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Clinical significance of the WHO grades of follicular lymphoma in a population-based cohort of 505 patients with long follow-up times

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Summary

The prognostic value of grading follicular lymphoma has been debated since the 1980s. There is consensus that World Health Organization (WHO) grades 1 and 2 are indolent, but not whether grades 3A or 3B are aggressive. We retrospectively reviewed the follicular lymphoma diagnoses according to the 2008 WHO classification in all diagnostic specimens from a population-based cohort of 505 patients with a median follow-up time of 10·0 years (range, 4·6–16·0). After excluding 43 patients with concomitant diffuse large B-cell lymphoma, 345 remained with grade 1–2, 94 with grade 3A, and 23 with grade 3B follicular lymphoma. Grades 1–2 and 3A seemed equally indolent, with indistinguishable clinical courses, even in patients receiving anthracyclines. Compared with grades 1–3A and independently of clinical factors, grade 3B correlated with higher mortality ($P = 0{\cdot}008$), but outcome was improved after upfront anthracycline-containing therapy ($P = 0{\cdot}015$). In contrast to grade 1–3A patients, grade 3B patients experienced no relapses or deaths beyond 5 years of follow-up. Furthermore, patients with grade 3B were predominantly male and seldom presented with bone-marrow involvement. We conclude that follicular lymphoma grade 1–3A is indolent and incurable with conventional therapy. Grade 3B appears to be an aggressive but curable disease.

Keywords: anthracyclines, follicular lymphoma, grade, rituximab, World Health Organization

Follicular lymphoma is the most common indolent nodal lymphoma. It is composed of small centrocytes and large centroblasts residing in follicles that also harbour non-malignant immune cells. Follicular lymphoma is morphologically graded, the value of which has been debated since the 1980s (Gallagher *et al*, 1986; Horning *et al*, 1987; Anderson *et al*, 1993; Bartlett *et al*, 1994; Martin *et al*, 1995; Miller *et al*, 1997; Wendum *et al*, 1997; Rodriguez *et al*, 1999; Chau *et al*, 2003; Hans *et al*, 2003; Hsi *et al*, 2004; Ganti *et al*, 2006; Klapper *et al*, 2007; Harris *et al*, 2008; Piccaluga *et al*, 2008; Shustik *et al*, 2011). Based on the number of centroblasts, the World Health Organization (WHO) classification divides follicular lymphoma into grades 1, 2, and 3 (Harris *et al*, 2008). Subdivision between follicular lymphoma grades 3A and 3B is determined according to the presence of centrocytes (grade 3A) or solid sheets or entire follicles comprised of centroblasts (grade 3B).

There is consensus that grades 1 and 2 are indolent and incurable with conventional therapy, although allogeneic stem cell transplantation may cure some patients (van Besien, 2009). Today, most international lymphoma groups also include patients with grade 3A in indolent B-cell lymphoma-trials, while others treat them aggressively. The rare grade 3B is mostly treated as an aggressive diffuse large B-cell lymphoma (DLBCL). However, none of these therapeutic approaches finds firm support in the conflicting data.

In smaller samples, we have previously shown that patients with follicular lymphoma grade 1–2 and grade 3A do not have

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different outcome (Wahlin *et al*, 2007, 2010). The aim of this study was to evaluate the WHO grades in a large cohort of patients with long follow-up times. Our study hypothesis was that the subdivision between grade 3A and 3B is the one most clinically important in the grading system, because grade 3B would be a disease clinically more similar to DLBCL – aggressive but curable with anthracyclines, while grade 3A would have similar outcome as grade 1–2 – indolent and incurable with anthracyclines.

Materials and methods

Patients

Diagnostic samples from all patients newly diagnosed with follicular lymphoma between January 1994 and January 2004 at two lymphoma centres were subjected to a central review. Nine cases diagnosed only with fine-needle aspiration were not included. In total, 505 follicular lymphoma cases were confirmed (188 at the Karolinska University Hospital at Huddinge, Stockholm, Sweden and 317 at the Norwegian Radium Hospital, Oslo, Norway). These patients constitute a heterogeneously treated, unselected, population-based cohort with long follow-up times (median 10 years). They were studied according to a protocol approved by appropriate ethics committees in Stockholm and Oslo (reference numbers 04-526/4 and 200805132, respectively). Baseline and follow-up clinical data were obtained from hospital files.

