The AA Genotype of the Regulatory *BCL2* Promoter Polymorphism (-938C>A) Is Associated with a Favorable Outcome in Lymph Node–Negative Invasive Breast Cancer Patients

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Abstract

Purpose: Expression of the antiapoptotic and antiproliferative protein Bcl-2 has been repeatedly shown to be associated with better clinical outcome in breast cancer. We recently showed a novel regulatory (-938C>A) single-nucleotide polymorphism (SNP) in the inhibitory P2 *BCL2* gene promoter generating significantly different *BCL2* promoter activities.

Experimental Design: Paraffin-embedded neoplastic and nonneoplastic tissues from 274 patients (161 still alive after a follow-up period of at least 80 months) with primary unilateral invasive breast carcinoma were investigated. Bcl-2 expression of tumor cells was shown by immunohistochemistry; nonneoplastic tissues were used for genotyping. Both the Bcl-2 expression and the (-938C>A) genotypes were correlated with the patients' survival.

Results: Kaplan-Meier curves revealed a significant association of the AA genotype with increased survival ($P = 0.030$) in lymph node–negative breast cancer patients, whereas no genotype effect could be observed in lymph node–positive cases. Ten-year survival rates were 88.6% for the AA genotype, 78.4% for the AC genotype, and 65.8% for the CC genotype. Multivariable Cox regression identified the *BCL2* (-938CC) genotype as an independent prognostic factor for cancer-related death in lymph node–negative breast carcinoma patients (hazard ratio, 3.59; $P = 0.032$). Immunohistochemical Bcl-2 expression was significantly associated with the clinical outcome of lymph node–positive but not of lymph node–negative breast cancer patients. In lymph node–negative cases, the (-938C>A) SNP was both significantly related with the immunohistochemically determined level of Bcl-2 expression ($P = 0.044$) and the survival of patients with Bcl-2–expressing carcinomas ($P = 0.006$).

Conclusions: These results suggest the (-938C>A) polymorphism as a survival prognosticator as well as indicator of a high-risk group within patients with lymph node–negative breast cancer.

Apoptosis and cellular proliferation play an important role during normal mammary development and carcinogenesis of the mammary gland (1). In normal tissues, the delicate homeostasis between proliferation and apoptosis is controlled by a variety of proteins of the Bcl-2 family. The Bcl-2 family of proteins consists of different apoptosis regulators that integrate diverse survival and death signals generated outside and inside the cell (2). This family is subdivided into two major classes, proapoptotic members such as Bax and Bak (BAX-like death factors), and antiapoptotic/antiproliferative members such as Bcl-2 and Bcl-xL (Bcl2-like survival factors), which protect cells from apoptosis by cell cycle arrest in G_0 (3). Until now, it remains obscure whether antiapoptosis and cell cycle inhibition by Bcl-2 and Bcl-xL are separate or identical functions (4). Interestingly, in tumorigenesis and cancer progression, these members of the Bcl-2 family can act in both oncogenic and tumor-suppressive manners. Which of the dual functions predominates seems to be tissue-specific as well as context-dependent; for example, increased Bcl-2 expression is associated with decreased survival in B-cell chronic lymphocytic leukemia (B-CLL; ref. 5), but with increased survival in colon cancer (6, 7). In breast cancer patients, various studies have shown that increased Bcl-2 expression is associated with a more favorable outcome (8, 9).

The *BCL2* gene is located on chromosome *18q21.3*. It consists of three exons and two promoters, both of which have different functional properties. The second promoter, P2, is located 1,400 bp upstream of the translation initiation site and its activation decreases the activity of the P1 promoter, thus functioning as a negative regulatory element (10, 11). Direct sequencing of DNA samples from a Caucasian population has led to the identification of a novel single nucleotide polymorphism (SNP;

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-938C>A) in the inhibitory P2 promoter of the *BCL2* gene (12). Recently, Nückel et al. (13) investigated the functional role of this SNP and showed that the -938C allele is significantly associated with increased P2 promoter activity and binding of nuclear proteins compared with the A-allele. Consistent with this suppressive effect of the P2 promoter, they found significantly increased Bcl-2 protein expression in B-cells from CLL patients carrying the -938AA genotype. In line with the finding that Bcl-2 overexpression is an unfavorable prognostic factor in B-CLL, this genotype was significantly associated with decreased survival. The aim of the present study was to elucidate a possible association of the (-938C>A) SNP with the clinical

Table 1. Clinicopathologic characteristics at primary diagnosis and genotype distribution in patients with breast cancer

