original article

Prognostic impact of proliferative index determined by quantitative image analysis and the International Prognostic Index in patients with mantle cell lymphoma

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Background: The proliferative index (PI) is a powerful prognostic factor in mantle cell lymphoma (MCL); however, its utility is hampered by interobserver variability. The mantle cell international prognostic index (MIPI) has been reported to have prognostic importance. In this study, we determined the prognostic value of the PI as determined by quantitative image analysis in MCL.

Patients and methods: Eighty-eight patients with adequate tissue were included in this analysis. Patients were treated with one of two treatment programs: sequential therapy with high-dose therapy consolidation or radioimmunotherapy followed by combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone. Patients were divided into four groups based on PI (<10%, 10%–29.9%, 30%–49.9%, and >50%), and outcomes were analyzed.

Results: Thirty percent was identified as the optimal cut-off for PI. By univariate analysis, intensive treatment and a low PI were associated with a superior progression-free survival (PFS); only PI was associated with overall survival. By multivariate analysis, both intensive treatment and PI correlated with PFS. The MIPI had no prognostic impact.

Conclusions: PI is the most important prognostic factor in MCL. The cut-off of 30% is clinically meaningful and can be used to tailor the intensity of therapy in future clinical trials.

Key words: mantle cell lymphoma, prognosis, proliferative index, quantitative image analysis

introduction

The management of patients with mantle cell lymphoma (MCL) lacks an accepted standard of care. CHOP (combination chemotherapy with cyclophosphamide, adriamycin, vincristine, and prednisone) or CHOP-like chemotherapy regimens are associated with a median overall survival (OS) between 3 and 5 years [1–3]. Adding rituximab to CHOP (R-CHOP) or fludarabine-based chemotherapy has improved the overall response, but progression-free survival (PFS) and OS are not improved [4, 5]. Intensive regimens have been evaluated including fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone alternating with high-dose methotrexate—cytarabine (R-HyperCVAD/R-MA) and sequential chemotherapy followed by consolidation with high-dose therapy and autologous stem-cell rescue (HDT/ASCR) [6, 7]. For those patients who could tolerate more intensified treatment regimens, event-free survival and OS at 5–6 years improves to 48%–56% and 65%–70%, respectively [7, 8]. In contrast, some advocate a less intensive 'watch and wait' approach to MCL, with treatment when clinically indicated [9]. A better understanding of MCL prognostic factors would identify patients for whom intensified treatment is most appropriate. Currently, the best defined prognostic factors are the proliferative index (PI) and the mantle cell international prognostic index (MIPI).

Several studies have demonstrated that PI estimated by immunohistochemistry (IHC) for Ki-67 predicts clinical outcome. The PI inversely correlates with OS irrespective of treatment type [7, 10–18]. However, the method of determining PI by IHC has varied from a semiquantitative estimation to direct counting of 1000 cells per case. Different groups have reported variable cut-offs between 10% and 60% [7, 10–12, 14, 16–18]. This variability likely arises at least in part from interobserver variation in the interpretation of IHC for Ki-67.

MIPI is a better predictor of OS than the International Prognostic Index in a population treated with a variety of approaches including anthracycline-based chemotherapy with or without rituximab, as well as some patients undergoing consolidation with HDT/ASCR [19]. However, the simplified

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Table 1. Comparison of treatment groups