Pathology review

All biopsies were reviewed by B.S, and all grade 3 cases were subject to an additional review together with B.C. Complicated cases were discussed with J.D. and C.S. The final diagnosis was always decided by B.S. The 2008 WHO criteria were used throughout (Harris *et al*, 2008). The reviewers were blinded to outcome. Grading was based on the average number of centroblasts per ×40 high-power microscopic field (hpf; 0·159 mm^2) in ≥10 randomly selected neoplastic follicles. Cases with ≤5 centroblasts/hpf were diagnosed as grade 1, 6–15 centroblasts/hpf as grade 2, and >15 centroblasts/hpf as grade 3. WHO defines grade 3B by 'solid sheets of centroblasts' but also says that 'grade 3B follicles are composed entirely of large blastic cells (centroblasts or immunoblasts)' (Harris *et al*, 2008). A solid sheet was defined as >15 intra-follicular centroblasts lumped together with no interspersed centrocytes (Fig 1). We examined the grade 3 cases using both the solid-sheet and the entire-follicle criteria, and one sheet or blastic follicle rendered a grade 3B diagnosis. In accordance with the WHO criteria, grades 1 and 2 were merged as grade 1–2, and diffuse components of any size in grade 3 biopsies were separately diagnosed as DLBCL. Cases with concomitant DLBCL were excluded from outcome-analysis, because concomitant DLBCL is a well-known adverse prognostic factor that could confound the impact of the grades (Hans *et al*, 2003). We also reviewed average numbers of centroblasts/hpf in each sample, proportions of follicular/diffuse components, and, when available, the proliferation marker Ki67 (always after grading).

Statistical methods

Overall survival was calculated from the date of primary follicular lymphoma diagnosis to the date of death, and time to treatment-failure from the date of starting first-line therapy to the date of starting second-line therapy or death. Patients were censored at last follow-up (January 2010). Additionally, survival was checked using the Swedish and Norwegian Population Registries. Associations with survival were evaluated using Kaplan-Meier curves and the log-rank test. Multivariate analysis was performed with forward stepwise Cox regression and the proportional hazards assumption was verified using Schoenfeld residuals. The predictors' associations with one another were estimated with χ^2, Wilcoxon and Spearman tests, depending on the nature of the variables. All *P* values are two-tailed and calculated using Stata 9.2 (StataCorp, College Station, TX, USA). $P < 0{\cdot}05$ was considered significant.

Results

Concomitant DLBCL was more common in higher grades ($P < 0{\cdot}0001$), seen in 9/32 grade 3B (28%), 22/116 grade 3A (19%), and 12/357 grade 1–2 cases (3%). These 43 patients

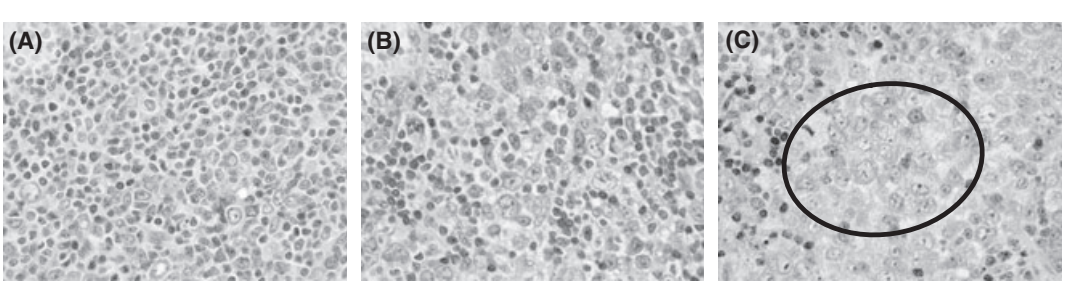

Fig 1. Centroblasts in follicular lymphoma. (A) Follicular lymphoma grade 1–2. (B) Follicular lymphoma grade 3A. (C) Follicular lymphoma grade 3B, with a solid sheet of centroblasts encircled.

with concomitant DLBCL were excluded from outcome-analysis. The pathology review data and therapeutic history of the remaining 462 patients are presented in Table I. Rituximab was given upfront to 13% and cumulatively to 42% of the patients. Of the patients with grade 3B, 70% received upfront anthracyclines, which thus constituted the only analysable treatment type in grade 3B. As shown in Table I, patients with grade 1–2 and 3A were similarly treated ($P = 0{\cdot}22$ for global heterogeneity of first-line therapy).

