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Original Article

Ki67-BCL2 index in prognosis of malignant peritoneal mesothelioma

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Abstract: Background: malignant peritoneal mesothelioma (MPM) is a rare peritoneal mesothelial neoplasm. Ki67 and BCL2 are established prognostic markers in several cancers. High Ki67 expression indicates tumour progression, whilst similar expression of BCL2 retards tumour replication. Traditionally, prognosis in MPM is gauged with a single biomarker assessed separately in a dichotomous manner. Here, we examine prognosis with dual biomarkers incorporated in a model to predict survival. Materials and methods: Forty two MPM archival patient tumours were screened for Ki67 and BCL2 by immunohistochemistry and evaluated using standard methods. Ki67 and BCL2 expression was incorporated into a prognostic model to develop Ki67-BCL2 index. Using this index, three hazard groups were identified (high, medium and low risk). Kaplan-Meier survival analysis was performed to assess the significance of these hazard groups in the various clinicopathological categories. Results: In all clinicopathological categories, high risk group showed poor prognosis compared to low risk group ($p = < 0.001$). Compared to medium risk, high risk group carried poor prognosis in all tumours, females, epitheloid tumours, peritoneal cancer index (PCI) < 20, ≥ 20, age at diagnosis (AAD) < 60, and ≥ 60 years. Independent of the Ki67-BCL2 index, male, sarcomatoid, PCI ≥ 20 and AAD ≥ 60 were poor prognostic factors. High risk group was an independent poor prognostic factor in all tumours, males, females and age < 60 years. The distribution of high risk: low risk group in male and female was 3: 2 and 2: 3, respectively, indicating a gender difference. Comparing hazard ratios generated by Ki67-BCL2 index to that of either Ki67 or BCL2, as a single prognostic biomarker, there was a reduction of HR values. Conclusion: Ki67-BCL2 index seems to suggest a more sensitive method of predicting prognosis. However, the current model needs further evaluation in an independent large cohort sample.

Keywords: Ki67, BCl2, prognosis, malignant peritoneal mesothelima

Introduction

Malignant peritoneal mesothelioma (MPM) is a rare disease and asbestos has been suggested to be a causative agent [1, 2]. However mesothelioma also occurs in 20% of patients without previous exposure to asbestos and in these patients, MPM seems to be more common. This suggests other agents to be involved such as mineral fibres, chronic peritonitis, remote abdominal radiation and simian virus 40 [3, 4]. Survival is poor after treatment and it ranges from 1-3 years [5, 6]. Treatment with rigorous cytoreductive surgery and hyperthermic intraperitoneal chemotherapy has improved survival beyond 3 years in selective patients and this may be due to other favourable molecular factors that are present in these cases [7, 8]. Hence, there is an urgent need for new therapies that may eventually lead to a curative modality.

Several prognostic factors have been identified in MPM and mostly they are clinical or surgical factors [5, 9-11]. Although these factors enable to predict prognosis with probable better patient management, they may not lead to the development of therapies. In many cancers such as breast [12, 13], prostate [14], colon [15] and ovarian [16], therapies have been developed from identification of molecular prognostic factors. Pinton et al. have identified estrogen receptor – β and epidermal growth factors as prognostic factors in pleural mesothelioma and have suggested that therapies may be developed by modulating this protein [17-19]. Hence, the identification of molecular markers in MPM may lead to future development of more effective therapies.

Table 1. Ki67-BCL2 index for various clinicopathological categories of 42 MPM patients

VARIABLES	TOTAL	LR	*P*	MR	*P*	HR	*P*
Patients	42	14 (33%)		13 (31%)		5 (36%)	
Median Age	57	50		49		50	
Range	23-71	(33-63)		(23-71)		(37-70)	
GENDER							
Male	20 (48%)	6 (14%)	0.049	5 (12%)	0.041	9 (21%)	0.049
Female	22 (52%)	8 (19%)		8 (19%)		6 (14%)	
HISTOLOGY							
Epithelial	35 (83%)	12 (28%)		11 (26%)		12 (28%)	
Male-Epithelial	16 (38%)	5 (12%)	0.063	4 (9.5%)	0.054	7 (17%)	0.062
Female	19 (45%)	7 (16%)		7 (16%)		5 (12%)	
Sarcomatoid	7 (17%)	2 (5%)		2 (5%)		3 (7%)	
Male	4 (10%)	1 (2.5%)		1 (2.5%)		2 (5%)	
Female	3 (7%)	1 (2.5%)		1 (2.5%)		1 (2.5%)	
PERITONEAL CANCER INDEX (PCI)							
PCI < 20	25 (59%)	7 (17%)	0.081	8 (19%)	0.042	10 (24%)	0.044
PCI > 20	17 (41%)	7 (17%)		5 (12%)		5 (12%)	
AGE AT DIAGNOSIS (AAD)							
AAD < 60 years	30 (71%)	12 (28%)	0.032	8 (19%)	0.039	10 (24%)	0.025
AAD > 60 years	12 (29%)	2 (5%)		5 (12%)		5 (12%)	

LOW RISK (LR) = Immunohistochemical score (IHCS) Of -2 to -1; MEDIUM RISK (MR) = IHCS of 0 to +1; HIGH RISK (HR) = IHCS of +2; χ^2 = Student T test or Mann Whitney U test when sample is small (*P* values < 0.05 are considered significant).

Owing to the rarity of the disease compared to pleural mesothelioma (PM), there has been less emphasis on studies related to MPM. This is partly due to the assumption that MPM and PM are similar diseases. Although, they both originate on the mesothelial lining, the anatomical differences may differentiate these two forms of mesothelioma. Our earlier studies (under review) have shown that PMP have specific cytoplasmic ER-β that portend poor survival. These have never been reported in PM. We have also examined the distribution of other biomarkers such as MUC1, Ki67 and BCL2, in relation to patient survival. These studies show a definite link between the expression of these biomarkers and survival (under review).

Although prognostic studies using single biomarkers are widely used in cancer, mainly for convenience and ease of application, the accuracy of predicting survival may be compromised [20]. The use of more than one marker in validating prognosis may be a more reliable method. Ki67 is an immunohistochemical biomarker that has been widely used in prognostic evaluation of a number of cancers [21-25]. It is an indicator of the proliferation status of the cell and is found to be expressed in only replicating cells [26]. B-cell lymphoma 2 protein (BCL2) is an anti-apoptotic protein which also possesses an anti-proliferative effect influencing cell cycle entry [27-30]. BCL2 has been shown to be predictive of favourable outcome, particularly in breast cancer [31]. Hence, the evaluation of BCL2 along with Ki67 could add valuable information to prognosis. In fact BCL2 has recently been combined with mitotic index as a method of modifying tumour grade [32]. Further, more recent work with breast cancer, has indicated the value of combining Ki67 and BCL2 as a prognostic index in breast cancer [20].

