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NEOPLASIA

Correlation of *Bcl-2* Rearrangement With Clinical Characteristics and Outcome in Indolent Follicular Lymphoma

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The t(14;18) translocation, which involves the *bcl-2* oncogene, occurs in follicular lymphomas (FL) at two common sites: the major breakpoint region (*MBR*) and the minor cluster region (*mcr*). The biological and clinical significance of these breakpoints is unknown. The *bcl-2* breakpoint site was determined in 247 previously untreated patients (49% men; median age 52 years) with indolent FL (155 grade I, 83 grade II, and 8 grade III) to correlate it with pretreatment characteristics, response, and outcome. The *bcl-2* breakpoint site was determined by a polymerase chain reaction method of peripheral blood (all cases), bone marrows (149 cases), and fresh lymph node biopsy specimens (68 cases). The breakpoint site occurred at *MBR* in 175 cases (71%) and at *mcr* in 27 (11%). In 45 cases (18%), no breakpoint was detected (germline). No significant relationship was found between the rearrangements and the expression of *BLC-2* and *BAX* proteins. Patients' germline for *MBR* and *mcr* tended to present more frequently with stage IV disease and higher β2-microglobulin (β2M) levels, whereas *mcr*-rearranged patients presented more frequently with early stage and normal β2M. The complete response rate of germline patients was significantly lower than that of *MBR* and *mcr* patients. An estimated 3-year failure-free survival (FFS) for *mcr*, MBR, and germline cases was 95%, 76%, and 57%, respectively ($P < .001$). The *bcl-2* breakpoint site was independent of serum β2M and lactate dehydrogenase in its correlation with FFS. In conclusion, the *bcl-2* rearrangement site is an important prognostic factor in indolent FL, useful to identify patients who may require different treatment.
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FOLLICULAR LYMPHOMAS (FLs) are characterized by the presence of the t(14;18)(q32;q21) translocation, which causes a rearrangement of the *bcl-2* oncogene. This rearrangement normally occurs in chromosome 18, with the immunoglobulin heavy-chain gene (*IgH*) in chromosome 14.[1,2] The consequence is an overexpression of a chimerical *bcl-2/IgH* mRNA.[3] Because the breakpoint is usually located outside the translated portion of the *bcl-2* gene, the protein product is identical to the normal *BCL-2* protein.[4] The function of the *BCL-2* protein is to block apoptosis, probably by means of its interaction with other *bcl-2*-family proteins such as *bax*.[5-8] The inhibition of apoptosis leads to accumulation of B lymphocytes, which might later acquire additional mutations that eventually result in the development of FL.

Approximately 70% of *bcl-2* rearrangements in FL occur at the major breakpoint region (*MBR*) located in the untranslated 3′ end of the last exon,[9-11] and in approximately 10% of cases, the rearrangement occurs in the minor cluster region (*mcr*) located approximately 30 kb downstream of the *bcl-2* gene.[12] In a few cases, rearrangements occur at other sites such as the variant cluster region at the 5′ end of the *bcl-2* gene. In approximately 15% of patients, no t(14;18) translocation can be detected by either cytogenetic, Southern blot analysis, or polymerase chain reaction (PCR) techniques[13] (germline configuration). Tight clustering of the breakpoints at *MBR* and *mcr*, as well as the availability of consensus sequences of the *JH* segments of the *IgH* make this a particularly favorable target for PCR amplification.[9-12] In fact, it is possible to use two universal primers (one for each breakpoint) along with a primer derived from JH region to amplify the majority of the translocations at *MBR* and *mcr*,[9,14,18] with less than 5% of translocations failing to be amplified.

The biological and clinical significance of these differences in *bcl-2* rearrangement sites in FL remain unclear. In fact, it has been suggested that the presence of the t(14;18) translocation in FL correlates with better,[16] worse,[17] and similar[18-21] clinical outcomes. However, these studies are based on a small number of cases and frequently included high-grade and intermediate-grade lymphomas. On the other hand, *BCL-2*-protein expression recently has been associated with poor prognosis in patients with diffuse large-cell lymphoma.[22-24] Currently, the treatment to indolent FL is based largely on the Ann Arbor stage of the disease at the time of diagnosis.[25] Management of patients with advanced stage includes observation and single agent or combination chemotherapy. A therapeutic approach based on the patient's prognostic risk could be of practical help in the decision-making process.[26] Furthermore, knowledge of these prognostic factors could lead to an improvement in the analysis of clinical trials and in the understanding of the biology of these disorders. Consequently, it is important to ascertain whether or not the *bcl-2* rearrangements are useful in predicting the outcome of FL patients.