Characteristics	Intensive therapy[a] (N = 69), No (%)	RIT-CHOP[b] (N = 19), No. (%)	P[c]
Age, years			0.01
<60	49 (71)	7 (37)	
≥60	20 (29)	12 (63)	
LDH			0.40
Normal	43 (66)	15 (79)	
High	22 (34)	4 (21)	
NA	04	0	
Performance status (ECOG)			0.68
0–1	60 (88)	18 (95)	
2	8 (12)	1 (5)	
NA	1	0	
Ann Arbor stage			0.99
I or II	3 (4)	0	
III or IV	66 (96)	19 (100)	
Bone marrow involvement			0.09
No	17 (25)	9 (47)	
Yes	52 (75)	10 (53)	
MIPI			0.98
Low	34 (55)	10 (53)	
Intermediate	18 (29)	6 (32)	
High	10 (16)	3 (16)	
NA	7		
sMIPI			0.28
Low	33 (52)	9 (47)	
Intermediate	20 (31)	9 (47)	
High	11 (17)	1 (6)	
NA	5		
MIPI-Ki-67			0.30
Low	11 (19)	3 (16)	
Intermediate	29 (49)	13 (68)	
High	19 (32)	3 (16)	
NA	10	–	
Ki-67, %			0.06
<10	15 (22)	7 (37)	
10–29.9	23 (33)	10 (53)	
30–49.9	17 (25)	1 (5)	
≥50	14 (20)	1 (5)	

[a]R-CHOP-14 + (R)ICE + autologous stem-cell transplant.
[b]Tositumomab followed by CHOP.
[c]Chi-square test.
RIT-CHOP, radioimmunotherapy + CHOP; CHOP, cyclophosphamide, adriamycin, vincristine, prednisone; LDH, lactose dehydrogenase; NA, not available; ECOG, Eastern Cooperative Oncology Group; MIPI, mantle cell international prognostic index; sMIPI, simplified MIPI; R-CHOP, rituximab to CHOP; (R)ICE, ifosfamide, carboplatin, and etoposide) ± rituximab chemotherapy.

Table 2. Univariate analysis of prognostic factors in mantle cell lymphoma

Treatment	% 4-Year PFS	P[a]	% 4-Year OS	P[a]
Type		<0.01		0.64
Intensive (n = 69)	65		84	
Conventional (n = 19)	26		84	
MIPI		0.83		0.13
Low (n = 44)	62		83	
Intermediate (n = 24)	51		94	
High (n = 13)	48		69	
sMIPI		0.86		0.92
Low (n = 42)	63		82	
Intermediate (n = 28)	54		92	
High (n = 11)	43		75	
MIPI-Ki-67		0.87		0.13
Low (n = 14)	58		100	
Intermediate (n = 42)	60		89	
High (n = 22)	46		80	

[a]Log-rank test.
PFS, progression-free survival; OS, overall survival; MIPI, mantle cell international prognostic index; sMIPI, simplified MIPI.

variability in determining PI. We sought to determine a cut point for PI that could distinguish outcome in patients with MCL. Furthermore, we sought to determine the prognostic value of the MIPI, sMIPI, and Ki-67-MIPI.

patients and methods

One hundred and eleven patients with a new diagnosis of MCL between 1998 and 2008 participated in a treatment plan of sequential therapy with four cycles of CHOP-14 ± rituximab induction followed by two or three cycles of ICE (ifosfamide, carboplatin, and etoposide) ± rituximab chemotherapy [(R)ICE]. The number of cycles of (R)ICE chemotherapy was determined by functional imaging after induction. Patients underwent consolidation with HDT with combination of carmustine, etoposide, cytarabine and melphalan followed by ASCR. Between April 2001 to December 2004, 24 patients were included in a prospective study of sequential ^{131}I-tositumomab followed by six cycles of CHOP-21 [radioimmunotherapy (RIT-CHOP)] [21]. The diagnosis of MCL was confirmed by review of diagnostic biopsies. A total of 88 patients with adequate tissue were included in the analysis (69 from the HDT/ASCR group and 19 from the RIT-CHOP group). This study was approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board under a waiver of authorization.

MIPI

The MIPI, MIPI combined with Ki-67, and the sMIPI were determined as originally described [19].

immunohistochemistry

Eighty-eight patients had formalin-fixed paraffin-embedded (FFPE) tumor tissue available for IHC for Ki-67 staining using the MIB1 mouse mAb (Immunotech, Westbrook, ME) at a 1:500 dilution. Standard avidin–biotin–peroxidase complex techniques were used for IHC carried out on 5-μm sections of FFPE tissue. Antigen retrieval in heated citrate buffer at pH 6.0 was applied for all antibodies. Positive control tissues were stained in parallel with the study cases. Slides were counterstained with hematoxylin.

mantle cell international prognostic index (sMIPI) did not predict outcome for patients treated with R-HyperCVAD/R-MA [20].