	All	*BCL2* -938 genotype			*P*
		AA	AC	CC	
Healthy controls (blood donors)	120	36 (30.0)	63 (52.5)	21 (17.5)	
n (%)	274	76 (27.7)	140 (51.1)	58 (21.2)	
Median follow-up, mo (range)	106 (4-155)	104.5 (4-154)	106.5 (10-155)	103.5 (10-152)	0.896*
Age at diagnosis (y)					
≤37	18 (6.6)	8 (44.4)	6 (33.3)	4 (22.2)	0.702
38-47	55 (20.1)	18 (32.7)	27 (49.1)	10 (18.2)	
48-57	82 (29.9)	22 (26.8)	46 (56.1)	14 (17.1)	
58-67	63 (23.0)	13 (20.6)	34 (54.0)	16 (25.4)	
68-77	46 (16.8)	12 (26.1)	22 (47.8)	12 (26.1)	
≥78	10 (3.6)	3 (30.0)	5 (50.0)	2 (20.0)	
Tumor type					
Ductal	196 (71.5)	55 (28.1)	99 (50.5)	42 (21.4)	0.939
Lobular	52 (19.0)	13 (25.0)	27 (51.9)	12 (23.1)	
Others	26 (9.5)	8 (30.8)	14 (53.8)	4 (15.4)	
Tumor size (mm ± SD)	25.23 ± 15.9	25.49 ± 14.8	26.04 ± 17.4	22.96 ± 13.4	0.417
Tumor stage					
pT_1	129 (47.4)	35 (27.1)	67 (51.9)	27 (20.9)	
pT_2	112 (41.2)	33 (29.5)	56 (50.0)	23 (20.5)	0.992
pT_{3+4}	31 (11.4)	8 (25.8)	16 (51.6)	7 (22.6)	
Lymph node status					
pN_0	141 (51.6)	35 (24.8)	71 (50.4)	35 (24.8)	0.256
pN_+	132 (48.4)	41 (31.1)	68 (51.5)	23 (17.4)	
Metastasis					
pM_0	268 (97.8)	72 (26.9)	138 (51.5)	58 (21.6)	0.081
pM_1	6 (2.2)	4 (66.7)	2 (33.3)	0	
UICC stage					
I	88 (32.4)	20 (22.7)	46 (52.3)	22 (25.0)	0.546
II	113 (41.5)	32 (28.3)	58 (51.1)	23 (20.4)	
III + IV	71 (26.1)	24 (33.8)	35 (49.3)	12 (16.8)	
Grade					
1	91 (34.3)	22 (24.2)	47 (51.6)	22 (24.2)	0.415
2	108 (40.8)	28 (25.9)	57 (52.8)	23 (21.3)	
3	66 (24.9)	23 (34.8)	34 (51.5)	9 (13.6)	
Immunohistochemistry					
Her2/*neu* status					
No overexpression	201 (86.3)	52 (25.9)	107 (53.2)	42 (20.9)	0.814
Overexpression	32 (13.7)	10 (31.3)	16 (50.0)	6 (18.8)	
ER status					
Negative	67 (29.1)	19 (28.4)	35 (53.8)	13 (19.4)	0.987
Positive	163 (70.9)	45 (27.6)	85 (52.1)	33 (20.3)	
Bcl-2 status					
Negative	47 (21.9)	10 (21.3)	29 (61.7)	8 (17.0)	
Weak	52 (24.2)	15 (28.8)	24 (46.2)	13 (25.0)	0.485
Positive	116 (53.9)	36 (31.0)	55 (47.4)	25 (21.6)	
Treatment					
Surgical treatment					
Breast conserving	54 (19.7)	17 (31.5)	25 (46.3)	12 (22.2)	0.712
Ablative	220 (80.3)	59 (26.8)	115 (52.3)	46 (20.9)	
Adjuvant therapy					
No adjuvant therapy	142 (51.8)	32 (22.5)	77 (54.2)	33 (23.2)	0.133
Tam and/or CMF	132 (48.2)	44 (33.3)	63 (47.7)	25 (18.9)	

NOTE: Data are numbers with percentages given in brackets. Categorical variables were analyzed by χ^2 statistics. *P* values were calculated using ANOVA for continuous variables.
Abbreviations: UICC, Unio Internationale Contra Cancrum; Tam, tamoxifen; CMF, cyclophosphamide, methotrexate, and 5 fluorouracil.
*Kruskal-Wallis test for nonparametric variables.