MATERIALS AND METHODS

Patients. The presence of *bcl-2* rearrangements was analyzed in the pretreatment peripheral blood (PB) and/or bone marrow (BM) aspirates

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of 247 patients diagnosed with indolent FL during a 7-year period. The median age was 52 years (range, 18 to 84 years); 120 patients (49%) were men and 127 (51%) were women. The distribution, according to the REAL classification, was follicular center cell lymphoma grade I, 155 cases; grade II, 83 cases; grade III, 8 cases, and unclassified, 1 case. Only eight patients had grade III histology and were included in the study because their tumors predominantly contained cleaved cells and for that reason were considered indolent. Twenty-two patients (9%) presented with Ann Arbor stage I disease, 28 (11%) stage II, 44 (18%) stage III, and 153 (62%) stage IV. Bulky disease was present in 21% of the cases, extranodal involvement in 68%, and BM infiltration in 56%. The proportion of patients with elevated serum lactate dehydrogenase (LDH) (top normal: 618 IU/L) and high β2-microglobulin (β2M) (top normal: 2 mg/L) was 16% and 42%, respectively.

Patients with stage IV disease received anthracycline-containing chemotherapy regimens depending on the protocol being used at the time they were diagnosed. The treatment consisted of an intensive alternating triple therapy (ATT) regimen[27] in 106 cases, FND (fludarabine, Novantrone, and dexamethasone)[28] in 34 cases, and standard cyclophosphamide doxorubicin, vincristine (oncovin), prednisone (CHOP) in 13 cases. Patients with stage III disease were treated with ATT chemotherapy in 16 cases, with CHOP in 13, and with total nodal radiotherapy in 15. Patients with early stage received either radiotherapy alone (9 cases) or a combined modality of chemotherapy plus radiotherapy to the involved fields (41 cases).

Histologic and immunophenotypic studies. The diagnosis of indolent FL was based on conventional examination of paraffin-embedded slides and whenever possible on immunohistologic staining of frozen sections, according to currently accepted criteria.[29] In 47 cases, the expression of *BCL-2* and *BAX* proteins was analyzed by an immunostaining method. The results were expressed in a semiquantitative manner by comparing *BCL-2* or *BAX*-tumor expression with that of contiguous interfollicular areas. Four patterns were distinguished: negative (0), no tumor *BCL-2* expression; weak (+), *BCL-2*-tumor expression present but weaker than in interfollicular areas; positive (++), positivity similar to interfollicular areas; and strongly positive (+++), *BCL-2*-tumor expression stronger than in the interfollicular areas.

PCR methods for detecting bcl-2 *rearrangements.* Baseline pretreatment PB samples were collected in 242 patients as part of a study aimed at assessing minimal residual disease in FL (in five cases, the PCR result was not assessable). In addition, a BM aspirate for PCR analysis was obtained in 154 cases. Finally, fresh material from the original lymph node biopsy specimen was available for *bcl-2* rearrangement studies in 68 patients.

DNA was isolated from PB, BM, and/or lymph node material by using conventional methods. A PCR technique was used to detect the *bcl-2* of *MBR* and *mcr*. PCR amplification was performed with 1 μg of purified DNA that was subjected to a 45-cycle PCR amplification.[14,15] The primers MBR^+, mcr^+, and JH^- have been previously described.[12,14,15] Twenty percent of the PCR products were size fractionated in a 2% NuSieve gel (FMC Bioproducts, Rockland, ME) and then transferred to a nylon membrane. Membranes were hybridized with 5′ end radiolabeled oligonucleotide probes *MBR* or *mcr*.[15]

To ensure the reliability of the PCR assay, we routinely included a weak, positive control (100 pg of positive DNA), a negative control (normal DNA), a reagent control, and an internal control. These controls helped us detect contamination, avoid false negativity caused by suboptimal PCR efficiency, and standardize the variation in PCR efficiency.

Statistical considerations. FFS was the outcome variable of major interest. FFS was defined as the time interval from the start of initial therapy to the first evidence of relapse or death from toxicity. Response to therapy and associations between *bcl-2*-breakpoint site and other pretreatment patient characteristics were also reported. Differences in patient characteristics and response were tested with the chi-square or Fisher's exact tests. Survival curves were estimated by using the Kaplan and Meier methods[30] and differences among curves were tested by using the Wilcoxon test.[31] A multivariate analysis was performed by the Cox's stepwise proportional hazard regression method.[32] However, because there was an apparent lack of proportionality of hazard rates, a stratified test was used to assess the independent prognostic value of the *bcl-2*-breakpoint site. For this purpose, because a standard score system for FLs does not exist, the patients were divided into three sets according to the most important prognostic variables in MDACC series, serum LDH, and β2M levels. We analyzed the prognostic value of the *bcl-2*-breakpoint sites in each group.

RESULTS

Distribution of the bcl-2 *rearrangement site.* The breakpoint site ocurred at *MBR* in 175 patients (71%), at the *mcr* in 27 patients (11%), and no rearrangement was detected for both *MBR* and *mcr* in the remaining 45 patients (18%). These results were obtained in PB and BM samples from the same patients in 149 cases and in PB only in 98 cases. In those patients in whom the *bcl-2* rearrangement was observed in blood or BM samples only, they were graded as positive.