Hence, in the current study, we set out to evaluate the prognostic value of combining Ki67 and BCL2 as an index to test our hypothesis that the combinatorial evaluation of these markers would provide a more robust method of measuring actively dividing cell is MPM.

Materials and method

Study population

This study was conducted with the approval of ethics committee of St. George Hospital

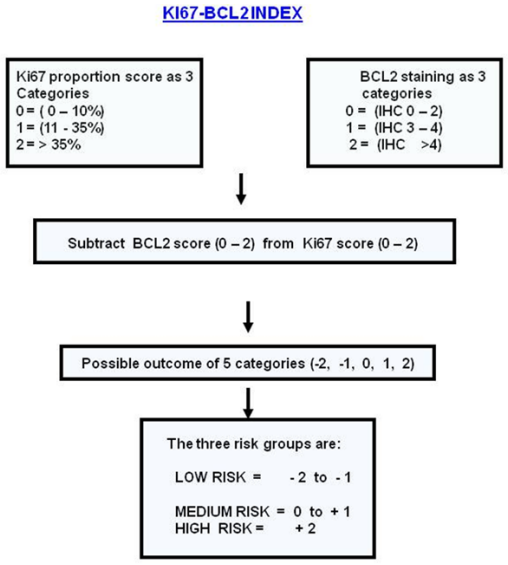

Figure 1. represents a schematic pathway for the generation of Ki67-BCL2 index.

(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, Jan 1999-Dec 2011. All participants were uniformly treated (within < weeks after diagnosis) with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (cisplatin and doxorubicin). Of the 42 patients, 40 (95%) had an optimal cytoreduction (CCRO/1) whilst 2 patients (5%) had incomplete cytoreduction (CCR2). The clinical features of these patients are summarized in **Table 1**.

Immunohistochemistry-BCL2

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 BCL2 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 mins, then washed again and incubated for 15 minutes with avidin – biotin – HRP complex as directed by Dako. Slides were the washed with PBS; and finally immunoprecipitation was visualized by treating with aminobenzidene tetrahydrochloride, Dako) for 30 minutes and counter staining with haematoxylin.

Immunohistochemical evaluation and controls

Three blinded and independent evaluation of the stained slides were carried out, without any knowledge of patient characteristics or other IHC markers. Discrepancies in evaluation were resolved by re-examination of the slides. Immunoreactivity was categorised as previously described [33-35] 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 4 and below, was classified as low score.

The final score was divided into three categories: 0 = None (IHC score 0 – 2); 1 = Moderate (IHC score 3 – 4); 2 = strong (IHC score > 4).

Immunohistochemistry (IHC), analysis & scoring of Ki67

For all IHC staining, 3 μm sections mounted on glass slides were used. Pre-treatment of slides before staining were adopted as in Rabiau et al [36], and for pKi67 from Fasching et al [37]. Briefly Ki67 index was determined by labelled streptavidin biotin method using the Ki67 antibody, MIB-1 (DAKO, K5001, Copenhagen, Denmark). Antigen retrieval was performed in a microwave oven in citrate buffer for 15 minutes. Slides were stained in TechMate 500 (DAKO) with an incubation time for 25 minutes at room temperature and with MIB-1 at 1: 200 dilutions. Diaminobenzidene was used as a chromogen. Negative controls were included with omission of MIB-1.

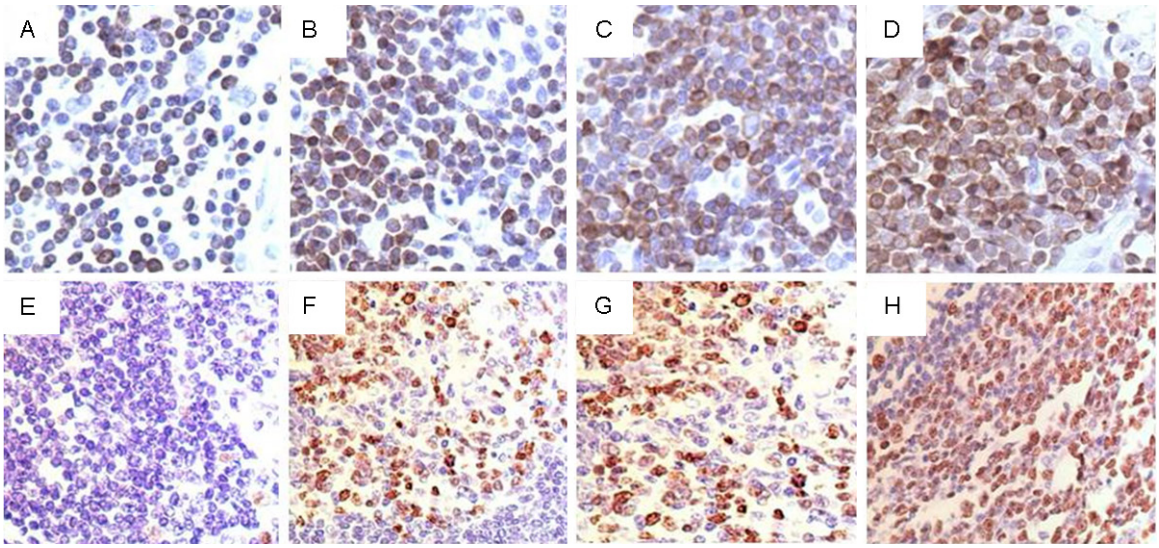

Figure 2. Represents immunohistochemical staining of tumour samples from different patient samples. A-D are BCL2 staining as described in methods section, the different samples show varying abundance and intensity of brown stain. E, F represents Ki67 staining from different patient tumour samples and E shows a complete absence of Ki67 staining whilst F-H have brown staining of varying abundance.

All image analysis was carried out using ZEISS AXIO VISION 3.1. Stained sections were examined using light microscope (ZEISS Axio-Vision) X 40 objective using a 10 X 10 eye piece graticule. Ki67 was defined as the percentage of total number of tumour cells (at least 1000) with nuclear staining over 10 high powered fields (X 40). Three independent evaluations were carried out blinded to patient outcome and any discrepancies were resolved by re-examination. Scoring was as follows: (< 10%), (≥ 10 - < 25%), (≥ 25 - < 35%), (≥ 35 - < 45%), (≥ 45 - < 55%), (≥ 55%). All expression below 25% was taken as low expression; the remaining was classified as high.

Ki67 IHC score was then divided into three categories: 0 = IHC score of 0-10%; 1 = IHC score of 11-35%; 2 = IHC score of > 35%. The final scores obtained for Ki67 and BCL2 were incorporated into a prognostic model as shown in **Figure 1**.