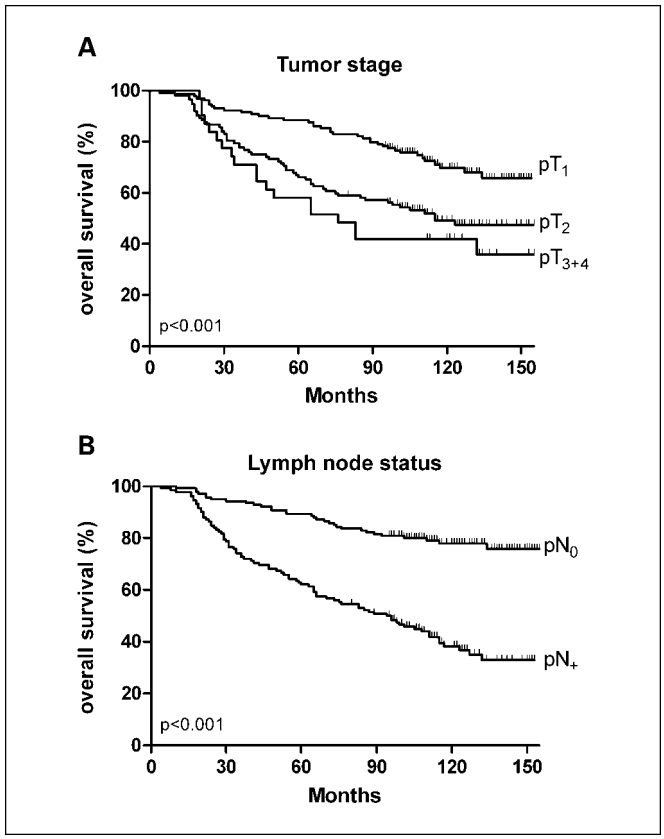

Fig. 1. Overall survival for the period of complete follow-up based on Kaplan-Meier curves for 274 patients with primary invasive breast cancer. *A,* based on tumor stage. *B,* based on lymph node status.

outcome of patients suffering from invasive breast carcinoma. In the light of previous Bcl-2 expression studies (9), one could expect that the AA-genotype, which is associated with higher Bcl-2 expression levels in B-CLL and poor prognosis, may be related with increased overall survival in breast cancer.

Materials and Methods

Patients and tumor assessment. The present study comprised 274 consecutive Caucasian patients of German ancestry who were operated for breast cancer at the Clinic of Obstetrics and Gynaecology (Medical Faculty, University of Duisburg-Essen, Essen, Germany). Entry criteria for this study consisted of clinical and histopathologic diagnosis of primary unilateral invasive breast cancer. Overall survival data of these patients were obtained from the patients' files or the local municipal registry. Of the 274 patients, 161 are still alive; the minimum follow-up period of patients still alive was 80 months. All tumors were classified and reassessed according to the WHO classification of tumors of the breast (14) and the tumor-node-metastasis classification (6th edition; ref. 15). The present study was done according to the Declaration of Helsinki and approved by the local Ethics Committee of the University Hospital of Essen.

Tissue microarray construction. One representative tumor tissue cylinder with a diameter of 3 mm was punched from paraffin blocks using a skin biopsy punch (PFM) and brought into overall 14 recipient blocks, each containing a maximum of 22 tumor samples. One cylinder of normal thyroid and liver tissue in each block served as control tissue.

Immunohistochemistry. Sections (5-μm thick) of tissue microarray blocks were transferred to SuperFrost Plus slides (Menzel). After antigen retrieval [waterbath at 95°C; 20 min in citrate buffer (pH 9.0) for Bcl-2], immunohistochemistry was done using the Dako Autostainer Plus (DakoCytomation). The slides were immunostained for Bcl-2 (clone 124, dilution 1:300, DakoCytomation). Antibody visualization was achieved using the anti-mouse IgG detection kit (EnVision+, DakoCytomation). Estrogen receptor (ER; clone SP1, dilution 1:300, DCS) and Her2/*neu* (clone SP3, dilution 1:100, DCS) immunostainings were done accordingly.

Evaluation of immunohistochemical staining. Stained sections were reviewed twice by one of the authors (F.O.) not knowing the *BCL2* -938 genotypes. For Bcl-2, the percentage of tumor cells showing a positive cytoplasmic staining was assessed. A complete lack of staining was scored as negative, an at least moderate staining of >50% of tumor cells was scored as strongly positive, and the remaining cases were scored as weakly positive. ER was scored positive if >10% of tumor cells showed