Assessment of bcl-2 *rearrangement in lymph node, PB, and BM samples.* To ascertain if the results obtained from PB and BM samples were representative of those obtained from the tumor itself, we studied by PCR a subset of 68 samples (from the 247 patients) obtained from the lymph nodes or masses involved by lymphoma. The results showed that 46 patients (68%) had *MBR* rearrangements, 4 (6%) had *mcr* rearrangements, and 18 (27%) were germline for both *MBR* and *mcr*. These results are similar to those obtained from blood and marrow analyses. When comparing tumor samples with blood or marrow, the only discrepancy found was in 4 of the 18 germline lymph node biopsy samples in which *MBR* (1) or *mcr* (3) rearrangements were found in PB, the BM, or both. In the remaining 64 cases (94.2% of all cases), there was agreement between the results of lymph node and PB/BM analyses. Of particular note, all patients germline for *MBR* or *mcr* in PB or BM also showed a germline pattern in lymph node tissue. Therefore, the rate of agreement between blood/marrow and tumor tissue was 94%.

On the other hand, we also compared the results obtained in PB and BM in 149 patients in whom paired blood and marrow were available (Table 1). *MBR* or *mcr* rearrangements were observed in 87 paired samples of blood and marrow, and no rearrangement was seen in another 31 paired samples. In 23 cases (15%), a *bcl-2* rearrangement was found in the PB sample and not in BM, whereas in 8 cases (5%) it was observed in BM but not in blood.

Expression of BCL-2 *and* BAX *proteins.* In an attempt to determine if there was any correlation between the *bcl-2*-breakpoint site and the expression of *BCL-2* and *BAX* proteins,

Table 1. Results of the PCR Analysis for *Bcl-2* Rearrangement Performed in 149 Patients With Both PB and BM Pretreatment

	Overall Series (n = 149)	*MBR* (n = 105)	*mcr* (n = 13)	Germline (n = 31)
PB^+ BM^+	87 (59%)	77	10	—
PB^- BM^-	31 (21%)	—	—	31
PB^+ BM^-	23 (15%)	21	2	—
PB^- BM^+	8 (5%)	7	1	—

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we analyzed such expression in a subset of 47 patients for whom tumor tissue was available. In 40 of 47 patients, *BCL-2*-protein expression was found in tumor tissue, whereas no expression was observed in 7 patients. Although not statistically significant, the proportion of cases expressing positive or strongly positive *BCL-2* protein was higher among germline cases (89%) than among *MBR* (73%) and *mcr* cases (60%). No differences were found regarding *BAX*-protein expression.

Bcl-2 *breakpoints and their correlation with pretreatment features.* The correlation of the *bcl-2*-breakpoint site with pretreatment features is summarized in Table 2. Germline patients tended to be older, to have higher LDH and β2M values, and to have a higher incidence of advanced stage. Patients with an *mcr* breakpoint tended to be younger, to have lower LDH and β2M values, and to have a lower rate of advanced stage. Differences in distribution of patient characteristics among breakpoint groups were not statistically significant at the .05 level with the exception of Ann Arbor stage ($P = .03$) and β2M values ($P = .02$). Differences between *mcr* and *MBR* patients did not reach statistical significance.

Response to therapy. Among the 228 patients with evaluable response, 200 (88%) achieved complete response (CR) and 26 (11%) achieved partial response CR rates for *mcr*, *MBR*, and germline groups were 96%, 90%, and 71%, respectively ($P < .01$). The trend for fewer responses among germline cases held true when consideration was restricted to patients with stage IV disease (Table 3). Differences between *mcr* and *MBR* patients did not reach statistical significance.

Failure-free survival. At the time of analysis, 56 patients (23%) had experienced relapse or progression; 7 patients experienced histologic transformation into large-cell lymphoma at relapse (5 cases *MBR*, 2 cases germline). Estimated FFS at 3 years after initiation of therapy was 0.74 (standard error: 0.04) (Fig 1).

Table 2. Characteristics of the 247 Patients With FL According to the Site of the *Bcl-2* Rearrangement

	Percent of Patients		
	mcr (n = 27)	*MBR* (n = 175)	G-*MBR*/*mcr* (n = 45)
Male gender (%)	44	48	55
Age ≥60 yr (%)	30	30	41
Histologic subtype (%)			
Grade I	67	63	60
Grade II	30	34	36
Grade III	4	3	4
Bulky disease (%)	23	22	16
Ann Arbor stage (%)			
I-II	33	20	11
III	11	17	27
IV	56	63	62
Extranodal involvement ≥1 site (%)	67	69	66
Bone marrow (+) (%)	52	57	53
Serum LDH level (median)	431	472	491
Serum β2M level (median)	1.7	1.9	2.2

Abbreviation: G-*MBR*/*mcr*, germline for *MBR* and *mcr*.