At the time of this analysis 247/462 patients (53%) had died, and 420/462 (91%) had received first-line and 330/420 (79%) second-line therapy. The median follow-up time was 10·0 years (range, 4·6–16·0). One patient was lost to follow-up. The median overall survival time was estimated to be 12·4 years, and the median time to treatment-failure was 2·2 years. Forty-two patients never received therapy: 18 died untreated, but 20 grade 1–2 and four grade 3A patients (in total, 5%) were untreated and alive with a median follow-up of 10·3 years.

Bone-marrow involvement was a stronger predictor of survival than stage III–IV disease (Table II), agreeing with a recent study that also included rituximab-treated patients (Federico *et al*, 2009). Patients with grade 3B had less stage III–IV disease and bone-marrow involvement, but showed trends towards an increased presence of B symptoms and male sex.

Grade 3B vs. 3A vs. 1–2

Patients with follicular lymphoma grade 1–2 and 3A showed similar median overall survival times: 12·4 and 12·2 years, respectively. There were no plateaux in their survival curves, which seemed to depict indolent and incurable conditions, in contrast to the curve of the grade 3B patients (Fig 2A). The 23 grade 3B patients had a median overall survival time of 4·4 years, with all 13 deaths occurring within the first 5 years, but no deaths or relapses thereafter. After multivariate adjustment for clinical factors, patients with grade 3B showed significantly inferior survival (Table II), even when compared separately with grades 1–2 ($P = 0{\cdot}012$) and 3A ($P = 0{\cdot}005$). Also independent in multivariate analysis were age, haemoglobin, lactate dehydrogenase, and bone-marrow involvement (Table II).

Table I. Morphological features and treatments.

		Distribution in grades			Difference between grade 3A and 1–2	Difference between grade 3B and 3A
Feature or treatment	Patients (%)	Grade 1–2 (%)	Grade 3A (%)	Grade 3B (%)	*P*-value	*P*-value
Type of biopsy	462 (100)	345 (75)	94 (20)	23 (5)		
Surgical	404 (84)	289 (84)	92 (98)	23 (100)		
Core needle	58 (16)	56 (16)	2 (2)	0 (0)		
Proportion of diffuse component						
<25%	360 (88)	244 (83)	93 (100)	23 (100)		
25–75%	35 (8)	35 (12)	0 (0)	0 (0)		
>75%	16 (4)	16 (5)	0 (0)	0 (0)		
Proportion of Ki67-positive cells					<0·0001	0·001
<5%	45 (18)	43 (24)	2 (3)	0 (0)		
5–25%	73 (29)	63 (36)	10 (17)	0 (0)		
26–50%	84 (33)	52 (29)	29 (49)	3 (18)		
51–75%	33 (13)	14 (8)	10 (17)	9 (53)		
>75%	18 (7)	5 (3)	8 (14)	5 (29)		
First-line treatment						
Rituximab without chemotherapy	31 (7)	25 (7)	6 (6)	0 (0)	0·77	0·21
Rituximab and anthracyclines	31 (7)	19 (6)	10 (11)	2 (9)	0·08	0·74
Anthracyclines without rituximab	127 (27)	83 (24)	30 (32)	14 (61)	0·12	0·010
Other regimen without rituximab	14 (3)	12 (3)	2 (2)	0 (0)	0·51	0·48
Single alkylator	130 (28)	104 (30)	23 (24)	3 (13)	0·28	0·24
Local radiation	87 (19)	65 (19)	18 (19)	4 (17)	0·95	0·85
Never treated patients	42 (9)	37 (11)	5 (5)	0 (0)	0·11	0·26
Cumulative treatment						
Ever received anthracyclines	292 (63)	211 (61)	62 (66)	20 (87)	0·41	0·049
Ever received fludarabine	59 (13)	46 (13)	12 (13)	1 (4)	0·88	0·25
Ever received rituximab	192 (42)	143 (41)	42 (45)	7 (30)	0·58	0·22
Stem-cell transplanted	61 (13)	39 (11)	16 (17)	6 (26)	0·14	0·32

Table II. Clinical characteristics and grades and their associations with survival.