Thus, both PI estimated by Ki-67 IHC and the MIPI have limitations in the prognostication of MCL. In this study, a highly reproducible computer-based quantitative image analysis (QIA) was employed to overcome the interobserver

quantitative image analysis

Ki-67-stained slides were scanned with the Aperio ScanScope XT (Aperio, Vista, CA) using a (20 objective. Whole slide images were analyzed with the Aperio nuclear IHC algorithm. Briefly, brown (3,3-diaminiobenzidine) and blue (hematoxylin) colors were separated spectrally. Blue cells that were also brown were scored as positive, and cells that were blue only were scored as negative. The percentage of positive cells was calculated as brown divided by the sum of brown and blue cells. The QIA algorithm counted a median of 571 358 cells per slide (1504–5 476 582).

cut-off determination

The sample size was not large enough to independently determine a clinically relevant cut point for the PI. We chose to validate the PI cut-offs of 10% and 30% as proposed by Determan et al. [10] and used in Geisler et al. [7]. Moreover, we tested a 50% cut-off to explore the impact of a higher PI on survival.

statistical analysis

Patient outcomes were evaluated by intention to treat. Patients who received any of the planned treatment (CHOP-14 for the HDT/ASCR group or radioimmunotherapy for the RIT-CHOP group) were included regardless of completing treatment. OS was calculated from diagnosis until death from any cause or the last follow-up visit. PFS was determined from the beginning of the treatment until progression, relapse, death from any cause, or last follow-up visit. Categorical data were analyzed by the chi-square test and continuous data by the Mann–Whitney test. Survival curves are per the method of Kaplan–Meier; comparative analyses were carried OUT by log-rank test. The Cox proportional hazards model was used for the multivariate analysis. Statistical analyses were conducted using SPSS version 17.0 (SPSS, Chicago, IL).

results

Patient characteristics ($N = 88$) are shown in Table 1, with a comparison of the HDT/ASCR and RIT-CHOP treatment groups. There was an expected male predominance (76%). The median age was 57 years (range 34–77 years); patients in the RIT-CHOP group were significantly older. Lactose dehydrogenase was abnormal in 26 of 84 available patients (31%). Bone marrow disease was present in 62 (71%) patients.
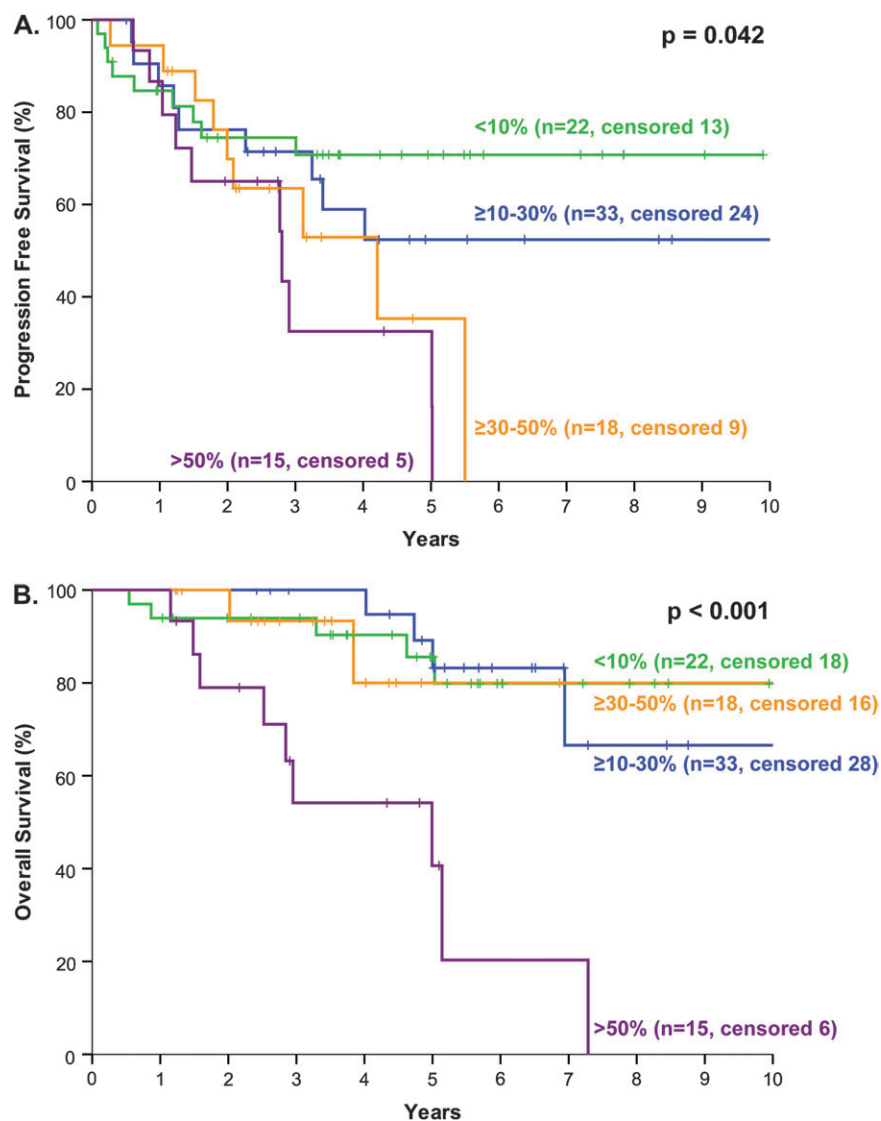