Table 3. Clinical Response to Therapy in 228 Assessable Patients According to the *Bcl-2* Breakpoint Site

	mcr (n = 27)	*MBR* (n = 163)	Germline (n = 38)
Whole series			
CR	26 (96%)	147 (90.5%)	27 (71%)
PR	1 (4%)	15 (9%)	10 (26%)
Failure	0	1 (0.5%)	1 (3%)
Stage IV disease	(n = 15)	(n = 104)	(n = 24)
CR	14 (93%)	92 (89%)	17 (71%)
PR	1 (7%)	11 (10%)	7 (29%)
Failure	0	1 (1%)	0

There was one relapse among 27 patients with *mcr* breakpoint, 42 among 175 patients with MBR breakpoint and 13 among 45 germline patients. An estimated 3-year FFS for these groups was 0.95 (standard error = 0.05), 0.76 (standard error = 0.04), and 0.57 (standard error = 0.10), respectively. The overall difference among FFS curves was statistically significant ($P < .001$; Fig 2). These results suggested a trend for superior FFS among patients with *mcr* breakpoint and unfavorable outcomes for germline cases. The large group of *MBR*-breakpoint cases was of particular interest because of the apparent low risk of failure in early follow-up, with a subsequent increase in the rate of failure. This observation was verified in an analysis of smoothed hazard rates (not shown), which indicated for the *MBR*-breakpoint group a gradually increasing risk of failure during the first 24 months after treatment. Although treatment failure among patients in the germline group was higher, there was no real evidence that the rate changed over time, at least through the first 36 months. The single failure in the *mcr* group did not allow characterization of their risk of failure over time.

Abnormal pretreatment LDH and β2M levels have been reported as poor prognostic factors in FL, and their association with FFS was verified in this patient group (results not shown). Because these poor features also occurred more commonly in the *MBR* group and particularly in the germline group, it was possible that these factors accounted for differences in FFS among the breakpoint groups. To investigate this question, patients were divided into three risk groups: those whose LDH and β2M were both normal, those in whom only one of these

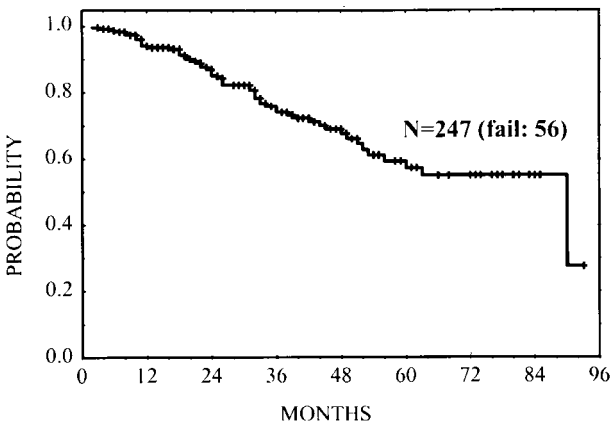

Fig 1. FFS in 247 patients with indolent FL.

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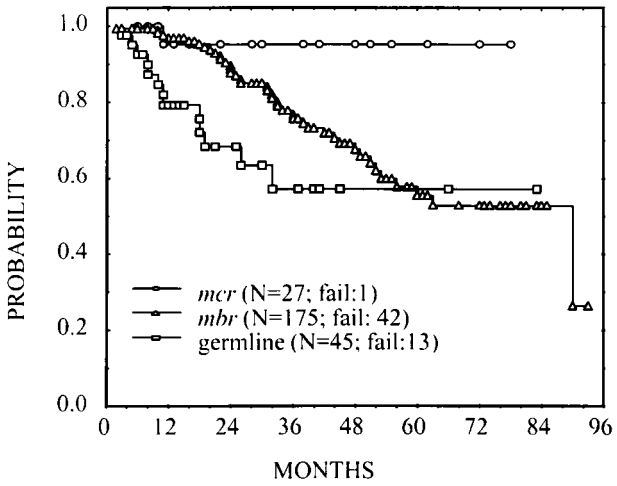
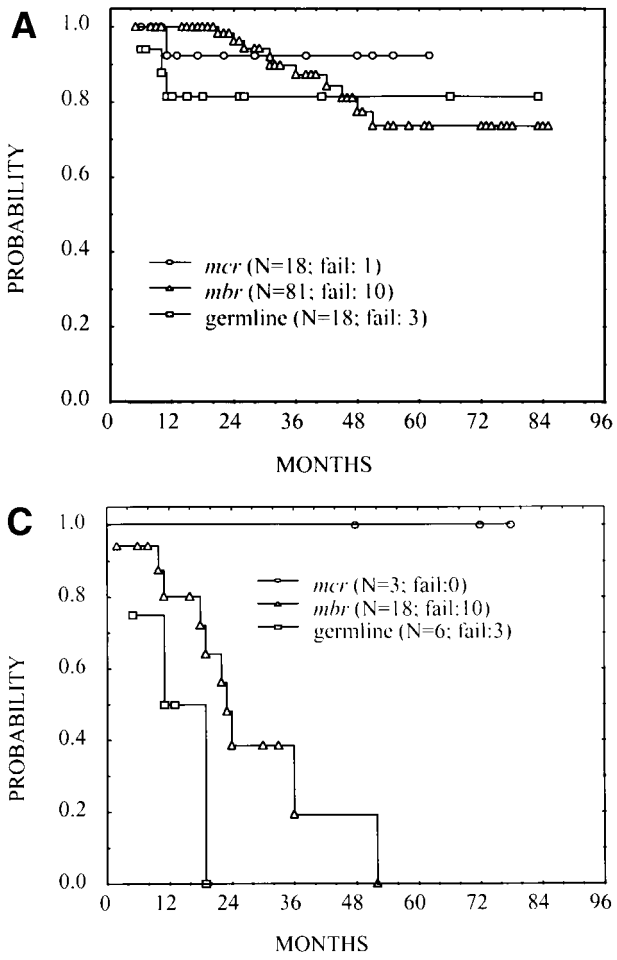