		Effect on overall survival			Distribution in grades			
Characteristic	Patients (%)	Univariate analysis *P*-value	Multivariate analysis *P*-value	HR (95% CI)	Grade 1–2 (%)	Grade 3A (%)	Grade 3B (%)	Difference between grade 3B and 1–3A *P*-value
All patients	462 (100)				345 (75)	94 (20)	23 (5)	
Male sex	244 (53)	0·80	NS		184 (53)	45 (48)	15 (65)	0·22
Median age (range), years	57 (25–89)				57 (25–89)	59 (29–85)	59 (34–84)	
Age >60 years	190 (41)	<0·0001	<0·0001	2·8 (2·1–3·7)	136 (39)	44 (47)	10 (44)	0·81
Nodal stations >4	113 (25)	0·001	NS		84 (25)	23 (24)	6 (26)	0·90
Hb <120 g/l	57 (12)	<0·0001	0·0002	2·0 (1·4–3·0)	35 (10)	19 (20)	3 (13)	0·92
LDH > ULN	99 (23)	<0·0001	0·004	1·6 (1·2–2·2)	65 (21)	28 (30)	6 (26)	0·72
Ann Arbor stage III–IV	296 (65)	0·0001	NS		226 (66)	61 (66)	9 (41)	0·016
FLIPI		<0·0001	NS					0·47
0–1: low risk	198 (46)				146 (46)	39 (42)	13 (57)	
2: intermediate risk	124 (29)				98 (31)	22 (24)	4 (17)	
3–5: high risk	112 (26)				75 (23)	31 (34)	6 (26)	
Bone-marrow involvement	175 (39)	<0·0001	<0·0001	2·1 (1·6–2·9)	134 (39)	37 (41)	4 (17)	0·032
B symptoms	80 (18)	<0·0001	NS		55 (16)	18 (19)	7 (30)	0·10
Grade 3A vs. grade 1–2	439 (95)	0·55	0·07	0·7 (0·5–1·0)				
Grade 3B vs. grade 1–3A	462 (100)	0·19	0·008	2·2 (1·2–3·9)				

HR, hazard ratio; CI, confidence interval; NS, not significant; Hb, haemoglobin; LDH, lactate dehydrogenase; ULN, upper limit of normal; FLIPI, the follicular lymphoma international prognostic index.

Treatment with first-line anthracyclines correlated with superior overall survival in grade 3B disease (Fig 2B; multivariate $P = 0{\cdot}015$). All grade 3B patients appeared to suffer from aggressive lymphoma, both those whose follicles were entirely populated with centroblasts ($n = 13$) and those who were only defined by the presence of solid sheets of centroblasts ($n = 10$). In patients who were not given any anthracyclines upfront, either of the subcategories of grade 3B correlated with worse overall survival compared with grade 1–3A ($P = 0{\cdot}0001$ and $P = 0{\cdot}020$, respectively). In contrast, patients with grade 1–2 and 3A follicular lymphoma did not seem to benefit from upfront anthracycline-containing therapy (Fig 2B). Even after intensified treatment with autologous stem-cell transplantation patients with grade 1–2 and 3A experienced late relapses and lymphoma-related deaths (data not shown). The curability of grade 3B was not only due to more cases of limited disease, because also in grade 3B patients with generalized disease a survival plateau was observed (Fig 2C). Patients' overall survival rates at 5 years were 71% (95% CI 66%–76%) for grade 1–2 disease, 71% (95% CI 61%–79%) for grade 3A and 43% (95% CI 23%–62%) for grade 3B. At 10 years, the survival rates had decreased in patients with grade 1–2 [53% (95% CI 47%–59%)] and grade 3A disease [60% (95% CI 48%–70%)], but were unchanged in grade 3B.

The number of centroblasts/hpf (median, nine) did not predict survival in grade 1–3A patients ($P = 0{\cdot}85$). Attempts with non-WHO grading thresholds between 1–2 and 3A such as 10, 20, or 25 centroblasts/hpf did not produce prognostic groups (data not shown). Increasing Ki67-positivity correlated with higher WHO grades (Table I) and numbers of centroblasts/hpf ($P = 0{\cdot}0001$) but had no bearing on survival in grade 1–3A patients (Fig 2D). Neither did the proportion of diffuse component in grade 1–2 patients ($P = 0{\cdot}67$).