Statistical analysis

Statistical analysis was performed using GRAPHPAD PRISM (version 5.0). Association between two clinicopathological categories was evaluated using the X^2 test and Mann-Whitney U test where applicable. Overall survival of patients expressing low and high Ki67 in different clinicopathological categories were compared according to Kaplan-Meier method. Overall survival was measured from the date of surgery to date of last follow up examination or death. Differences between the survival curves were tested using the log-rank test. Univariate and multivariate analysis were performed using the Cox proportional hazard regression model. For multivariate model, 0.05 (95% confidence interval) was used as the cut off *P* value to select the analysed factors as significant.

Results

Immunohistochemistry

There was a wide variability of IHC staining in the tumour samples for both the BCL2 and Ki67 antigens. A few samples did not show any staining at all for the antigens that was examined and they were classified as negative (0). This may not be correct as the expression of antigens may vary with the tumour location. The classification of BCL2 and Ki67 expression has been outlined earlier in the methods section (**Figure 2**).

Distribution of Ki67-BCL2 index

Examining the total tumour samples, the percentage distribution of tumours in the Ki67-BCL2 index for the three groups, Low risk (LR), Medium risk (MR) & High risk (HR) are almost

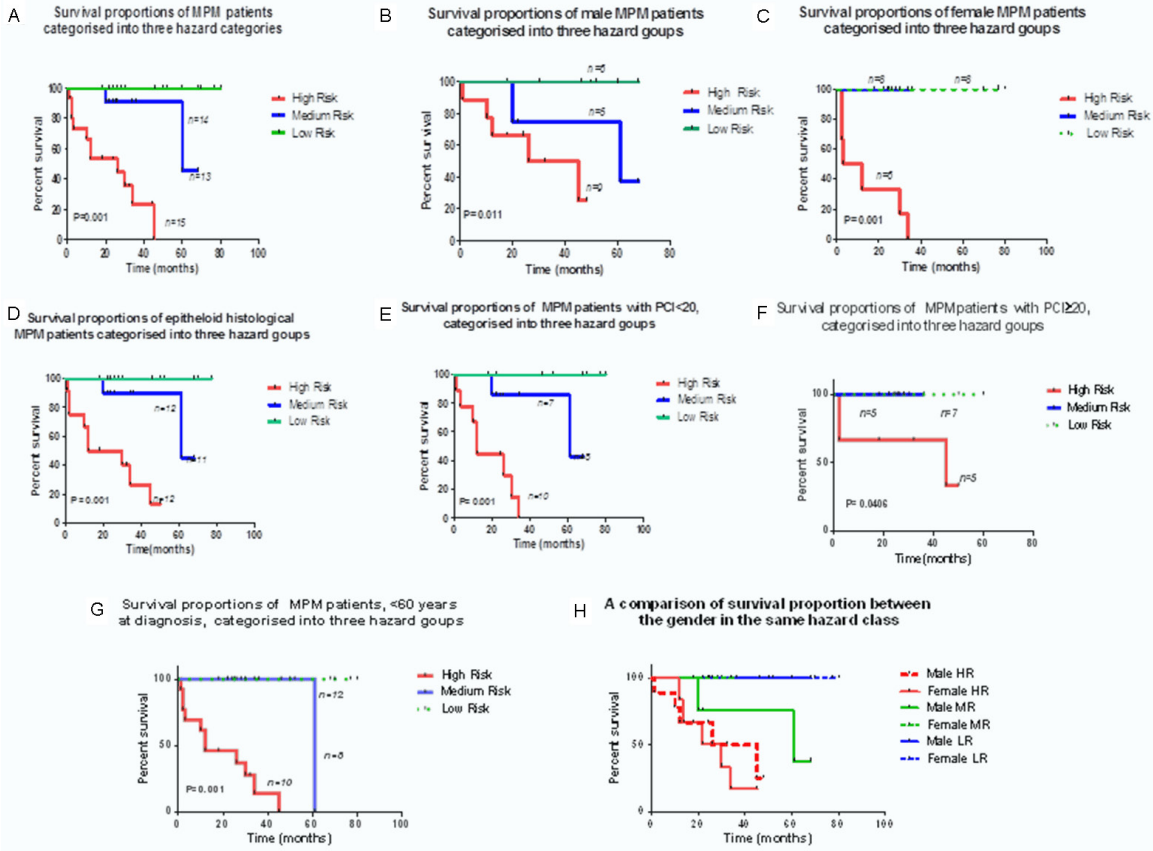

Figure 3. Are Kaplan-Meier Survival Curves, a depicting survival trends in total patient samples in three categories (High Risk, Medium Risk & Low Risk); B in male samples; C in female; D in epitheloid histological category; E For PCI < 20; F for PCI ≥ 20; G in age group at diagnosis < 60 years and H indicates survival within the three hazard categories between the gender.

similar (33, 31 and 36% respectively). There was also a similar median age between the three risk groups (50, 49 and 50). The youngest patient was found in MR group. (**Table 1** and **Figure 3**).

There were almost an equal percentage of males and females (48% vs. 52%, respectively) in the total sample. In the males out of 48% tumours, 14% fell in LR group, whilst 12% in MR group, the remaining 21% was in HR group, indicating that a high percentage of tumours in the males belonged to the High risk group. In the female, out 52% tumours, equal percentage (19%) belonged to LR and MR group, whilst a slightly smaller percentage (14%) fell into HR group, indicating that the females were favourably distributed within the less hazardous group.

The histology was mainly epitheloid (83%) with a minority of sarcomatoid histology (17%). There was an even distribution of tumours in the three risk groups for epitheloid histology. However, in the male epithelial category, there was a higher percentage of tumours in HR group (17%), compared to the female HR – 12%).

In the sarcomatoid category, the percentage of males: females was almost similar (4: 3), however, there were twice the percentage of tumours for males in the HR group when compared to females (2: 1).

Majority of the patients 59% belonged to PCI < 20 and of which 17% fell into LR, whilst 19% belonged to MR group. The remaining 24% fell into the HR group. For the PCI ≥ 20, out of 41% patients, 17% fell into LR and 12% in MR group, the remaining (12%) went into HR group. In the high risk group, PCI < 20 had a higher percentage of tumours, compared to ≥ 20 (24%: 12%).