Fig 2. FFS in 247 patients with FL according to the *bcl-2*-breakpoint site *MBR*, *mcr*, and germline for both *MBR* and *mcr* ($P < .001$).

factors was normal, and those in whom both factors were abnormal. FFS for patients with *mcr* and *MBR* breakpoints and those with germline rearrangements are plotted in Fig 3 separately within the three risk groups. Although the number of patients was small, the same pattern of FFS differences by breakpoint was noted within each risk group as observed in the overall group. A Wilcoxon test stratified by risk group resulted in a *P* value of .08. These results suggest that the breakpoint site is an independent prognostic variable from LDH and β2M values in predicting FFS. Although there was an apparent lack of proportionality of hazard rates, we performed a proportional hazard model analysis[32] that confirmed the same results as the stratified test previously reported; the presence of bcl-2 rearrangement (at *mcr* or *MBR*) maintained its prognostic importance along with serum β2M and LDH levels.

Patients with stage IV disease treated with the intensive ATT regimen fared somewhat better than those who received the FND regimen. In an attempt to consider the possibility that a treatment effect could have influenced the FFS as related to the breakpoint site, FFS was compared for those patients with stage IV disease treated with ATT and FND. Although the number of patients was small, the same ordering of FFS curves was preserved within the homogeneously treated subsets of ATT and FND.

Overall survival. There have been 17 deaths so far in this group of patients. The cause of death was directly or indirectly related to lymphoma in 11 cases (progressive disease in 9 cases, toxicity in 2). The remaining six patients died in CR from other causes such as concurrent tumors in two cases (glioblastoma



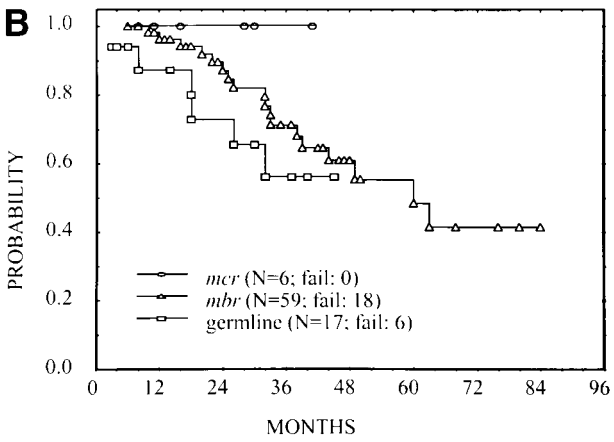


Fig 3. FFS according to the *bcl-2*-breakpoint site (*mcr, MBR,* and germline) in three subsets of patients with different risks: (A) patients with normal serum LDH and β2M (no significant difference among breakpoints), (B) patients with high LDH or β2M ($P = .02$), and (C) patients with high LDH and β2M ($P = .03$).

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multiforme, disseminated breast cancer), myocardial infarction in one case, gunshot wound in one case, and unknown (probably cardiovascular disease) in two cases.

DISCUSSION

Most patients with FL, even those in early stages, have circulating cells that carry the *bcl-2* rearrangement providing a unique opportunity to identify and classify them according to their *bcl-2* rearrangement without necessarily having to directly study their tumor tissue. However, in a considerable number of cases (one third of the whole series) we performed the same analysis directly on tissue involved by the malignant lymphoma to ascertain if the results obtained from PB and BM samples were representative of those obtained from the tumor. The agreement between the results from PB/BM and lymph node was 94.2%. No patient with a *bcl-2* rearrangement assessed in lymph-node tissue failed to be detected when combining PB and BM determinations. Of note is the fact that in 5.8% of the cases, a *bcl-2* rearrangement was detected in PB or BM by PCR but was not observed in tumor tissue. Possible explanations for this discrepancy are (1) the tumor sample studied was only partially infiltrated by lymphoma, (2) some technical problems could have occurred in extracting DNA from tumor tissue, (3) the blood/marrow results could be spurious and could perhaps represent contamination, or (4) the *bcl-2* rearrangement detected was real but did not correspond to the tumor tissue. This latter alternative cannot be completely ruled out in these four cases, because *bcl-2* rearrangements have been described in PB of healthy individuals.[33-35] However, when only 1 μg of DNA is loaded, as we have done in this study, the *bcl-2* rearrangement in blood is only detected in 6% of normal individuals.[33] When whole PB white cells are tested and not B lymphocytes, as in our study, this proportion is expected to be much lower.