Also with other types of upfront treatment, follicular lymphoma grade 3A appeared as indolent as grade 1–2. Outcome was almost identical in grades 1–2 and 3A in patients treated upfront with single alkylators (Fig 3A; $P = 0{\cdot}96$), and there was likewise little difference in patients treated upfront with local radiation ($P = 0{\cdot}70$). Grade 3A showed a non-significant trend for better overall survival after upfront rituximab-containing therapy (Fig 3B; $P = 0{\cdot}12$). Overall survival was similar in grade 1–2 and 3A patients given anthracyclines ($P = 0{\cdot}71$), fludarabine ($P = 0{\cdot}82$), or rituximab ($P = 0{\cdot}22$) at any time, and in those never treated ($P = 0{\cdot}38$). In multivariate analyses stratified by grade 1–2 and 3A, the different therapeutic approaches showed similar effects, with the possible exception that grade 3A patients appeared to have an additional benefit from rituximab (Table III).

Other immunostainings

For a descriptive analysis, the pathology review also included an assessment of all available slides immunostained for BCL2, BCL6, CD10, and TP53 (p53). Cases were considered positive or negative according to clinical routine decision cut-off levels. BCL2 was positive in 84/89 (94%) of grade 1–2, 28/32 (88%)

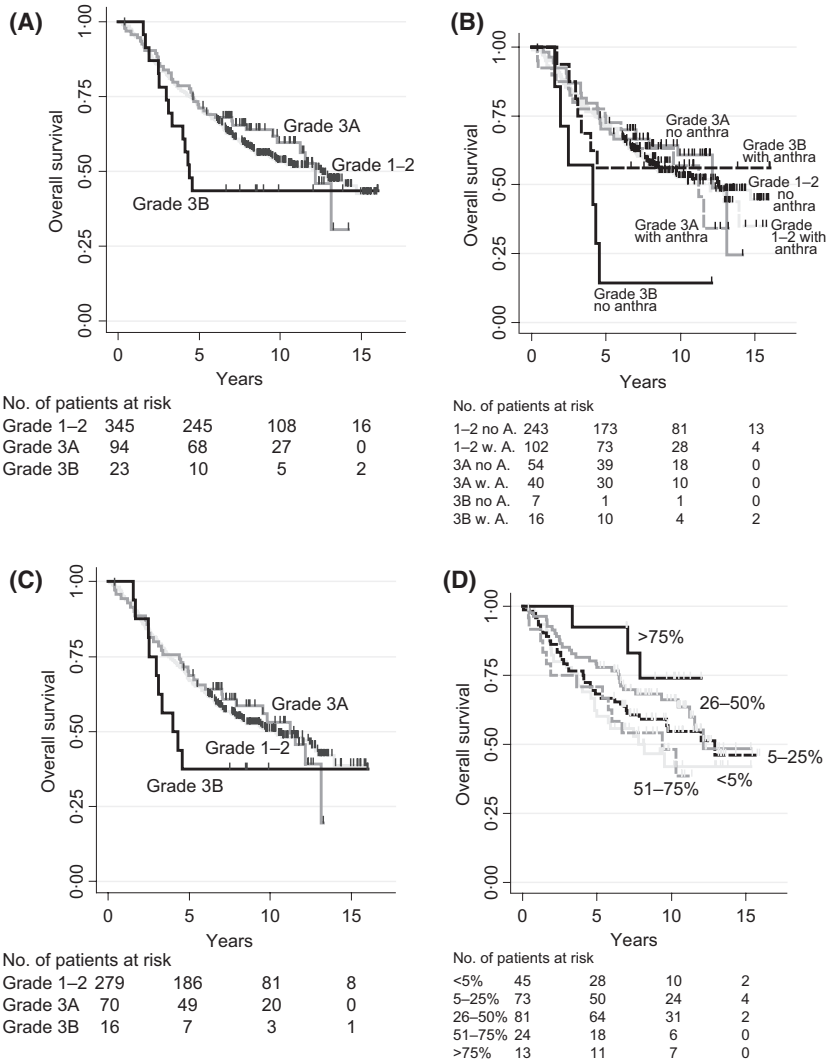

Fig 2. Overall survival curves. (A) Overall survival according to WHO grades 1–2, 3A, and 3B. (B) Overall survival according to grades 1–2, 3A, and 3B, stratified by upfront anthracycline-containing treatment (anthra). no A., no anthracycline; w. A., with anthracycline. (C) Overall survival according to grades 1–2, 3A, and 3B in patients with stage II–IV disease. (D) Overall survival according to Ki67-positivity in patients with grade 1–3A follicular lymphoma.

of grade 3A, and 16/21 (76%) of grade 3B slides. The difference in BCL2 between grade 1–3A and 3B was significant ($P = 0{\cdot}020$). Conversely, TP53 was more common with increasing grades of 1–2, 3A, and 3B: 4%, 7%, and 40%, respectively. Again, the difference between grade 3B and the rest was significant ($P < 0{\cdot}001$). Differences in CD10 positivity did not reach statistical significance (grade 1–3A, 94%; grade 3B, 87%). BCL6 was equally positive in grade 1–3A and 3B (97% and 100%, respectively).