Table 2. Univariate analysis and prognosis in different clinicopthological categories of malignant peritoneal mesothelioma (N = 42)

VARIABLES	HR (CI 95%)	*P*
ALL TUMORS		
High Risk vs. Low Risk	3.13 (0.89-11.2)	0.001
Medium Risk vs.“	3.0 (0.91-24.6)	0.057 NS
High Risk vs. Medium Risk	2.3 (4.7-14.6)	0.001
GENDER		
Male vs. Female	2.0 (0.64-7.1)	0.007
Male – High Risk vs. Low Risk	1.82 (0.3-11.11)	0.016
Male – Medium Risk vs.“	1.53 (0.42-26.6)	0.169 NS
Male – High Risk vs. Medium Risk	1.1 (0.11-3.10)	0.225 NS
Female – High Risk vs. Low Risk	4.36 (0.77-24.6)	0.005
Female – Medium Risk vs. “	-	-
Female – High Risk vs. Medium risk	3.14 (0.56-17.0)	0.001
HISTOLOGY		
Sarcomatoid vs. Epitheloid	2.0 (0.64-7.10)	0.001
Epitheloid – High Risk vs. Low Risk	2.6 (0.67-10.10)	0.002
Epithelod – Medium Risk vs.“	2.1 (0.63-14.6)	0.101 NS
Epitheloid – High risk vs. Medium Risk	1.3 (0.37-4.64)	0.004
PERITONEAL CANCER INDEX (PCI)		
PCI ≥ 20 vs. <20	1.1 (0.3-3.8)	0.013
PCI < 20 – High Risk vs. Low Risk	3.1 (0.7-13.7)	0.003
“ Medium Risk vs. Low Risk	2.37 (0.68-20.5)	0.089 NS
“ High Risk vs. Medium Risk	1.47 (0.38-5.69)	0.004
PCI ≥ 20 – High Risk vs. Low Risk	2.2 (1.0-20.7)	0.049
“ Medium Risk vs. Low Risk	-	-
“ High Risk vs. Medium Risk	1.5 (0.08-28.84)	0.173 NS
AGE AT DIAGNOSIS (AAD)		
AAD ≥ 60 years vs. < 60 years	3.1 (1.0-9.1)	0.001
AAD < 60 – High Risk vs. Low Risk	2.9 (0.22-19.2)	0.001
AAD < 60 – Medium Risk vs. Low Risk	1.5 (0.9-12.1)	0.045
AAD < 60 – High Risk vs. Medium Risk	1.3 (0.41-4.58)	0.002

LOW RISK = Immunohistochemical score (ICHS) of -2 to -1; MEDIUM RISK = IHCS of 0 to 1; HIGH RISK = ICHS of +2. NS = Not Significant; CI = 95% confidence Interval; *P* values < 0.05 are considered significant.

The age at diagnosis (AAD) shows that majority of the patients 71% were < 60 years and the remaining 29% were above 60 years. In the age group < 60 years, majority (28%) fell into LR group, whilst another 19% in the MR group and the remaining 24% in the HR group. The difference in distribution of tumours between the LR and HR was not very significant (28 vs. 24%, respectively), a difference of 4% in favour of age group < 60 years.

In the AAD ≥ 60 years, the total percentage of tumours was almost half that found in AAD < 60 years. (71 vs. 29%). More noticeably, the percentage of LR tumours were much lower compared to MR and HR tumours ratio being 5: 12: 12%, indicating that this category of age may carry a higher risk of death.

Clinical outcome

The clinical outcome in this study is measured by survival in patients as monitored from the date of diagnosis and treatment to death or survival within the last 10 years. Using Kaplan-Meier analysis, with log-Rank and Cox-proportional Hazard Tests, we were able to determine how the Ki67-BCL2 index affect prognosis in the three classification of patient groups (LR, MR & HR). Both univariate and multivariate analysis were carried out.

Univariate analysis

Of the clinicopathological variables examined by univariate analysis for Ki67-BCL2 index, high risk groups were correlated to poor survival when compared to low risk group in the following categories: All tumours *(p = 0.002)*; Male *(p = 0.016);* Female *(p = 0.005);* Epitheloid histology (p = 0.008); PCI < 20 *(p = 0.003);* PCI ≥ 20 *(p = 0.049),* and AAD < 60 years *(p = 0.001).*

When medium risk group was compared to low risk group for survival difference, there was no significant difference, excepting in age at diagnosis < 60 years *(p = 0.045).*

For other variables independent of Ki67-BCL2 index, the following carried poor prognosis: Males *(p = 0.007);* Sarcomatoid histology *(p = 0.001);* PCI ≥ 20 *(p = 0.013)* and AAD ≥ 60 years *(p = 0.001)* (**Table 2**).

Further comparisons of survival between the genders belonging to the same hazard class

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

VARIABLES	HR (CI 95%)	*P*
ALL TUMORS		
High Risk vs. Low Risk	4.8 (1.2-14.2)	0.016
GENDER		
Male vs. Female	2.1 (0.7-6.6)	0.166 NS
Male – High Risk vs. Low Risk	2.4 (0.9-14.6)	0.049
Female – High Risk vs. Low Risk	5.7 (1.2-30.9)	0.045
Female – High Risk vs. Medium Risk	3.22 (0.66-19.0)	0.065 NS
HISTOLOGY		
Sarcomatoid vs. Epitheloid	3.3 (0.1-10)	0.117 NS
Epitheloid – High Risk vs. Low Risk	4.6 (1.6-12.8)	0.058 NS
Epitheloid – High risk vs. Medium Risk	1.9 (0.7-5.6)	0.062 NS
PERITONEAL CANCER INDEX (PCI)		
PCI ≥ 20 vs. < 20	1.0 (0.3-3.8)	0.998 NS
PCI < 20 – High Risk vs. Low Risk	3.8 (1.7-23.9)	0.116 NS
PCI ≥ 20 – High Risk vs. Low Risk	4.2 (2.62-30.7)	0.086 NS
Medium Risk vs. Low Risk	-	-
AGE AT DIAGNOSIS (AAD)	3.3 (1.6-16.4)	0.049
AAD ≥ 60 years vs. < 60 years		
AAD < 60 – High Risk vs. Low Risk	3.4 (1.34-26.4)	0.001
AAD < 60 – Medium Risk vs. Low Risk	2.8 (1.2-15.6)	0.089 NS
AAD < 60 – High Risk vs. Medium Risk	1.5 (0.53-6.44)	0.036

LOW RISK = Immunohistochemical score (ICHS) of -2 to -1; MEDIUM RISK = IHCS of 0 to 1; HIGH RISK = ICHS of +2. NS = Not Significant; CI = 95% confidence Interval; *P* values < 0.05 are considered significant. For all categories, only those that were significant (P = < 0.05) were entered into multivariate analysis.

(**Figure 3H**) showed no statistical difference in survival between the sexes in high risk, low risk and medium risk category, indicating that no gender difference exists using the current model.