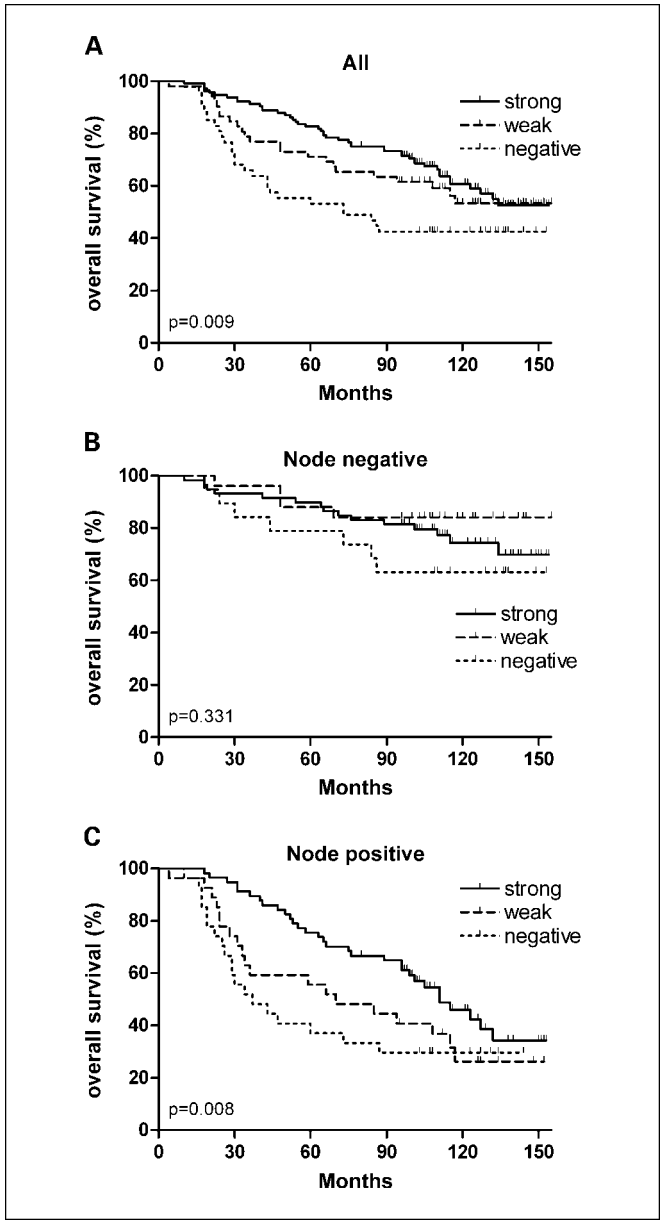

Fig. 2. Overall survival for the period of complete follow-up based on Kaplan-Meier curves for 215 patients with primary invasive breast cancer on different Bcl-2 expression levels. *A,* all patients. *B,* lymph node – negative patients. *C,* lymph node – positive patients. *P* values for log-rank statistics were calculated for linear comparison of all expression levels.

Table 2. Factors influencing the risk of death by univariate analysis

Variable	Univariate analysis					
	All		pN_0		pN_+	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
-938C>A						
AA	1*		1*		1*	
AC	0.961 (0.62-1.50)	0.862	1.909 (0.63-5.75)	0.251	0.826 (0.50-1.36)	0.450
CC	1.167 (0.70-1.95)	0.556	3.202 (1.03-9.93)	0.044	0.984 (0.53-1.86)	0.959
Bcl-2 expression						
Negative	1*		1*		1*	
Weak	0.643 (0.37-1.12)	0.120	0.405 (0.12-1.39)	0.150	0.793 (0.42-1.50)	0.476
Strong	0.521 (0.32-0.84)	0.007	0.650 (0.27-1.60)	0.348	0.483 (0.27-0.86)	0.013
Her2 expression						
No overexpression	1*		1*		1*	
Overexpression	0.802 (0.43-1.50)	0.491	0.732 (0.22-2.41)	0.608	0.664 (0.30-1.46)	0.308
ER expression						
Negative	1*		1*		1*	
Positive	0.550 (0.37-0.83)	0.004	0.542 (0.26-1.13)	0.100	0.507 (0.31-0.83)	0.007
Age (y)						
≤47	1*		1*		1*	
48-67	1.286 (0.81-2.03)	0.282	0.856 (0.35-2.09)	0.733	1.382 (0.81-2.37)	0.241
≥68	1.286 (0.74-2.23)	0.370	1.688 (0.68-4.20)	0.260	1.217 (0.60-2.45)	0.582
Grade						
1	1*		1*		1*	
2	1.373 (0.86-2.20)	0.185	0.513 (0.21-1.25)	0.140	1.685 (0.90-3.17)	0.106
3	2.316 (1.42-3.78)	0.001	1.456 (0.62-3.40)	0.386	2.091 (1.08-4.06)	0.029
Tumor type						
Ductal	1*		1*		1*	
Lobular	1.078 (0.67-1.73)	0.757	1.087 (0.43-2.72)	0.858	1.199 (0.69-2.09)	0.522
Others	1.712 (0.98-2.98)	0.057	2.979 (1.19-7.48)	0.020	1.241 (0.61-2.51)	0.548
Tumor size	1.022 (1.01-1.03)	<0.001	1.010 (0.99-1.04)	0.410	1.017 (1.01-1.03)	0.002

*Reference group.

nuclear staining irrespective of its staining intensity. Her2/*neu* was assessed according to the DAKO score. Any complete membranous staining of >10% of tumor cells (DAKO score 2+ and 3+) was interpreted as Her2/*neu* overexpression.

Determination of BCL2 -938 genotypes. DNA was extracted from routinely processed paraffin-embedded nonneoplastic breast tissue or tumor-free lymph node tissue. Dewaxing and isolation of genomic DNA were done using a commercially available kit (QIAamp, Qiagen) following the manufacturer's instructions. Genotypes of the -938C>A polymorphism were determined by the "slowdown" PCR (16). The slowdown PCR was done using Eppendorf *Taq* PCR Mastermix by 48 cycles with 30-s denaturation at 95°C, 30-s annealing with a progressively lowered temperature from 70°C to 53°C at a rate of 1°C every third cycle, and a primer extension of 40 s, followed by 15 additional cycles with an annealing temperature of 58°C. Slowdown PCR was conducted with a generally reduced ramp rate at 2.5°C and specifically a small cooling rate for reaching annealing temperature at 1.5°C. Previously described primers were used (13): forward primer 5′-CAGCAGCTTTTCGGAAAATG-3′ and biotinylated reverse primer 5′-TATCCACGGGACCGCTTCAC-3′. The 134-bp PCR products were analyzed by Pyrosequencing using the primer 5′-TCCCCGGCTC-CTTCATCGTC-3′ on the PSQ96 system according to the manufacturer's instructions (Biotage). Results were analyzed using the PSQ96 SNP software. Regenotyping of 94 randomly selected samples to control for genotype failures revealed 100% concordance with the previously obtained results.