Discussion

This is, to date, the largest comprehensive investigation of the WHO's grading system for follicular lymphoma. Patient follow-up times are long (median 10·0 years). Furthermore, 42% of the 462 analysed patients have received rituximab.

We showed that grade 3B is clinically different from grade 3A (Fig 2). Although previous reports often showed that the two grade 3 subtypes had diverging Kaplan-Meier curves, the differences were not statistically significant, probably because of few grade 3B patients and short follow-up. The study by Hsi *et al* (2004) included 10 grade 3B patients and a median follow-up time of 2·0 years and that of Chau *et al* (2003) comprised 11 grade 3B patients and a median follow-up time of 6·6 years. Chau *et al* (2003) noted that none of the grade 3B patients relapsed after anthracycline-containing therapy. Hans *et al* (2003) reported 25 grade 3B patients without concomitant DLBCL; the study's median follow-up time was 5·9 years (range, 1·0–17·6). Two of these reports (Hans *et al*, 2003; Hsi *et al* (2004) showed a trend for inferior survival in grade 3B patients: median overall survival was shown by Hans *et al* (2003) to be about 10·0 years in grade 3A and about 6·5 years

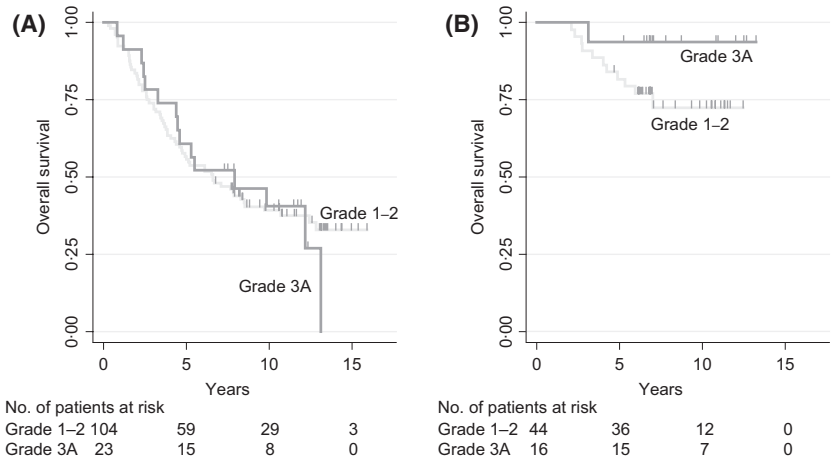

Fig 3. Overall survival according to follicular lymphoma grades 1–2 and 3A with different treatments. Overall survival in grade 1–2 and 3A patients treated upfront with (A) single alkylators and (B) rituximab-containing therapy.

Table III. Multivariate analyses of treatment types in grades 1–2 and 3A.

	Overall survival				Time to treatment-failure			
	Grade 1–2		Grade 3A		Grade 1–2		Grade 3A	
Therapy	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Upfront								
Anthracycline-containing	0·9 (0·6–1·3)	0·65	0·9 (0·4–1·7)	0·67	1·2 (0·9–1·6)	0·18	1·1 (0·7–1·8)	0·69
Anthracyclines without rituximab	1·1 (0·8–1·6)	0·64	1·6 (0·8–3·2)	0·14	1·3 (1·0–1·8)	0·06	2·0 (1·2–3·3)	0·011
Rituximab-containing	0·4 (0·2–0·7)	0·003	0·1 (0·0–0·5)	0·008	0·8 (0·5–1·1)	0·17	0·3 (0·1–0·6)	0·002
Rituximab without chemotherapy	0·4 (0·2–0·9)	0·022	*		0·8 (0·5–1.3)	0·35	0·4 (0·1–1·2)	0·10
Single alkylator	1·6 (1·2–2·3)	0·004	1·8 (0·9–3·5)	0·11	1·6 (1·2–2.1)	0·001	2·1 (1·2–2·1)	0·011
Local radiation	0·7 (0·4–1·2)	0·22	2·3 (0·6–7·8)	0·19	0·4 (0·3–0·6)	<0·0001	0·4 (0·3–0·6)	0·029
Never treated	0·9 (0·5–1·6)	0·69	0·6 (0·1–4·6)	0·64				
Cumulative treatments								
Ever anthracyclines	1·2 (0·8–1·8)	0·26	0·9 (0·4–2·0)	0·77				
Ever fludarabine	1·1 (0·7–1·7)	0·68	1·2 (0·5–2·8)	0·67				
Ever rituximab	0·6 (0·4–0·8)	0·002	0·3 (0·1–0·6)	0·001				
Stem-cell transplanted	0·7 (0·4–1·2)	0·24	0·7 (0·3–1·6)	0·38				