Multivariate analysis

Only variables that tested to be significant in univariate analysis were entered into multivariate analysis. Of the variables tested, high risk group in Ki67-BCL2 index were correlated with poor survival in the following categories: All tumours, HR 4.8 (95% CI 1.2-14.2), p = 0.016; Males, HR 2.4 (CI 0.9-14.6), *p = 0.049*; Females, HR 5.7 (CI 1.2-30.9), *p = 0.045*; AAD < 60 years, HR 3.4 (CI 1.34-26.4), *p = 0.001.* Amongst the variables tested independent of Ki67-BCL2 index, only AAD ≥ 60 years was a poor prognostic factor, HR 3.3 (CI 1.6-16.4), *p = 0.049* (**Table 3**).

Although Ki67 detection by immunohistochemistry has been used to gauge prognosis in several cancers [21-25], its utility in malignant peritoneal mesothelioma is totally lacking. One of the main reasons for not using a biomarker to determine proliferation index in MPM is that the disease is normally confined to the abdominal cavity, with very little spread beyond [38, 39]. However, a group has recently recommended the use of Ki67 for prognostication in malignant mesothelioma, based on their case studies [40]. We have examined the expression of Ki67 in 42 MPM tumours and have concluded that high expression of this antigen correlates with poor survival and that its expression determines survival in all the individual clinicopathological categories that we have examined.

It is also known that high Ki67 expression is a an indicator of good prognosis in breast cancer since the ER positive tumours respond better to ER antagonist, when they have high expression of Ki67 [41, 42]. Other reports indicate that pleural mesothelioma expressing ER-β respond to agonist (in vitro studies) [18] with evidence to suggest that ER is linked functionally to EGFR and that the proliferative action of EGFR can be silenced with an antagonist, perifosine [19]. Although the latter studies did not examine Ki67 expression, there is a strong likelihood that these tumour cells may have had high expression of Ki67. The response to treatment modality of high Ki67 expressing tumour cells is an indication of the predictive nature of the antigen, rather than prognosis since in the absence of treatment, it may generally lead to poor prognosis [43]. Fast replicating cells (indicated by High expression of Ki67) are generally more receptive to treatment and hence response may generally be better in these tumours [41, 42, 44]. Hence, Ki67 seem to hold both a prognostic and predictive role in tumour cells [45, 46].

BCL2 is an anti-apoptotic oncoprotein that accelerates cell proliferation and tumour growth [47]. Over expression is found in a variety of tumours and lymphomas [48]. However, in many solid organ tumours, including breast and colon cancers, BCL2 appears to exert a tumour suppressive function [49-51]. One of the early studies that investigated expression of BCL2 in malignant pleural mesothelioma, came to the conclusion that BCL2 did not add any useful information to prognosis [52]. However, our earlier studies on BCL2 in MPM seems to contradict this finding and this may be due to several factors, one factor being the anatomical difference between malignant peritoneal and malignant pleural mesothelioma. In addition the heterogeneity of tumour tissue sampling may add to the difference. Hence, we found that the expression of BCL2 to be a useful prognostic marker (unpublished data).

Since, our earlier studies has indicated the prognostic significance of Ki67 and BCL2, with high expression of Ki67 leading to poor prognosis whilst the opposite was the case with BCL2, we decided to combine the expression of these two proteins in such a way as to give a new model for evaluating prognosis. A similar model has been demonstrated in breast cancer [20]. In the current study with MPM, based on their hazard scores, we were able to divide them into three groups, low risk, median risk and high risk. Generally patients belonging to high risk group had poor survival compared to low risk group in all the clinicopathological categories. Comparison of high risk to median risk groups indicated that the median risk group had better prognosis in all tumours, in females, PCI < 20 and age at diagnosis < 60 years by univariate analysis, however only age at diagnosis < 60 years persisted by multivariate analysis. Survival difference existed for only age at diagnosis < 60 years when medium risk group was compared to low risk by univariate analysis. Hence, using the Ki67/bcl2 index, there is a clear indication of poor prognosis in high risk group compared to low risk group or medium risk groups.

When we examined the percentage distribution of gender within the high and low risk groups, we found that a very much higher percentage of males belonged to the high risk group (HR), ratio of low to high (2: 3) as compared to females (3: 2 approximately). In the epitheloid histological category, there existed a higher percentage of males in the HR group compared to females, ratio being 3: 2. In the sarcomatoid category twice the amount of tumours belonged to high risk category for the males, compared to the females. Although without using the Ki67-BCL2 index, a gender difference is observed by univariate analysis, it did not persist by multivariate analysis. However, using the Ki67-BCL2 index, both in the males and the females, high risk group predisposed the patients to poorer prognosis compared to low risks and this finding persisted by multivariate analysis, indicating that the current model is able to pick up gender difference that is observed in MPM, as reported in other studies [11, 53].

In MPM, sarcomatoid histology generally predicts poor patient survival, as compared to epitheloid histology [54]. Independent of Ki67-BCL2 index, the comparison of survival between sarcomatoid and epitheloid tumours seems to indicate that the former histology carries poor prognosis, by univariate analysis that did not persist by multivariate analysis. However, using the Ki67-BCL2 index, we were able to show in the epitheloid category, that high risk patients had poorer survival compared to low risk patients. A similar finding existed when we compared high risk to medium risk, however this did not persist in multivariate analysis. Hence, although prognosis as predicted by the current model seems to only persist by univariate analysis, it indicates that Ki67-BCL2 index within the epitheloid category may decide on survival. We did not examine prognosis in sarcomatoid category using this model since the patient number was very small.

PCI has been proposed as a prognostic factor in MPM, in several studies. Our earlier study comparing survival between PCI ≥ 20 to < 20 did not show any significance by multivariate analysis. In the present study with Ki67/BCL2 index, high risk patients in both the PCI categories had poor prognosis, however, it did not persist by multivariate analysis. Further, our analysis indicated that PCI score of < 20 had a greater percentage of tumours with higher risk as compared to PCI ≥ 20. High PCI has been shown to be a poor prognostic factor in MPM in some studies [9, 10]. PCI is an indicator of the spread of tumours within the thirteen quadrants in the abdominal cavity [55]. MPM are

Table 4. shows the hazard ratios (HR) with 95% confidence interval for three biomarker models

Prognostic variable	Ki67/BCL2 index	Ki67	BCL2
	HR vs. LR	HE vs. LE	LE vs. HE
All Tumours	3.13 (0.89-11.2)	4.55 (1.3-15.1)	6 (2.0-47)
Male	1.82 (0.3-11.11)	3.54 (0.7-17)	4.3 (1.15-15.9)
Female	4.36 (0.77-24.1)	2.77 (0.48-15.8)	7.0 (1.6330.25)
Epitheloid	2.6 (0.67-10.1)	6.23 (3.78-57.2)	5.5 (1.57-19.62)
PCI < 20	3.1 (0.7-13.7)	3.2 (0.8-12.7)	4.1 (1.0-16.4)
PCI ≥ 20	2.2 (1.0-20.7)	10 (1.5-58)	4.2 (0.56-31.6)
Age < 60 years	2.9 (0.22-19.2)	4.3 (0.33-57.4)	5.9 (1.72-20.47)