Figure 1. Progression-free survival (A) and overall survival (B) of patients with mantle cell lymphoma according to the proliferative index (Ki-67): <10% (blue); 10%–29.9% (green); 30%–49.9% (yellow); ≥50% (pink).

The majority had advanced-stage disease (stage III 8%; IV 77%). The MIPI and its variants were balanced between treatments. The PI was higher in patients receiving HDT/ASCR (median 29% versus 17%, $P = 0.002$).

Sixty-nine patients were treated according to the intensive protocol, and 64 were submitted to autogeneic stem-cell transplantation (93%). The reasons for not proceeding to HDT/ASCR were disease progression (one), allogeneic SCT (one), heart disease (one), renal disease (one), and one death from sepsis during ICE.

survival analysis

The median follow-up of surviving patients was 4.8 years (1.0–11.5 years). Thirty-seven patients relapsed, and 20 died during this period. The median PFS was 5 years; the median OS has not been reached (5-year OS 76%). There was no difference in PFS or OS with respect to rituximab use in the patients undergoing HDT/ASCR (4-year PFS 58% versus 50%, $P = 0.66$, and 4-year OS 87% versus 77%, $P = 0.17$). PFS was superior for patients in the HDT/ASCR group compared with the RIT-CHOP group (65% versus 26% at 4 years, $P < 0.01$); there was no difference in the OS between treatment groups (84% at 4 years in both groups) (Table 2).

proliferative index

The distribution of patients according to PI subgroups is summarized in Table 1; survival is depicted in Figure 1. The patients were divided into four groups based on PI: 0–9.9, 10–29.9, 30–49.9, and >50%) (Figure 1A). However, as there was no difference in PFS or OS in patients with PI <10% and PI 10%–29.9%, the prognostic groups could be reduced to two based on the cut-off of 30% for PFS and 50% for OS (Figure 1B). A PI cut-off of 50% performed no better than a cut-off of 30% for PFS. Furthermore, the 30% cut-off split the sample into two subgroups of a reasonable number of patients (low PI, 53 patients; high PI, 33 patients) with prognostic relevance to both PFS and OS and was chosen as the cut-off for the multivariate analysis (Figure 2A and C). More importantly, there is a plateau in the PFS for patients with a PI <30% (Figure 2A). The results were similar when only the HDT/ASCR patients were analyzed (data not shown).