HR, hazard ratio and CI, confidence interval.

*Not calculable because there were no deaths among grade 3A patients given upfront rituximab without chemotherapy. The multivariate analyses were adjusted for the follicular lymphoma international prognostic index.

in grade 3B. The corresponding numbers reported by Hsi *et al* (2004) were 3·8 and 1·8 years. The survival curves for grade 3A and (22 cases of) grade 3B did not diverge in another study (Shustik *et al*, 2011). However, this study was also hampered by short follow-up times [median 3·8 years (range, <1·0–19·9)], and possibly by an underrepresentation of grade 3B cases (only 14% of all grade 3 were 3B) (Shustik *et al*, 2011). The WHO classification states that in unselected series, grade 3B is 20%–25% of purely follicular grade 3 (Harris *et al*, 2008). This expected frequency of grade 3B is matched in the present (20%) and most previous studies: 22% (Hsi *et al*, 2004), 20% (Chau *et al*, 2003), 25% (Hans *et al*, 2003), 27% (Ott *et al*, 2002), 18% (Katzenberger *et al*, 2004), and 14% (Shustik *et al*, 2011), in total 100/522 (19%). Thus, our study broadly agrees with most of the previous results. That notwithstanding, it is the first to show a plateau in the survival curve of grade 3B patients, which is only possible to do when patients have been followed for long times. Long-term observation is therefore crucial for appreciating the different natures of grades 3A and 3B. In the present study, the grade 3B patients had a median follow-up time of 9·4 years (range, 6·7–16·0), which is by far the longest period reported. In fact, the *minimal* follow-up time of 6·7 years in the present study is longer than the longest previously published *median* follow-up time for purely follicular grade 3 patients (6·6 years, Chau *et al*, 2003). If the follow-up had been shorter in our report, the survival curves

would probably have looked more like those presented by Hans *et al* (2003) and Hsi *et al* (2004).

In the present report, the survival curve for the grade 3B patients reached a plateau after 5 years and no relapses were observed thereafter. With upfront anthracyclines their chances for long-term survival increased from 14% to 56% (Fig 2B). The one grade 3B patient who survived without anthracyclines received curative radiation against localized disease. The grade 3B patients who did not receive upfront anthracyclines were all diagnosed with follicular lymphoma before this millennium and thus also before grade 3B was recognized as a diagnostic entity. Although they showed a trend for more limited disease, they did not otherwise present different clinical characteristics or follicular lymphoma international prognostic index (FLIPI) scores (Solal-Celigny *et al*, 2004) compared with those who received anthracyclines. Taken together, our data support the conclusion that the clinical course of grade 3B is similar to that of DLBCL. Agreeing with previous reports (Chau *et al*, 2003; Shustik *et al*, 2011), upfront anthracyclines were not associated with favourable outcome in grade 3A (or grade 1–2) disease. Because high-risk clinical factors indicate anthracycline-containing therapy in follicular lymphoma, selection-bias must be taken into account. Anthracycline-treated grade 1–3A patients showed significantly higher FLIPI scores than others ($P < 0{\cdot}0001$), even within each grade (grade 1, $P = 0{\cdot}038$; grade 2, $P = 0{\cdot}008$; grade 3A, $P = 0{\cdot}011$). However, grade 3A patients did not appear to benefit more from anthracyclines than grade 1–2 patients did. With all kinds of upfront therapy (and also without any therapy), the outcome was similar in grades 1–2 and 3A, even after multivariate adjustment for clinical factors, although patients with grade 3A seemed to benefit more from rituximab than those with grade 1–2 did. This finding needs to be further explored in datasets with more rituximab-treated patients. There seem to be fundamentally different levels of aggressiveness and curability between the two subtypes of grade 3. Patients with grade 3A may safely be recruited to indolent lymphoma trials. Grade 3B appears to be an aggressive lymphoma.