Statistical analysis. Kaplan-Meier plots and the log-rank test for trend were used to retrospectively evaluate the relationship between tumor stage, lymph node status, Bcl-2 expression, *BCL2* genotypes, as well as outcome from the date of primary diagnosis to the end of follow-up. The effect of age, grade, tumor type, tumor size, ER-status, Her2/*neu*-status, Bcl-2 status, and *BCL2* genotype as well-established or widely discussed prognostic factors (17, 18) and/or significantly associated factors were analyzed for the clinical outcome by univariate analysis and stepwise multivariate Cox regression analysis. Complete immunohistochemistry with Bcl-2 status, Her2/*neu* status, and ER status was available only in 100 of 141 lymph node–negative patients. Due to this fact, a second multivariate Cox regression analysis was computed without immunohistochemical data but with the complete study population. Hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated from the Cox regression model, including all factors for multivariate analysis. Contingency tables and Pearson's χ^2 test were used to compare categorical variables using *BCL2* genotypes as indicated. Postulating a gene-dose effect of the *BCL2* -938C>A polymorphism, linear ANOVA was used for comparison of continuous variables where appropriate. Linear χ^2 test was used for comparison of Bcl-2 expression levels with *BCL2* -938C>A alleles. Control for deviation from the Hardy-Weinberg equilibrium was conducted with the public domain program Hardy-Weinberg equilibrium by J. Ott.[7] Differences were regarded significant at $P < 0.05$. All statistical analysis were done using SPSS 13.0 (SPSS) or GraphPad Prism 4.0 (GraphPad Software).

Results

Genotype distribution and subject characteristics. Demographic characteristics as well as tumor type, grade, pathologic tumor-node-metastasis and Unio Internationale Contra Cancrum

[7] http://www.genemapping.cn/util.htm

stage, size, treatment regimens; as well as the immunohistochemical ER, Her2/*neu*, and the Bcl-2 status in relation to the *BCL2* (-938C>A) genotypes are summarized in Table 1. Median follow-up time was 106 months (range 4-155 months). Distributions of tumor stage, grade, and ER status of the entire study group were compatible with those reported in the literature (19). The genotype distribution (75 AA, 140 AC, 58 CC) was compatible with Hardy-Weinberg equilibrium. C-allele frequency (53%) as well as genotype distribution were not significantly different from the published genotype distribution of healthy Caucasian blood donors. Age, tumor size, stage, grade, lymph node status, Her2/*neu*-status, ER status, surgical treatment, and adjuvant therapy were not associated with *BCL2* genotypes. To confirm that our study group was representative for patients with breast cancer, we calculated Kaplan-Meier curves for overall survival depending on the pathologic stage and the lymph node status. As shown in Fig. 1A and B, respectively, overall survival was significantly dependent on pathologic stage ($P < 0.001$) and lymph node status ($P < 0.001$), and computed values were compatible with published data.

Immunohistochemical Bcl-2 expression—association with patient survival and (-938C>A) genotypes. Representative tumor material for the immunohistochemical assessment of Bcl-2 expression was available in 215 patients. Bcl-2 expression was significantly associated with survival of these 215 patients ($P = 0.009$; Fig. 2A) and the subgroup of lymph node–positive patients ($P = 0.008$; Fig. 2B) but failed to reach significance in the subgroup of node-negative cases ($P = 0.331$; Fig. 2C). This was confirmed in univariate analysis (Table 2). However, Bcl-2 expression was no longer an independent predictor in the multivariable Cox regression analysis (Table 3). *BCL2* genotypes were not significantly associated with immunohistochemically determined Bcl-2 expression (Table 1). Interestingly, in node-negative breast cancer patients, Bcl-2 expression was significantly different between (-938C>A) alleles ($P = 0.044$) with higher expression associated with the A-allele (Bcl-2 expression comparing A:C alleles: negative 17:25, weak 21:31, strong Bcl-2 expression 70:56). In node-negative cases showing (either weak or strong) Bcl-2 expression, favorable clinical outcome of patients was associated both with (-938)AA/AC genotypes ($P = 0.021$; Fig. 3A) and combined carriers of the A allele ($P = 0.006$; Fig. 3B).