MIPI

The MIPI, sMIPI, and MIPI-Ki-67 failed to show prognostic significance for OS or PFS (Table 2, Figure 3).
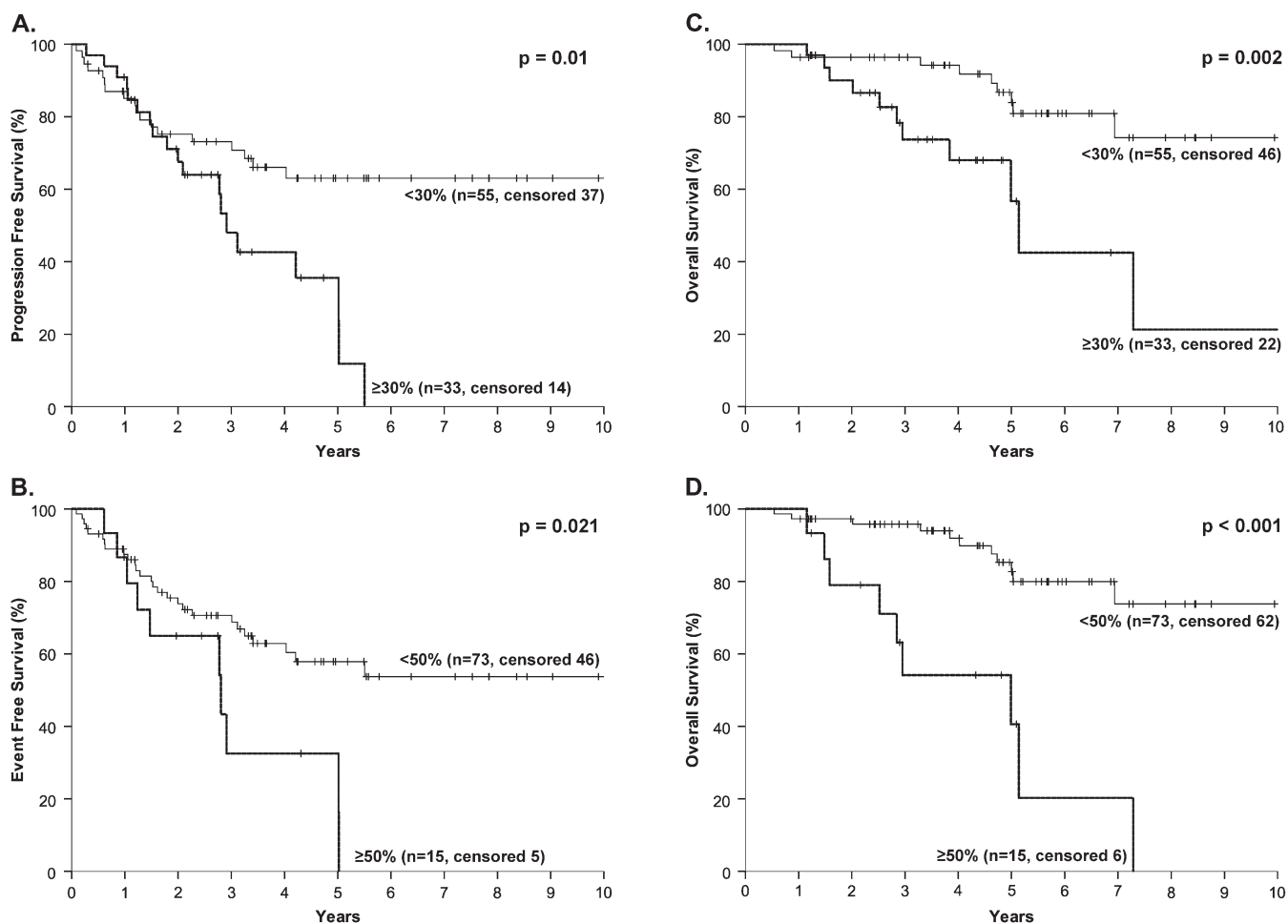