HR = High Risk; LR = Low Risk; HE = High Expression (immunohistochemical score = 5-8); LE = Low Expression (immunohistochemical score = 0-4); PCI = peritoneal cancer index. The *p* values for all HR are < 0.05.

generally diagnosed very late in the disease and hence progression of the disease within the abdominal cavity may be expected, however, progression or spread within the cavity may not be an indicator of tumour aggression or potency. Ki67 is an indicator of tumour replication, high level of Ki67 is indicative of active tumour turnover and hence may also reflect on cancer spread [56, 57]. Therefore, using the current model, our findings of a negative correlation of PCI with prognosis is probably a true reflection of the nature of the disease. PCI may indicate the extent of local spread of the disease although it does not indicate the rate of progression of the disease. Ki67 expression or Ki67-BCL2 index would probably give an accurate indication of progression and prognosis when compared to PCI. This may be clarified if a study is conducted with a large sample size.

MPM patients are normally diagnosed in their late 5th or 6th decade of life and hence, studies have indicated that older patients generally have poor prognosis. This is true of many other cancers such as bladder, lung and renal [58-60]. This finding may be related to co-morbidities that are more common with the older patients [61, 62]. In the case of MPM, several studies have indicated that age is a significant prognostic factor [63, 64]. All our earlier studies have indicated that age at diagnosis is correlated with survival. In the current study using the Ki67-BCL2 index, we further confirm that age at diagnosis is an independent factor that predicts survival. High risk groups in this category are greater at risk of death compared to either low or medium risk groups.

Before developing the Ki67-BCL2 index, we have conducted biomarker study on individual biomarkers. Hence, we made a comparison of the hazard ratios (HR), as determined by univariate analysis, in various clinicopathological categories with the three models as shown in **Table 4**. Based on the observation of hazard ratio values, the Ki67-BCL2 index seems to have a more conservative value for the various HRs. This seems to indicate that the high HRs of individual biomarker has been toned down owing to the interactive action of the two biomarkers that are working in opposition to each other. This is an indication of attenuation most probably to produce a more reliable and accurate value for prognosis.

The use of combinatorial prognostic markers such as Ki67-BCL2 index have been found to be more superior compared to the use of single biomarker [20] since validation of the model carried out on a different large cohort sample showed robust performance. Other studies on combinatorial biomarkers in prognosis also indicate the advantage of using more than one biomarker [65, 66]. The use of Ki67-BCL2 index has never been reported in either MPM or pleural malignant mesothelioma, hence we are the first to evaluate such a model. However, we were unable to validate the model further because we do not have another independent sample to validate the performance of the current biomarker model. This is mainly due to the rarity of the disease and hence forth a small population sample. Hence, a multi-institutional study involving a substantial population is warranted.

Disclosure of conflict of interest

All authors declare that there is no conflict of interest.

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Reference

[1] Robinson BW, Musk AW and Lake RA. Malignant mesothelioma. Lancet 2005; 366: 397-408.

[2] Suzuki Y, Yuen SR and Ashley R. Short, thin asbestos fibers contribute to the development of human malignant mesothelioma: pathological evidence. Int J Hyg Environ Health 2005; 208: 201-210.

[3] Manzini Vde P, Recchia L, Cafferata M, Porta C, Siena S, Giannetta L, Morelli F, Oniga F, Bearz A, Torri V and Cinquini M. Malignant peritoneal mesothelioma: a multicenter study on 81 cases. Ann Oncol 2010; 21: 348-353.

[4] Kinoshita Y, Takasu K, Yuri T, Yoshizawa K, Uehara N, Kimura A, Miki H, Tsubura A and Shikata N. Two cases of malignant peritoneal mesothelioma without asbestos exposure: cytologic and immunohistochemical features. Ann Diagn Pathol 2013; 17: 99-103.

[5] Baratti D, Kusamura S, Cabras AD, Laterza B, Balestra MR and Deraco M. Lymph node metastases in diffuse malignant peritoneal mesothelioma. Ann Surg Oncol 2010; 17: 45-53.

[6] Sugarbaker PH, Welch LS, Mohamed F and Glehen O. A review of peritoneal mesothelioma at the Washington Cancer Institute. Surg Oncol Clin N Am 2003; 12: 605-21, xi.

[7] Pillai K, Akhter J, Pourgholami MH and Morris DL. Peritoneal mesothelioma in a woman who has survived for seven years: a case report. J Med Case Rep 2011; 5: 36.

[8] Sugarbaker DJ, Wolf AS, Chirieac LR, Godleski JJ, Tilleman TR, Jaklitsch MT, Bueno R and Richards WG. Clinical and pathological features of three-year survivors of malignant pleural mesothelioma following extrapleural pneumonectomy. Eur J Cardiothorac Surg 2011; 40: 298-303.

[9] Yan TD, Deraco M, Elias D, Glehen O, Levine EA, Moran BJ, Morris DL, Chua TC, Piso P, Sugarbaker PH and Peritoneal Surface Oncology G. A novel tumor-node-metastasis (TNM) staging system of diffuse malignant peritoneal mesothelioma using outcome analysis of a multi-institutional database*. Cancer 2011; 117: 1855-63.

[10] Yan TD, Brun EA, Cerruto CA, Haveric N, Chang D and Sugarbaker PH. Prognostic indicators for patients undergoing cytoreductive surgery and perioperative intraperitoneal chemotherapy for diffuse malignant peritoneal mesothelioma. Ann Surg Oncol 2007; 14: 41-49.

[11] Cao C, Yan TD, Deraco M, Elias D, Glehen O, Levine EA, Moran BJ, Morris DL, Chua TC, Piso P, Sugarbaker PH and Peritoneal Surface Malignancy G. Importance of gender in diffuse malignant peritoneal mesothelioma. Ann Oncol 2012; 23: 1494-1498.

[12] Osborne CK and Schiff R. Estrogen-receptor biology: continuing progress and therapeutic implications. J Clin Oncol 2005; 23: 1616-1622.

[13] Ring A and Dowsett M. Mechanisms of tamoxifen resistance. Endocr Relat Cancer 2004; 11: 643-658.

[14] Joshi MD, Ahmad R, Yin L, Raina D, Rajabi H, Bubley G, Kharbanda S and Kufe D. MUC1 oncoprotein is a druggable target in human prostate cancer cells. Mol Cancer Ther 2009; 8: 3056-3065.

[15] Kulasingam V and Diamandis EP. Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. Nat Clin Pract Oncol 2008; 5: 588-599.

[16] Herbst RS. Erlotinib (Tarceva): an update on the clinical trial program. Semin Oncol 2003; 30: 34-46.