Clinical outcome by (-938C>A) genotypes. Overall survival was analyzed for dependency on (-938C>A) genotypes using Kaplan-Meier survival curves (Fig. 4A-C). Survival was not significantly associated with (-938C>A) genotypes when all patients were analyzed (Fig. 4A) or in the subgroup of lymph node–positive cases (Fig. 4C). However, survival was significantly dependent on the (-938C>A) genotype with an apparent gene dose effect in lymph node–negative patients (Fig. 4B; $P = 0.030$). In univariate analysis, *BCL2* -938C homozygous patients had a higher risk for death than -938A homozygous patients, with heterozygous patients being at intermediate risk (Table 2). The following HRs were calculated: CC versus

Table 3. Factors influencing the risk of death by multivariate Cox regression analysis

Variable	Multivariate analysis					
	All		pN_0		pN_+	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
-938C>A						
AA	1*		1*		1*	
AC	1.058 (0.63-1.79)	0.833	2.775 (0.69-11.16)	0.151	0.861 (0.47-1.58)	0.628
CC	1.231 (0.66-2.28)	0.509	4.915 (1.18-20.43)	0.028	0.936 (0.41-2.14)	0.875
Bcl-2 expression						
Negative	1*		1*		1*	
Weak	0.724 (0.36-1.44)	0.358	0.355 (0.07-1.86)	0.221	0.732 (0.33-1.61)	0.438
Strong	0.779 (0.40-1.51)	0.460	0.849 (0.20-3.70)	0.827	0.557 (0.26-1.18)	0.128
Her2 expression						
No overexpression	1*		1*		1*	
Overexpression	0.552 (0.26-1.16)	0.115	0.560 (0.12-2.73)	0.474	0.342 (0.13-0.88)	0.025
ER expression						
Negative	1*		1*		1*	
Positive	0.635 (0.35-1.14)	0.129	0.719 (0.19-2.72)	0.626	0.541 (0.29-0.99)	0.048
Age (y)						
≤47	1*		1*		1*	
48-67	1.447 (0.82-2.56)	0.203	0.723 (0.22-2.35)	0.590	1.334 (0.67-2.66)	0.413
≥68	1.729 (0.90-3.34)	0.103	1.371 (0.40-4.67)	0.614	1.620 (0.69-3.78)	0.265
Grade						
1	1*		1*		1*	
2	1.204 (0.69-2.09)	0.508	0.493 (0.17-1.45)	0.200	1.771 (0.78-4.04)	0.174
3	2.121 (1.11-4.04)	0.022	1.857 (0.45-7.72)	0.395	2.494 (0.99-6.28)	0.052
Tumor type						
Ductal	1*		1*		1*	
Lobular	1.402 (0.77-2.56)	0.272	1.442 (0.49-4.24)	0.506	1.494 (0.65-3.43)	0.343
Others	1.796 (0.93-3.48)	0.083	1.500 (0.38-5.86)	0.560	1.328 (0.59-3.00)	0.494
Tumor size	1.017 (1.01-1.03)	0.004	1.009 (0.98-1.04)	0.589	1.014 (1.00-1.03)	0.056

NOTE: Model A, with results of immunohistochemistry, $n = 205$.
*Reference group.

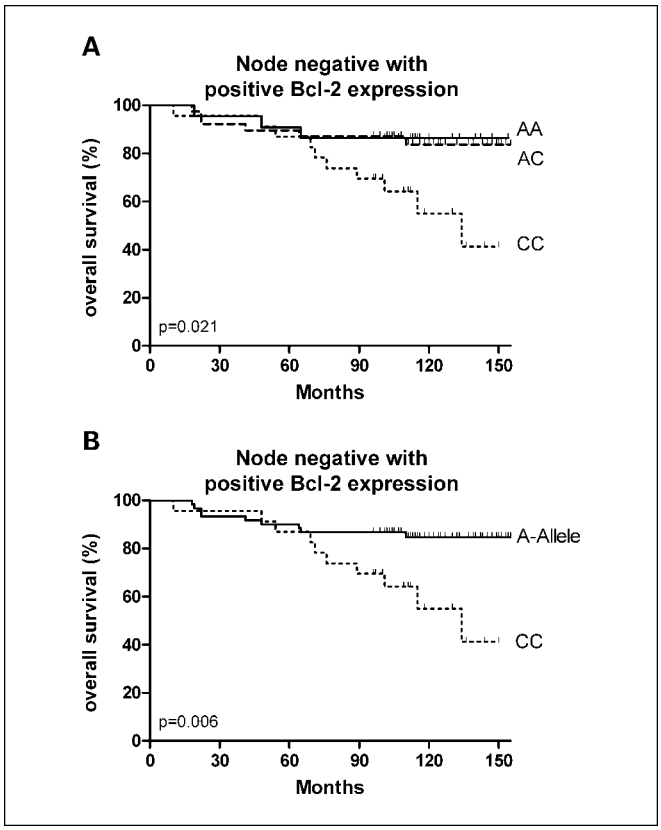

Fig. 3. Overall survival for the period of complete follow-up based on Kaplan-Meier curves for node-negative breast cancer patients with positive Bcl-2 expression. *A,* on different -938C>A genotypes. *B,* A allele versus CC genotype. *P* values for log-rank statistics were calculated for linear comparison of all genotypes and for comparison of A allele versus CC genotype, respectively.