Relatively good concordance between the detection of lymphoma cells in blood and BM at diagnosis has been previously observed by different groups.[36-38] However, in one fifth of the patients tested, the *bcl-2* rearrangement was detected only in the PB or in BM but not in both. Two thirds of these cases were positive in blood, but negative in BM. Because BM infiltration in FL is characteristically patchy and frequently not observed in marrow aspirates, this finding is not viewed as unusual. In fact, Gribben et al[39] previously observed that the results of PCR from BM biopsy specimens varied according to the site where the biopsy was performed.

The most significant and interesting observation in this study is the intriguing correlation between the *bcl-2* breakpoint site and the clinical outcome. As a group, the mcr^+ patients so far have shown an excellent prognosis. Their pretreatment prognostic variables were more favorable than those of the other groups (earlier Ann Arbor stage and lower serum β2M levels); in addition, they also had a higher CR rate and superior FFS. So far, only 1 of 27 patients have relapsed at a median follow-up of 27 months. On the other hand, those patients with germline *bcl-2* had a poor prognosis. Their response to therapy was suboptimal (<70% of CR rate), and their relapse rate was highest of all. It is interesting to note that the relapse pattern of MBR^+ cases appears different when compared with germline cases. The MBR^+ cases display the typical FFS curve of FL, which consists of a slow but relentless relapse pattern, without any hint of a plateau. Interestingly, the germline cases have a FFS curve that mimics that of aggressive lymphomas: early and frequent relapses for the first 3 years but no more relapses. A longer follow-up will be necessary to confirm this observation.

The type of treatment delivered to patients with advanced stage was associated with some differences in FFS. Those who received the intensive ATT regimen experienced a better FFS than those treated with FND. However, the two groups of patients might not be strictly comparable because the median follow-up is different. This is because of the fact that FND recently has been introduced for advanced, previously untreated FL in our institution.[40] In fact, in a current prospective randomized study comparing FND with ATT, no statistically significant differences have been found between these two regimens.[40]

Table 4 summarizes previous studies[16-21] on the prognostic importance of *bcl-2* rearrangements in FL. In most of these series, the number of patients was small. Furthermore, most authors included all FL-cell types, whereas Yunis et al[17] only considered mixed and large cell FL, and one half of Johnson's cases[16] were of ''high-grade'' histology. This indicates a predominance of large cell as well as a diffuse pattern on the biopsy specimen. These subgroups are not comparable with typical indolent FL and probably a large number of FL transformed to large cell lymphoma have been included in these series. In large

Table 4. Previous Series Regarding the Prognostic Importance of *Bcl-2* Rearrangement in FL

Reference	No. of Patients	Histology	Technique to Assess t(14;18)	Prognostic Value of *bcl-2* Rearrangement		
				CR Rate	DFS	OS
Levine,[18] 1988	30	FL	Cytogenetics	NS	NS	NS
Yunis,[17] 1989	20	FL mixed/large cell	SB	*MBR*(+) > others	NA	*MBR*(+) > others
Pezzella,[19] 1992	70	FL	SB/PCR	NA	NA	NS
Tilly,[20] 1994	66	FL	Cytogenetics	NA	NA	NS
Johnson,[16] 1995	102	FL (52 high-grade)	PCR	NA	NA	*MBR*(+) > others (low-grade)
Louie,[21] 1996	79	FL	SB/cytogenetics	NA	NA	NS
This study, 1998	247	Indolent FL	PCR	mcr(+)/MBR(+) > Others	*mcr*(+)/*MBR*(+) > Others	NS

Abbreviations: DFS, disease-free survival; OS, overall survival; SB, Southern blot; NS, not significant; NA, not assessed; PCR, polymerase chain reaction.

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cell lymphomas, the presence of a *bcl-2* rearrangement has been related to a poor outcome,[41] supposedly because these patients correspond to transformed FL, which traditionally have had a poor outcome. On the other hand, Johnson et al[16] found *bcl-2* rearrangement to be a favorable factor for survival, but only for patients with the indolent cell types.

Finally, it is difficult to explain the differences in outcome of the patients according to the *bcl-2* rearrangement. In fact, the expression of *BCL-2* and *BAX* proteins, assessed by a semiquantitative immunostaining technique in a significant number of patients, did not show significant differences according to the *bcl-2* rearrangement, although the proportion of germline cases with positive or strongly positive *bcl-2* expression was higher than that of *MBR* and *mcr* cases. Because the number of patients tested is small, we have to be cautious in the interpretation of these results. On the other hand, p53 mutations,[42] c-myc rearrangements[43] or, more recently, different p16 alterations[44] have been related to aggressive behavior in FL, mostly by histologic transformation of FL. However, there is no proved link between such alterations and the *bcl-2* rearrangement so far.