[17] Pinton G, Brunelli E, Murer B, Puntoni R, Puntoni M, Fennell DA, Gaudino G, Mutti L and Moro L. Estrogen receptor-beta affects the prognosis of human malignant mesothelioma. Cancer Res 2009; 69: 4598-4604.

[18] Pinton G, Thomas W, Bellini P, Manente AG, Favoni RE, Harvey BJ, Mutti L and Moro L. Estrogen receptor beta exerts tumor repressive functions in human malignant pleural mesothelioma via EGFR inactivation and affects response to gefitinib. PLoS One 2010; 5: e14110.

[19] Pinton G, Manente AG, Angeli G, Mutti L and Moro L. Perifosine as a potential novel anticancer agent inhibits EGFR/MET-AKT axis in malignant pleural mesothelioma. PLoS One 2012; 7: e36856.

[20] Ali HR, Dawson SJ, Blows FM, Provenzano E, Leung S, Nielsen T, Pharoah PD and Caldas C. A Ki67/BCL2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer. J Pathol 2012; 226: 97-107.

[21] Viale G, Giobbie-Hurder A, Regan MM, Coates AS, Mastropasqua MG, Dell'Orto P, Maiorano E, MacGrogan G, Braye SG, Ohlschlegel C, Neven P, Orosz Z, Olszewski WP, Knox F, Thurlimann B, Price KN, Castiglione-Gertsch M, Gelber RD, Gusterson BA, Goldhirsch A and Breast International Group T. Prognostic and predictive value of centrally reviewed Ki-67 labeling index in postmenopausal women with endocrine-responsive breast cancer: results from Breast International Group Trial 1-98 comparing adjuvant tamoxifen with letrozole. J Clin Oncol 2008; 26: 5569-5575.

[22] Lazar D, Taban S, Sporea I, Dema A, Cornianu M, Lazar E, Goldis A and Vernic C. Ki-67 expression in gastric cancer. Results from a prospective study with long-term follow-up. Rom J Morphol Embryol 2010; 51: 655-661.

[23] Yang J, Ramnath N, Moysich KB, Asch HL, Swede H, Alrawi SJ, Huberman J, Geradts J, Brooks JS and Tan D. Prognostic significance of MCM2, Ki-67 and gelsolin in non-small cell lung cancer. BMC Cancer 2006; 6: 203.

[24] Aune G, Stunes AK, Tingulstad S, Salvesen O, Syversen U and Torp SH. The proliferation markers Ki-67/MIB-1, phosphohistone H3, and survivin may contribute in the identification of aggressive ovarian carcinomas. Int J Clin Exp Pathol 2011; 4: 444-453.

[25] Berney DM, Gopalan A, Kudahetti S, Fisher G, Ambroisine L, Foster CS, Reuter V, Eastham J, Moller H, Kattan MW, Gerald W, Cooper C, Scardino P and Cuzick J. Ki-67 and outcome in clinically localised prostate cancer: analysis of conservatively treated prostate cancer patients from the Trans-Atlantic Prostate Group study. Br J Cancer 2009; 100: 888-893.

[26] Gerdes J, Schwab U, Lemke H and Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983; 31: 13-20.

[27] Zinkel S, Gross A and Yang E. BCL2 family in DNA damage and cell cycle control. Cell Death Differ 2006; 13: 1351-1359.

[28] Greider C, Chattopadhyay A, Parkhurst C and Yang E. BCL-x(L) and BCL2 delay Myc-induced cell cycle entry through elevation of p27 and inhibition of G1 cyclin-dependent kinases. Oncogene 2002; 21: 7765-7775.

[29] Pietenpol JA, Papadopoulos N, Markowitz S, Willson JK, Kinzler KW and Vogelstein B. Paradoxical inhibition of solid tumor cell growth by bcl2. Cancer Res 1994; 54: 3714-3717.

[30] Hockenbery D, Nunez G, Milliman C, Schreiber RD and Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 1990; 348: 334-336.

[31] Dawson SJ, Makretsov N, Blows FM, Driver KE, Provenzano E, Le Quesne J, Baglietto L, Severi G, Giles GG, McLean CA, Callagy G, Green AR, Ellis I, Gelmon K, Turashvili G, Leung S, Aparicio S, Huntsman D, Caldas C and Pharoah P. BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. Br J Cancer 2010; 103: 668-675.

[32] Abdel-Fatah TM, Powe DG, Ball G, Lopez-Garcia MA, Habashy HO, Green AR, Reis-Filho JS and Ellis IO. Proposal for a modified grading system based on mitotic index and Bcl2 provides objective determination of clinical outcome for patients with breast cancer. J Pathol 2010; 222: 388-399.

[33] Harvey JM, Clark GM, Osborne CK and Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999; 17: 1474-1481.

[34] Allred DC, Harvey JM, Berardo M and Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998; 11: 155-168.

[35] Allred DC, Clark GM, Elledge R, Fuqua SA, Brown RW, Chamness GC, Osborne CK and McGuire WL. Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. J Natl Cancer Inst 1993; 85: 200-206.

[36] Rabiau N, Dechelotte P, Guy L, Satih S, Bosviel R, Fontana L, Kemeny JL, Boiteux JP, Bignon YJ and Bernard-Gallon D. Immunohistochemical staining of mucin 1 in prostate tissues. In Vivo 2009; 23: 203-207.

[37] Fasching PA, Heusinger K, Haeberle L, Niklos M, Hein A, Bayer CM, Rauh C, Schulz-Wendtland R, Bani MR, Schrauder M, Kahmann L, Lux MP, Strehl JD, Hartmann A, Dimmler A, Beckmann MW and Wachter DL. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer 2011; 11: 486.

[38] Bridda A, Padoan I, Mencarelli R and Frego M. Peritoneal mesothelioma: a review. MedGenMed 2007; 9: 32.

[39] Yan TD, Stuart OA, Yoo D and Sugarbaker PH. Perioperative intraperitoneal chemotherapy for peritoneal surface malignancy. J Transl Med 2006; 4: 17.

[40] Hirano H, Fujisawa T, Maekawa K, Ohkubo E, Okimura A, Kuribayashi K, Nakano T, Nakasho K and Nishigami T. Malignant mesothelioma of the peritoneum: case reports and immunohistochemical findings including Ki-67 expression. Med Mol Morphol 2010; 43: 53-59.

[41] Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R, Salter J, Detre S, Hills M, Walsh G and Group IT. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J Natl Cancer Inst 2007; 99: 167-170.