More patients with follicular lymphoma, but not with DLBCL, are women (58% compared with 45%) (The Non-Hodgkin's Lymphoma Classification Project., 1997). Interestingly, there is a similar gender difference between grades 3A and 3B. In this cohort of 505, 62/116 (53%) of grade 3A and 11/32 (34%) of grade 3B patients were female. In previous studies where gender was reported, the proportions of women with grades 3A and 3B were 57% and 40% (Hsi *et al*, 2004), 59% and 43% (Hans *et al*, 2003), and 52% and 54% (Shustik *et al*, 2011), respectively. An analysis of the aggregated numbers from these four studies shows a gender difference: women represented 218/397 (55%) of grade 3A and 50/117 (43%) of grade 3B patients ($P = 0{\cdot}020$). Furthermore, patients with grade 3B had less bone-marrow and stage III–IV disease, as previously shown in entities of older nomenclatures corresponding to grade 3B (Miller *et al*, 1997; Rodriguez *et al*, 1999). In our study, 44% of grade 1–3A and 17% of grade 3B patients showed bone-marrow involvement; the reported frequency in DLBCL is 17% (The Non-Hodgkin's Lymphoma Classification Project., 1997).

Recent data suggest that there are underlying genetic differences between grades 1–3A and 3B. The hallmark of follicular lymphoma, the *BCL2*-upregulating translocation t(14;18), has been detected in 73% of grade 3A but only in 13% of grade 3B cases, whereas chromosomal breaks at 3q27, rearranging *BCL6*, are more common in grade 3B (Ott *et al*, 2002). It has been suggested that $CD10^{+}$grade 3B cases with t(14;18) are more related to grades 1–3A, while those with 3q27 breaks are a separate entity (Bosga-Bouwer *et al*, 2006). However, 3q27 breaks have predominantly been found in grade 3B cases with concomitant DLBCL, whereas pure grade 3B cases typically lack either abnormality (Katzenberger *et al*, 2004). In pure grade 3B, positivity for IRF4 (MUM1) and *MYC* breaks are more prevalent than in grades 1–3A (Horn *et al*, 2011). Recently, also gene expression in grade 3B was shown to be distinct from that in both grades 1–3A and DLBCL (Piccaluga *et al*, 2008). Taken together, grade 3B has not only a divergent clinical course compared with grades 1–3A, but is also different with respect to epidemiology (more males), clinical characteristics (less bone-marrow involvement), and genetic features. Our clinical results contribute substantially to previous findings and suggest that follicular lymphoma grade 3B is both clinically and biologically separate from grade 1–3A. Fewer cases of grade 3B were positive for BCL2 and more were positive for TP53. These distinct features of grade 3B argue against its present WHO sub-classification under follicular lymphoma, suggesting that grade 3B is an entity of its own (*cf.* the Kiel classification (Feller *et al*, 2004)).

The limitations of our study should be noted. Grade 3B is a rare entity, and among the 462 patients without transformation there were 23 with grade 3B, a number similar to those in the two largest previous studies [25 and 22 (Hans *et al*, 2003; Shustik *et al*, 2011)]. These low numbers limit the possibility of firm statements about the relationship between grade 3A and 3B (but not about that between grade 1–2 and 3A). However, the present study contains more information on each patient, because the follow-up times are much longer than those of the previous reports. Another potential concern would be the heterogeneity in treatment. Still, this study provides externally valid data from two similar Scandinavian regions. This unselected, population-based cohort allows for an estimation of the distributions and prevalence rates of relevant variables, and we found not only differences in clinical outcome between grade 3A and 3B but also in gender distribution, protein expression and disease stage. Although a randomized trial would be valuable for identifying clinical differences between grade 3A and 3B, it would probably be impossible to perform. Recruiting sufficient numbers of grade 3B patients would require many years, and the follow-up would extend into decades. We believe that a meta-analysis of the published clinical grade 3 studies, especially with updated follow-up times, would be of great interest.

Follicular lymphoma grade 3A, like grade 1–2, is indolent and incurable with conventional therapy. In contrast to grade 1–3A, grade 3B did not relapse beyond 5 years of follow-up in our population-based cohort. This study supports the hypothesis that grade 3B might be an aggressive neoplasm, but if it is treated aggressively, some patients are probably cured.

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Competing Interests

The authors have no competing interests.

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