In conclusion, the type of *bcl-2* rearrangement appears to be an important biological feature that correlates well with the outcome in patients with FL, especially when combined with other classical prognostic factors such LDH and β2M. The biological explanation for the clinical differences we have identified remains unclear. More studies on *bcl-2*, *bax*, and other related molecules, including a more detailed analysis of such proteins and their mRNA would be useful to better understand the pathogenesis of FL.

REFERENCES

1. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A: Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. N Engl J Med 307:1231, 1982

2. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM: Cloning of the chromosome breakpoint of neoplastic B cell with the t(14;18) chromosome translocation. Science 226:1097, 1984

3. Graninger WB, Seto M, Boutain B, Goldman P, Korsmeyer SJ: Expression of *bcl-2* and *bcl-2*-Ig fusion transcripts in normal and neoplastic cells. J Clin Invest 80:1512, 1987

4. Tsujimoto Y, Croce C: Analysis of the structure, transcripts, and protein products of *bcl-2*, the gene involved in human follicular lymphoma. Proc Natl Acad Sci USA 83:5214, 1986

5. Vaux DL, Cory S, Adams JM: *Bcl-2* gene promotes haematopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335:440, 1988

6. Oltvai ZN, Milliman CL, Korsmeyer SJ: *Bcl-2* heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74:609, 1993

7. Cory S: Regulation of lymphocyte survival by the *bcl-2* gene family. Annu Rev Immunol 13:513, 1995

8. Yang E, Korsmeyer SJ: Molecular thanatopsis: A discourse on the *BCL2* family and cell death. Blood 88:386, 1996

9. Lee MS: Molecular aspects of chromosomal translocation t(14; 18). Semin Hematol 30:297, 1993

10. Cleary ML, Sklar J: Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci USA 82:7439, 1985

11. Tsujimoto Y, Cossman J, Jaffe E, Croce CM: Involvement of the *bcl-2* gene in human follicular lymphoma. Science 228:1440, 1986

12. Cleary ML, Galili N, Sklar J: Detection of a second t(14;18) breakpoint cluster region in human follicular lymphomas. J Exp Med 164:315, 1986

13. Horsman DE, Gascoyne RD, Coupland RW, Coldman AJ, Adomat SA: Comparison of cytogenetic analysis, southern analysis, and polymerase chain reaction for the detection of t(14;18) in follicular lymphoma. Am J Clin Pathol 103:472, 1995

14. Saiki RK, Gelfand DH, Stoffel S, Scharf S, Higuchi R, Horn G, Mullis K, Ehrlich H: Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487, 1988

15. Lee MS, Chang KS, Cabanillas F, Freireich EJ, Trujillo JM, Stass SA: Detection of minimal residual cells carrying the t(14;18) by DNA sequence amplification. Science 237:175, 1987

16. Johnson A, Brun A, Dictor M, Rambech E, Akerman M, Anderson H: Incidence and prognostic significance of t(14;18) translocation in follicle center cell lymphoma of low and high grade. Ann Oncol 6:789, 1995

17. Yunis JJ, Mayer MG, Arnesen MA, Aeppli DP, Oken MM, Frizzera G: *Bcl-2* and other genomic alterations in the prognosis of large cell lymphoma. N Engl J Med 320:1047, 1989

18. Levine EG, Arthur DC, Frizzera G, Peterson BA, Hurd DD, Bloomfield CD: Cytogenetic abnormalities predict clinical outcome in non-Hodgkin lymphoma. Ann Intern Med 108:14, 1988

19. Pezzella F, Jones M, Ralfkiaer E, Ersboll J, Gatter KC, Mason DY: Evaluation of *bcl-2* protein expression and 14;18 translocation as prognostic markers in follicular lymphoma. Br J Cancer 65:87, 1992

20. Tilly H, Rossi A, Stamatoullas A, Lenormand B, Bigorgne C, Kunlin A, Monconduit M, Bastard C: Prognostic value of chromosomal abnormalities in follicular lymphomas. Blood 84:1043, 1994

21. Louie DC, Hochauser D, Schluger A, Jaslow R, Roy P, Oliver J, Offit K, Chaganti RSK: *BCL6* and *BCL2* rearrangement and p53 overexpression as prognostic factors in follicular lymphoma. Ann Oncol 7:16 (051), 1996 (suppl 3)

22. Hermine O, Haioun C, Lepage E, d'Agay MF, Briere J, Lavignac C, Fillet G, Salles G, Marolleau JP, Diebold J, Reyes F, Gaulard P, for the GELA: Prognostic significance of *bcl-2* protein expression in aggressive non-Hodgkin's lymphoma. Blood 87:265, 1996

23. Hill ME, MacLennan KA, Cunningham DC, Vaughan Hudson B, Burke M, Clarke P, Di Stefano F, Anderson L, Vaughan Hudson G, Mason D, Selby P, Linch DC: Prognostic significance of *bcl-2* expression and *bcl-2* major breakpoint region rearrangement in diffuse large cell non-Hodgkin's lymphoma: A British national lymphoma investigation study. Blood 88:1046, 1996