Figure 2. Progression-free survival (A, B) and overall survival (C, D) according to the proliferative index (PI) higher or lower than 30% (A, C) or 50% (B, D) in 88 patients with mantle cell lymphoma.
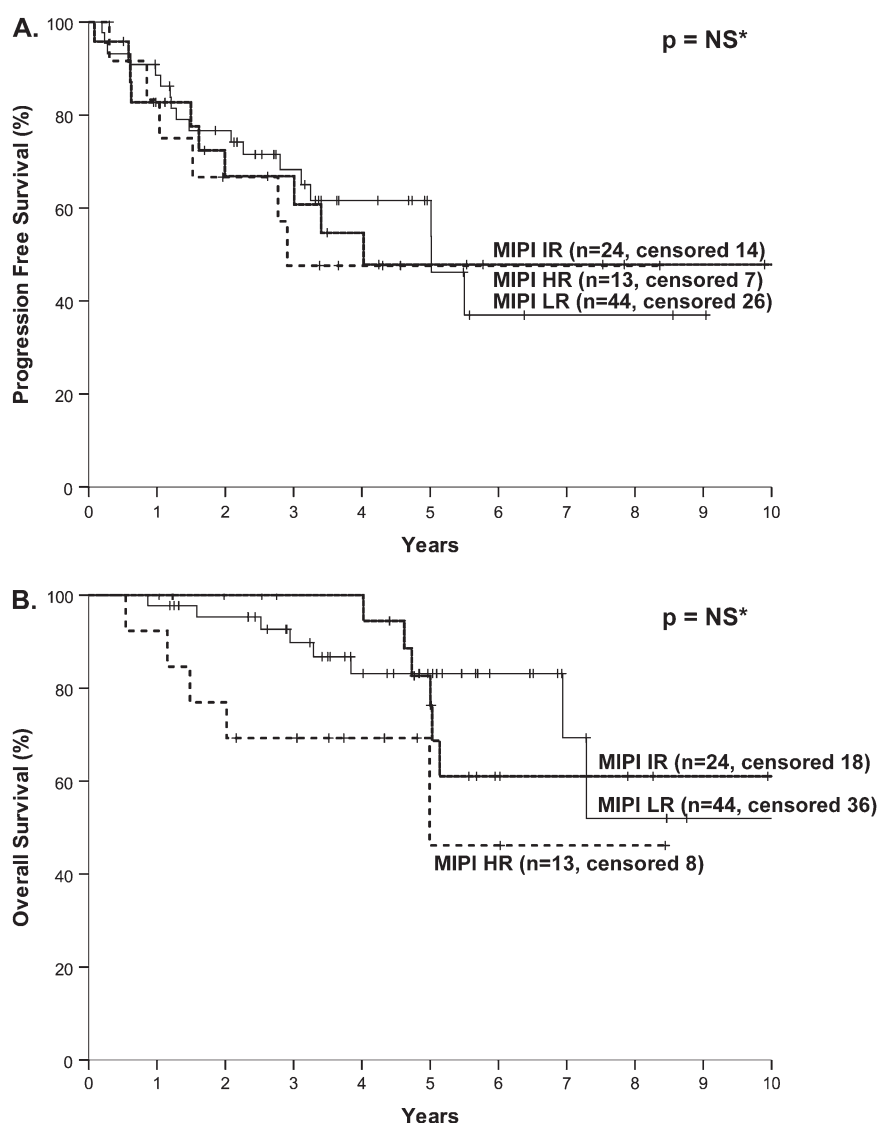
Figure 3. Progression-free survival (A) and overall survival (B) of patients with mantle cell lymphoma according to mantle cell international prognostic index score.