[42] Baum M, Budzar AU, Cuzick J, Forbes J, Houghton JH, Klijn JG, Sahmoud T and Group AT. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC

randomised trial. Lancet 2002; 359: 2131-2139.
[43] Urruticoechea A, Smith IE and Dowsett M. Proliferation marker Ki-67 in early breast cancer. J Clin Oncol 2005; 23: 7212-7220.
[44] Luporsi E, Andre F, Spyratos F, Martin PM, Jacquemier J, Penault-Llorca F, Tubiana-Mathieu N, Sigal-Zafrani B, Arnould L, Gompel A, Egele C, Poulet B, Clough KB, Crouet H, Fourquet A, Lefranc JP, Mathelin C, Rouyer N, Serin D, Spielmann M, Haugh M, Chenard MP, Brain E, de Cremoux P and Bellocq JP. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: analytical and critical review. Breast Cancer Res Treat 2012; 132: 895-915.
[45] Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, McShane L, Paik S, Penault-Llorca F, Prudkin L, Regan M, Salter J, Sotiriou C, Smith IE, Viale G, Zujewski JA, Hayes DF and International Ki-67 in Breast Cancer Working G. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011; 103: 1656-1664.
[46] Viale G, Regan MM, Mastropasqua MG, Maffini F, Maiorano E, Colleoni M, Price KN, Golouh R, Perin T, Brown RW, Kovacs A, Pillay K, Ohlschlegel C, Gusterson BA, Castiglione-Gertsch M, Gelber RD, Goldhirsch A, Coates AS and International Breast Cancer Study G. Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Natl Cancer Inst 2008; 100: 207-212.
[47] McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LW, Hsieh JT, Tu SM and Campbell ML. Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. Cancer Res 1992; 52: 6940-6944.
[48] Johnson NA, Savage KJ, Ludkovski O, Ben-Neriah S, Woods R, Steidl C, Dyer MJ, Siebert R, Kuruvilla J, Klasa R, Connors JM, Gascoyne RD and Horsman DE. Lymphomas with concurrent BCL2 and MYC translocations: the critical factors associated with survival. Blood 2009; 114: 2273-2279.
[49] Callagy GM, Webber MJ, Pharoah PD and Caldas C. Meta-analysis confirms BCL2 is an independent prognostic marker in breast cancer. BMC Cancer 2008; 8: 153.
[50] Hwang KT, Woo JW, Shin HC, Kim HS, Ahn SK, Moon HG, Han W, Park IA and Noh DY. Prognostic influence of BCL2 expression in breast cancer. Int J Cancer 2012; 131: E1109-1119.
[51] Poincloux L, Durando X, Seitz JF, Thivat E, Bardou VJ, Giovannini MH, Parriaux D, Barriere N, Giovannini M, Delpero JR and Monges G. Loss of Bcl-2 expression in colon cancer: a prognostic factor for recurrence in stage II colon cancer. Surg Oncol 2009; 18: 357-365.
[52] Segers K, Ramael M, Singh SK, Weyler J, Van Meerbeeck J, Vermeire P and Van Marck E. Immunoreactivity for bcl-2 protein in malignant mesothelioma and non-neoplastic mesothelium. Virchows Arch 1994; 424: 631-634.
[53] Yan TD, Popa E, Brun EA, Cerruto CA and Sugarbaker PH. Sex difference in diffuse malignant peritoneal mesothelioma. Br J Surg 2006; 93: 1536-1542.
[54] Klebe S, Brownlee NA, Mahar A, Burchette JL, Sporn TA, Vollmer RT and Roggli VL. Sarcomatoid mesothelioma: a clinical-pathologic correlation of 326 cases. Mod Pathol 2010; 23: 470-479.
[55] Elias D, Souadka A, Fayard F, Mauguen A, Dumont F, Honore C and Goere D. Variation in the peritoneal cancer index scores between surgeons and according to when they are determined (before or after cytoreductive surgery). Eur J Surg Oncol 2012; 38: 503-508.
[56] Chen JX, Deng N, Chen X, Chen LW, Qiu SP, Li XF and Li JP. A novel molecular grading model: combination of Ki67 and VEGF in predicting tumor recurrence and progression in non-invasive urothelial bladder cancer. Asian Pac J Cancer Prev 2012; 13: 2229-2234.
[57] Mian C, Pennelli G, Barollo S, Cavedon E, Nacamulli D, Vianello F, Negro I, Pozza G, Boschin IM, Pelizzo MR, Rugge M, Mantero F, Girelli ME and Opocher G. Combined RET and Ki-67 assessment in sporadic medullary thyroid carcinoma: a useful tool for patient risk stratification. Eur J Endocrinol 2011; 164: 971-976.
[58] Tseng CH, Chong CK, Tseng CP and Chan TT. Age-related risk of mortality from bladder cancer in diabetic patients: a 12-year follow-up of a national cohort in Taiwan. Ann Med 2009; 41: 371-379.
[59] Knoke JD, Shanks TG, Vaughn JW, Thun MJ and Burns DM. Lung cancer mortality is related to age in addition to duration and intensity of cigarette smoking: an analysis of CPS-I data. Cancer Epidemiol Biomarkers Prev 2004; 13: 949-957.
[60] Komai Y, Fujii Y, Iimura Y, Tatokoro M, Saito K, Otsuka Y, Koga F, Arisawa C, Kawakami S, Okuno T, Tsujii T, Kageyama Y, Morimoto S, Toma T, Higashi Y, Fukui I and Kihara K. Young age as favorable prognostic factor for cancer-specific survival in localized renal cell carcinoma. Urology 2011; 77: 842-847.

[61] Yancik R, Ganz PA, Varricchio CG and Conley B. Perspectives on comorbidity and cancer in older patients: approaches to expand the knowledge base. J Clin Oncol 2001; 19: 1147-1151.

[62] Janssen-Heijnen ML, Houterman S, Lemmens VE, Louwman MW, Maas HA and Coebergh JW. Prognostic impact of increasing age and comorbidity in cancer patients: a population-based approach. Crit Rev Oncol Hematol 2005; 55: 231-240.

[63] Antman K, Shemin R, Ryan L, Klegar K, Osteen R, Herman T, Lederman G and Corson J. Malignant mesothelioma: prognostic variables in a registry of 180 patients, the Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965-1985. J Clin Oncol 1988; 6: 147-153.

[64] Kerrigan SA, Turnnir RT, Clement PB, Young RH and Churg A. Diffuse malignant epithelial mesotheliomas of the peritoneum in women: a clinicopathologic study of 25 patients. Cancer 2002; 94: 378-385.

[65] Edgell T, Martin-Roussety G, Barker G, Autelitano DJ, Allen D, Grant P and Rice GE. Phase II biomarker trial of a multimarker diagnostic for ovarian cancer. J Cancer Res Clin Oncol 2010; 136: 1079-1088.

[66] Rakha EA, Reis-Filho JS and Ellis IO. Combinatorial biomarker expression in breast cancer. Breast Cancer Res Treat 2010; 120: 293-308.