24. Seite P, Hillion J, d'Agay MF, Gaulard P, Cazals D, Badoux F, Berger R, Larsen CJ: *BCL2* gene activation and protein expression in follicular lymphoma: A report on 64 cases. Leukemia 7:410, 1993

25. Lister AT: The management of follicular lymphoma. Ann Oncol 2:131, 1991 (suppl 2)

26. López-Guillermo A, Montserrat E, Bosch F, Terol MJ, Campo E, Rozman C: Applicability of the International Index for aggressive lymphomas to patients with low-grade lymphoma. J Clin Oncol 12:1343, 1994

27. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JKC, Cleary ML, Delsol G, DeWolf-Peeters C, Falini B, Gatter KC, Grogan TM, Isaacson PG, Knowles DM, Mason DY, Muller-Hermelink HK, Pileri SA, Piris MA, Ralfkiaer E, Warnke RA: A revised European-American classification of lymphoid neoplasms: A proposal from the International Lymphoma Study Group. Blood 84:1361, 1994

28. McLaughlin P, Hagemeister FB, Swan F, Cabanillas F, Romaguera J, Rodriguez MA, Lee MS, Pate O, Sarris A, Younes A: Intensive conventional-dose chemotherapy for stage IV low-grade lymphoma: High remission rates and reversion to negative of peripheral blood bcl-2 rearrangement. Ann Oncol 5:S73, 1994 (suppl 2)

29. McLaughlin P, Hagemeister FB, Romaguera JE, Sarris AH, Pate O, Younes A, Swan F, Keating M, Cabanillas F: Fludarabine, mitoxan-

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trone and dexamethasone: An effective new regimen for indolent lymphoma. J Clin Oncol 14:1262, 1996

30. Kaplan EL, Meier P: Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457, 1958

31. Gehan EA: A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203, 1965

32. Cox DR. Regression models and life tables. J Royal Stat Soc 34:187, 1972

33. Dolken G, Illerhaus G, Hirt C, Mertelsmann R: BCL-2/Jh rearrangements in circulating B cells of healthy blood donors and patients with nonmalignant diseases. J Clin Oncol 14:1333, 1996

34. Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen GJB, Schuuring E, Kluin PM: Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood 85:2528, 1995

35. Liu Y, Hernandez AM, Shibata D, Cortopassi GA: Bcl-2 translocation frequency rises with age in humans. Proc Natl Acad Sci USA 91:8910, 1994

36. Yuan R, Dowling P, Zucca E, Diggelmann H, Cavalli F: Detection of *bcl-2*/JH rearrangement in follicular and diffuse lymphoma: concordant results of peripheral blood and bone marrow analysis at diagnosis. Br J Cancer 67:922, 1993

37. Berinstein NL, Reis MD, Ngan BY, Sawka CA, Jamal HH, Kuzniar B: Detection of occult lymphoma in the peripheral blood and bone marrow of patients with untreated early stage and advanced stage follicular lymphoma. J Clin Oncol 11:1344, 1993

38. Berinstein NL, Jamal HH, Kuzniar B, Klock RJ, Reis MD: Sensitive and reproducible detection of occult disease in patients with follicular lymphoma by PCR amplification of t(14;18) both pre- and post-treatment. Leukemia 7:113, 1993

39. Gribben JG, Neuberg D, Freedman AS, Gimmi CD, Pesek KW, Barber M, Saporito L, Woo SD, Coral F, Spector N, Rabinowe SN, Grossbard ML, Ritz J, Nadler LM: Detection by polymerase chain reaction of residual cells with the *bcl-2* translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. Blood 81:3449, 1993

40. McLaughlin P, Cabanillas F, Younis A, Lee MS, Rodriguez MA, Hagemeter FB, Sarris A, Preti A: Stage IV low grade lymphoma (LGL): Randomized trial of two innovative regimens, with monitoring of *BCL-2* by PCR. Ann Oncol 7:34, 1996 (suppl 3)

41. Offit K, LoCoco F, Loure DC, Parsa NZ, Leung D, Portlock C, Ye BH, Lista F, Filippa DA, Rosenbaum A, Ladanyi M, Jhanwar S, Della-Favera R, Chaganti RSK: Rearrangement of the *BCL-6* gene as a prognostic marker in diffuse large-cell lymphoma. N Engl J Med 331:74, 1994

42. Sander CA, Yano T, Clark HM, Harris C, Longo DL, Jaffe ES, Raffeld M: p53 mutation is associated with progression in follicular lymphomas. Blood 82:1994, 1993

43. Yano T, Jaffe ES, Longo DL, Raffeld M: Myc rearrangements in histologically progressed follicular lymphomas. Blood 80:758, 1992

44. Pinyol N, Cobo F, Beá S, Jares P, Nayach I, Fernández PL, Montserrat E, Cardesa A, Campo E: Association of deletions, mutations and hypermethylation of the $p16^{INK4A}$ gene with the aggressive variant of non-Hodgkin's lymphomas. Blood 91:2977, 1998

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