AA: 3.20; 95% CI, 1.03-9.93; $P = 0.044$; AC versus AA: 1.91; 95% CI, 0.63-5.75; $P = 0.251$. Ten-year survival rates were 88.6% for AA, 78.4% for AC, and 65.8% for CC genotypes.

Age, ER status, Her2/*neu* status, Bcl-2 status, *BCL2* genotype, grade, tumor type, and tumor size were included into two multivariate analysis models with (Table 3) as well as without results of immunohistochemistry (Table 4), which indicated that the (-938C>A) polymorphism was an independent risk factor for tumor-related death in lymph node–negative patients. Compared with the reference group consisting of -938A homozygous individuals, heterozygous patients had a HR of 2.78 (95% CI, 0.69-11.16; $P = 0.151$) in model A and a HR of 1.80 (95% CI, 0.57-5.66; $P = 0.317$) in model B, respectively. In contrast, patients harboring the CC genotype showed a significantly higher risk with a HR of 4.92 (95% CI, 1.18-20.43; $P = 0.028$) in model A and a HR of 3.59 (95% CI, 1.12-11.58; $P = 0.032$) in model B, respectively.

Discussion

Patients suffering from invasive breast carcinoma with a negative lymph node status generally have a favorable prognosis. Particularly in patients with early-stage invasive breast cancer, the presence or absence of axillary lymph node involvement is the most significant prognostic indicator (17). Nevertheless, some of these patients develop tumor recurrence and 20% to 30% of lymph node–negative breast carcinoma patients will die from their disease. Prospective randomized clinical trials have shown the beneficial effects of an adjuvant therapy in these high-risk patients (20–22), despite its increased morbidity and mortality (23). Therefore, it would be clinically most desirable to accurately identify this high-risk group of patients.

Several studies have analyzed the prognostic effect of Bcl-2 expression in breast cancer. Most of these have reported an association of Bcl-2 expression with better outcome (24–26), both in node-negative (27) and in node-positive disease (28), although its independent prognostic effect has been shown only in few studies (24–26, 28). In the present study, Bcl-2 expression was significantly associated with patient survival in

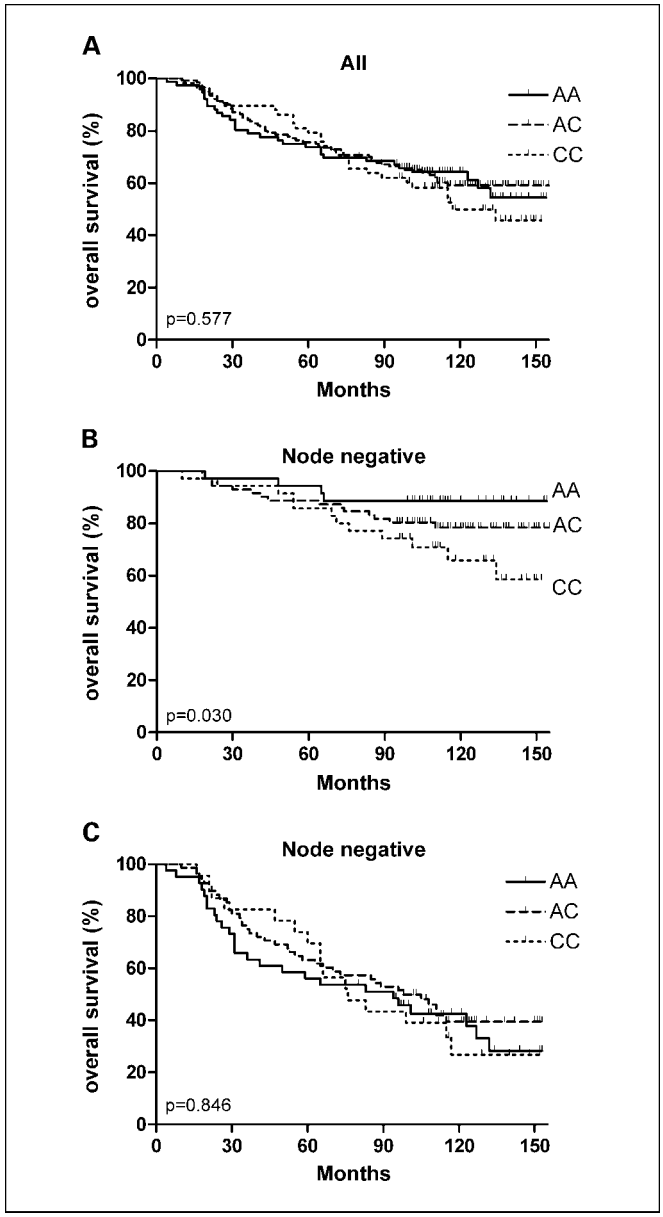

Fig. 4. Overall survival for the period of complete follow-up based on Kaplan-Meier curves for 274 patients with primary invasive breast cancer on different -938C>A genotypes. *A,* all patients. *B,* lymph node negative. *C,* lymph node – positive patients. *P* values for log-rank statistics were calculated for linear comparison of all genotypes.