multivariate analysis

In the Cox regression analysis, the PI [hazard ratio (HR) 2.75, 95% confidence interval (CI) 1.43–5.30)] and the treatment group (HR 4.52, 95% CI 2.32–8.80) retained their prognostic significance for PFS. We did not carry out multivariate analysis for OS because only PI showed prognostic importance in the univariate analysis.

impact of treatment type according to PI

Among the low-PI patients, treatment type had a profound impact on PFS ($P < 0.001$) but not OS ($P = 0.10$) (Figure 4). At 5 years, 82% of patients who underwent HDT/ASCR remained free of progression as compared with 24% after RIT-CHOP. Furthermore, there were no relapses after 5 years among the patients who underwent HDT/ASCR ($N = 15$). There were not enough patients with high PI treated with RIT-CHOP to analyze the impact of treatment.

discussion

Gene expression profiling has identified proliferation signature as a powerful predictor of outcome in MCL [22]. Several studies have shown that the PI estimated by percent of Ki-67-positive cells can be used to predict outcome [7, 10–18]. However, determination of PI by Ki-67 IHC is subject to interobserver variation limiting study-to-study comparison (R. Schaffel et al., unpublished data) [23]. Approaches have included manual counting and QIA. We applied QIA to Ki-67 determination in MCL and found it to be robust and reproducible (Schaffel et al., unpublished data) [10, 12, 15, 18].

In the current study, only PI determined by QIA was a prognostic factor for OS. Patients could be divided into two groups based on PI with the best cut-off value for PFS (30%) and for OS (50%). However, for practical purposes, a single cut-off was chosen for both PFS and OS using the 30% value. This generated two groups that retained prognostic significance
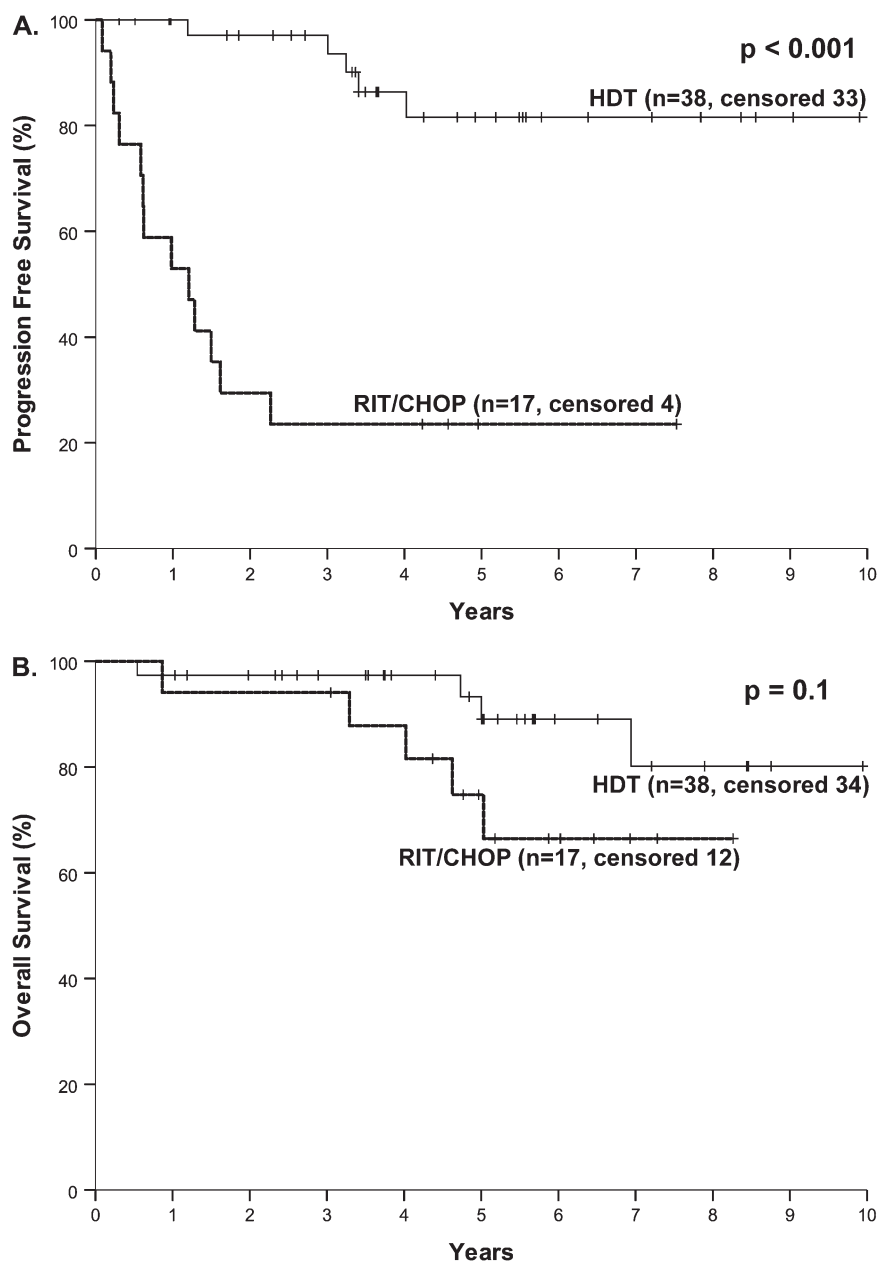
Figure 4. Progression-free survival (A) and overall survival (B) of patients with mantle cell lymphoma and a proliferative index <30% according to the planned therapy [high-dose chemotherapy with autologous stem-cell rescue (HDT/ASCR) or radioimmunotherapy followed by combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone (RIT-CHOP)].

