 3. Multivariate analysis and prognosis in different clinicopathological categories of malignant peritoneal mesothelioma (n=42)

	MULTIVARIATE ANALYSIS		
VARIABLES	HR	(CI 95%)	*P*
ALL TUMOURS			
High vs Low BCL2 Expression	6.4	2.32-17.61	0.038
GENDER			
Male vs Female	2.1	0.7-6.6	0.166 NS
Male - Low vs High BCL2 Expression	4.7	1.41-16.2	0.038
Female - Low vs High BCL2 Expression	7.3	1.72-38.6	0.048
HISTOLOGY			
Sarcomatoid vs Epithelial	3.3	0.1-10.0	0.117 NS
Epithelial (Low vs High BCL2 Expression)	5.7	1.4-26.9	0.052 NS
Female Epithelial (Low vs High BCL2 expression)	6.8	6.1-22.8	0.071 NS
PERITONEAL CANCER INDEX (PCI)			
PCI ≥ 20 VS < 20	1.0	0.3-3.8	0.998 NS
PCI < 20 (Low vs High BCL2 Expression)	4.6	1.8-19.3	0.052 NS
PCI ≥ 20 (Low vs High BCL2 Expression)	4.9	1.2-38.4	0.056 NS
AGE AT DIAGNOS = IS (AAD)			
AAD ≥ 60 vs < 60 years	3.3	1.6-16.4	0.049
AAD < 60 (Low vs High BCL2 Expression)	6.2	1.9-29.9	0.056 NS

Low BCL2 = BCL2 immunohistochemical score (IHC) = 0-4; High BCL2 = BCL2 IHC score of 5-8; NS = Not significant; CI = 95% confidence interval; *P* values < 0.05 are considered significant. For all categories of variables, only those that were significant by univariate analysis (P = < 0.05), were entered into multivariate analysis.

Generally, patients with epithelial tumours have been found to have better prognosis compared to sarcomatoid tumours [38]. In the present study, the majority (84%) of tumours belonged to epithelial type and high expression of BCL2 was correlated to better survival (*p = 0.008*). The ratio of tumours in group 1 : group 2 is 2:1 suggesting that majority of epithelial tumours tend to have low expression of BCL2 and hence poor survival. On closely examining the gender distribution, 38% were males whilst 48% were females, suggesting that there were a higher percentage of females with this favourable histology. However, when we examined the distribution of BCL2 within the gender, the ratio of low to high expression of BCL2 in the males versus the females, it was 1.9:1 vs 1.5:1, in favour of the females. We did not validate the data from sarcomatoid tumours as the number of tumours was too small to have statistical significance.

Although some studies have shown that high peritoneal cancer index (PCI) is correlated to poor prognosis [39], there are contentions [26, 40]. The current study shows a correlation for PCI with survival, by univariate analysis, but did not prove to be an independent variable. However, on examining the expression of BCL2 within the two categories of (PCI), in the category of PCI < 20, there was a correlation. Further, we also noticed that the number of tumours in the two groups of BCL2 (High and Low) were quite similar in the two categories of PCI, indicating that the expression of BCL2 was not dependant on PCI category. Also the ratio of Low:High BCL2 expression within the two categories of PCI was similar i.e. 2:1 indicating that Low BCL2 was preferentially found in these two categories of patients. This further indicates that the expression of BCL2 in the PCI category does not support the poor prognosis that has been proposed in the high PCI category as cited earlier.

In cancers of prostate and colon, age at diagnosis is a determinant of prognosis, patients in later decades of their lives tend to have poor prognosis [41, 42]. In the current study, age at diagnosis is an independent prognostic factor (*p = 0.049*), those above 60 years at diagnosis (AAD) tend to have poor outcome. Further, amongst the 42 tumour samples, the majority (74%) belonged to AAD < 60 years. On examin-

ing the BCL2 distribution within the two categories of AAD, the ratio of Low:High BCL2 expression was 2:1. This indicates that there is again no preferential distribution of BCL2 within the two categories of AAD, to support poor prognosis that is observed in older patients, as reported in a few studies [43, 44]. Generally older patients may have other co-morbidities that may influence their survival after undergoing treatment and this has been demonstrated in other cancers [45, 46]. Hence biomarkers such as BCL2 may not preferentially be down regulated in older patients to enhance death as indicated in this study.

High expression of BCL2 is considered as a good prognostic factor in cancer since it has been associated with improved survival in patients with breast cancer [47, 48]. Other reports have also indicated that high expression of BCL2 have been observed in Estrogen Receptor (ER), Progesterone Receptor (PR) positive breast cancers which have good prognosis [49, 50]. Further triple negative breast cancers express less BCL2 with consequential higher tumour grade and poor survival [37]. These findings in breast cancer indicate that regardless of the presence or the absence of steroid receptors, the expression of BCL2 plays an important role in promoting survival. More importantly, we were able to show for the first time, that in MPM, the status of BCL2 plays an important role in determining survival, regardless of the clinicopathological variables. The BCL2 protein with its anti-proliferative property may play an important role in regulating the growth of the tumours and hence slow down the progression of the cancer in these terminally ill patients. In conclusion, we recommend the routine screening of BCL2 in patients to determine their prognostic status. However, the current study may require further validation with a larger sample and hence a multi-institutional study is highly recommended.

Disclosure of conflict of interest

The authors fully declare that there are no competing interests.

Address correspondence to: Dr. David L Morris, Department of Surgery, St. George Hospital, University of New South Wales, Kogarah, NSW 2217, Australia. Phone: +612 – 9112070; Fax: +612 – 91133997; E-mail: david.morris@unsw.edu.au.

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