Table 4. Factors influencing the risk of death by multivariate Cox regression analysis

Variable	Multivariate analysis					
	All		pN_0		pN_+	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
-938C>A						
AA	1*		1*		1*	
AC	0.904 (0.56-1.46)	0.678	1.797 (0.57-5.66)	0.317	0.849 (0.50-1.45)	0.549
CC	1.421 (0.83-2.44)	0.203	3.593 (1.12-11.58)	0.032	1.352 (0.69-2.65)	0.382
Age (y)						
≤47	1*		1*		1*	
48-67	1.339 (0.82-2.18)	0.239	0.854 (0.33-2.20)	0.744	1.374 (0.78-2.43)	0.276
≥68	1.498 (0.84-2.68)	0.173	1.576 (0.53-4.66)	0.411	1.410 (0.66-3.00)	0.372
Grade						
1	1*		1*		1*	
2	1.406 (0.86-2.30)	0.174	0.588 (0.23-1.53)	0.275	2.116 (1.06-4.23)	0.034
3	2.428 (1.38-4.26)	0.002	2.100 (0.72-6.10)	0.173	2.744 (1.24-6.06)	0.013
Tumor type						
Ductal	1*		1*		1*	
Lobular	1.287 (0.75-2.20)	0.356	1.088 (0.40-2.98)	0.869	1.543 (0.77-3.10)	0.222
Others	2.003 (1.13-3.55)	0.017	2.976 (1.06-8.37)	0.039	1.273 (0.61-2.64)	0.517
Tumor size	1.022 (1.01-1.03)	<0.001	1.015 (0.99-1.04)	0.275	1.017 (1.01-1.03)	0.005

NOTE: Model B, without results of immunohistochemistry.
*Reference group.

lymph node–positive cases, but not in lymph node–negative ones. However, in the latter subgroup, the common -938C>A polymorphism in the inhibitory P2 *BCL2* gene promoter was significantly related with patient survival, suggesting this SNP as a candidate to identify node-negative high-risk breast carcinoma patients who may benefit from adjuvant therapy regimens. Multivariate analysis revealed that carriers of the homozygous CC genotype suffering from lymph node–negative breast carcinoma are, independently from other relevant factors, at an increased risk to die from their disease.

The protein Bcl-2 plays an important role in regulating apoptosis and cell cycle delay. Experiments done on different cell lines have clearly shown an influence of the (-938C>A) SNP on the protein expression of Bcl-2 (13). As shown by the results of the present study, in lymph node–negative breast carcinoma, alleles of the (-938C>A) SNP are significantly related with the immunohistochemically determined level of Bcl-2 expression as well as survival in Bcl-2–expressing lymph node–negative carcinomas, which further supports the concept of a functional relevance of this SNP on Bcl-2 expression and ultimately regulation of apoptosis. However, it has to be emphasized that the biological effect of a SNP associated with differential tumor behavior not necessarily has to involve the tumor cells directly; this effect may equally well be mediated via other factors, for example, angiogenesis, immune response, etc.

Our finding that the homozygous AA genotype is associated with a more favorable outcome is principally in contrast to the findings of the study on B-CLL patients by Nückel et al. (13). However, in both studies, an increased Bcl-2 expression was significantly associated with the *BCL2* -938A allele; in the literature, Bcl-2 overexpression in B-CLL (5) was consistently related with a poor, in breast carcinoma with a better clinical course (19, 25). Thus, the opposing results found in the two studies rather support than weakens our concept of a functional role of the (-938C>A) SNP of the *BCL2* gene. Additionally, the fact that this genetic host factor plays a significant role in the more favorable prognostic group of node-negative breast cancer patients supports the hypothesis that common genetic polymorphisms modifying normal regulation and expression of genes (e.g., those involved in apoptosis and tumorigenesis) are preferentially influencing the course of a disease in its localized but not advanced stages.

Finally, our results may have a putative effect on antisense strategies targeting *BCL2* by oligonucleotides (29). In breast cancer therapy, the use of this relatively novel therapeutic option is controversially discussed due to the dual antiapoptotic/antiproliferative role of Bcl-2 in breast cancer (30). Nevertheless, cell line experiments, xenograft models, and phase I/II studies in patients with metastatic breast cancer have shown promising results for *BCL2* antisense oligonucleotides. However, (prospective) clinical trials would be necessary to evaluate whether genotypes of the *BCL2* polymorphism described in the present study are able to affect the outcome of patients treated with these drugs, and could therefore potentially serve as a marker to identify responders and nonresponders to Bcl-2 antisense therapy.

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