for OS. Moreover, this cut-off has had prognostic importance in two other large studies [7, 10]. In multivariate analysis, the PI was strongly associated with PFS and was the only factor that predicted OS.

The possibility that a subgroup of patients with MCL could have a prolonged disease-free survival (DFS) was first described by Geisler et al. and has recently been confirmed [7, 24]. In our study, we corroborated this finding and showed that all patients who achieved long-term DFS (>5 years) had a PI of <30%. Moreover, this subset of patients could be found in both treatment programs, although the proportion was greater in the group consolidated with HDT/ASCR. The hypothesis that those patients may be cured is intriguing and requires longer follow-up.

Our study had some limitations. The relatively small number of patients precluded an independent determination of the PI cut-off. Rather, we sought to confirm cut-off values that had been previously reported. Furthermore, the sample size may have been too small to detect differences in outcome for PI cut-offs of <10% and 10%–29.9%. The subgroup of patients treated with RIT-CHOP included an insufficient number of patients with high-risk MIPI or high PI.

We also sought to determine whether the MIPI, sMIPI, or Ki-67-MIPI applied to our patient population. The MIPI and its variants were based on a population of 455 patients treated predominantly with CHOP ± R (87%), with only 80 patients (18%) treated with HDT/ASCR consolidation [19]. In that

study, application of the MIPI to the subset of patients undergoing HDT/ASCR consolidation was not shown. Application of the sMIPI to 97 patients receiving intensive treatment with R-HyperCVAD/R-MA failed to demonstrate the ability of this model to predict outcome [20].

In the current study, we also could not confirm that MIPI and its variants predicted outcome. There are several possible explanations. First, the median follow-up is longer in the current study; the early separation in the curves disappears beyond 4.5 years (Figure 3B). Second, in the current study, median OS was longer than in the original dataset for the intermediate (not reached versus 51 months) and high-risk groups (not reached versus 29 months) [19]. This may be the consequence of more effective therapy available after first line than was available for patients in the original model. Thus, with the treatment regimens used in this study, we are unable to confirm the utility of the MIPI and its variants to predict outcome.

In conclusion, PI is the most important prognostic factor in MCL, and the 30% cut-off was confirmed in our study. In future clinical trials, 30% PI may be a useful parameter. QIA may help overcome the center-to-center variability in the determination of PI and increase general applicability.

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disclosure

None of the investigators in this study have a conflict of interest pertaining to the findings or outcomes of this study.

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