IGHV gene usage and mutational status in follicular lymphoma: Correlations with prognosis and patient age

Ellen Berget a, b, *, Anders Molven a, b, Turid Løkeland c, Lars Helgeland a, b, Olav Karsten Vintermyr a, b

a The Gade Laboratory for Pathology, Department of Clinical Medicine, University of Bergen, 5021 Bergen, Norway
b Department of Pathology, Haukeland University Hospital, 5021 Bergen, Norway
c Department of Oncology, Haukeland University Hospital, 5021 Bergen, Norway

Abstract

Follicular lymphoma (FL) is a heterogeneous disease with some patients developing progressively or transformed disease early, whereas others follow an indolent clinical course. We evaluated the prognostic value of immunoglobulin heavy chain variable (IGHV) gene usage and mutational status in FL patients. One hundred and four IGH sequences were obtained in tumour samples from 99 patients. The IGHV3 subgroup had the highest usage frequency (57.7%) with IGHV3-23 being the most common sequence. Patients with the IGHV5 subgroup or IGH sequences from more than one subgroup had significantly less favourable prognosis with an estimated 5-year survival of 62.5% and 50.0%, respectively, as compared with a 5-year survival of 95.1% for patients with other IGHV groups (P = 0.013 and P < 0.001, log-rank). The poor survival associated with IGHV5 or >1 IGHV subgroup usage was an independent prognostic factor in Cox multivariate analysis (P = 0.005). IGHV genes were unmutated showing >98% homology in 15.2% of cases. Contrasting the situation in chronic lymphocytic leukaemia (CLL), the presence of unmutated sequences did not yield prognostic information, although unmutated sequences were associated with age at diagnosis >60 years (P = 0.022, Fisher’s exact). In conclusion, our results indicate that analysis of IGHV gene usage might aid in predicting prognosis for FL patients.

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Follicular lymphoma (FL) is the second most common B cell lymphoma after diffuse large B cell lymphoma (DLBCL) in Western countries [1]. FL is clinically characterised by a median survival of more than 10 years and a continuous pattern of relapse that often is associated with progression to treatment-resistant disease and sometimes is associated with histological transformation into DLBCL [2,3]. The translocation t(14;18)(q32;q21) deregulating BCL2 expression is considered the primary oncogenic event, but is also considered insufficient for FL development due to the frequent occurrence of this translocation in B cells of healthy human individuals [4-6].

It has long been assumed that FL is derived from the malignant transformation of germinal centre B cells. Morphologically, FLs are composed of tumour cells that appear in a predominantly follicular pattern with cytological and architectural features of normal germinal centres, and that usually express germinal centre B cells’ markers such as BCL6 and CD10 [7]. The germinal centre B cell origin has been supported by several previous studies of the immunoglobulin heavy variable (IGHV) and light chain genes demonstrating heavily mutated genes [8-12]. In comparison to normal germinal centre B cells, FLs contain significantly more mutations in the IGHV genes, an observation compatible with prolonged expansion in a germinal centre-like environment [12,13].

The analysis of IGHV genes of other B cell lymphomas, including chronic lymphocytic leukaemia (CLL), splenic marginal zone lymphoma (SMZL) and mantle cell lymphoma (MCL) has revealed an unexpected heterogeneity in mutational status [14-18]. This heterogeneity has also been related to prognosis particularly in CLL, in which IGHV sequence analysis now has become widely used for the purpose of prognostication [19,20]. Furthermore, the increasing use of IGHV sequence analysis in CLL patients has
allowed the recognition of specific IGHV genes that are associated with unfavourable prognosis, such as the IGHV3–21 gene [21–24]. Although most studies have shown that FL is characterised by mutated IGHV genes [8–12], a recent investigation has revealed that unmutated genes could also be present in FL [25]. Moreover, there have been only occasional reports on the distribution of IGHV subtypes in FL [8,10,12,26], and to our knowledge there has been no study attempting to correlate IGHV sequence analysis with clinical outcome. In the present study, the aim was, therefore, to analyse the use of IGHV genes and mutational status in relation to clinicopathological characteristics and prognosis in FL.

2. Materials and methods

2.1. Patients

The 106 patients included in the study had FL grade 1–3B at time of diagnosis, and all had a bone marrow biopsy evaluated for lymphoma involvement at the Department of Pathology, Haukeland University Hospital, Bergen, Norway in the period between February 2003 and July 2011. Patients with transformed FL at diagnosis were excluded. Bone marrow status for 86 cases was reported previously [27]. Diagnoses according to the WHO classification were based on morphological and immunohistochemical assessment of formalin-fixed, paraffin-embedded (FFPE) tumour biopsies from lymph nodes and extranodal sites [7]. Medical records were reviewed to determine age, sex, clinical stage, follicular lymphoma international prognostic index (FLIPI) score, management and disease course. FLIPI score was also determined for the patients with 3B FL, although clinically regarded as equivalent to the diagnosis of DLBCL. The study was approved by the Regional Committee for Ethics in Research (2013/211) and performed in accordance with the Declaration of Helsinki.

2.2. PCR amplification and sequencing

DNA was prepared from FFPE tumour material. In most cases, two 10-μm sections were cut from the paraffin blocks and deparaffinised with Deparaffinisation Solution (Qagen, Hilden, Germany). In one case, tumour material was successfully dissected from a haematoxylin and eosin stained slide before deparaffinisation. Automated DNA isolation was performed on a QIAasympson SP pipetting robot (Qagen) in combination with the QIAasympson DSP DNA Mini Kit (Qagen) according to the manufacturer’s instructions. PCR was performed in six separate reactions with the IGH framework region 1 (FR1) primers combined with the JH consensus primer as designed for the BIOMED-2 protocol [28]. The BIOMED-2 procedure was adapted for FFPE tissue by using the QAGEN Multiplex PCR Kit (Qagen) with HotStarTag DNA Polymerase as previously described [29]. All reactions were carried out in a total reaction volume of 25 μl containing 2.5 μl of DNA template, 2.5 μl of primer mix (2 μM of each primer), 12.5 μl of the Multiplex buffer and 7.5 μl ddH2O. PCR conditions included an initial activation step (95°C, 15 min), 38 cycles consisting of denaturation (95°C, 45 s), annealing of PCR primers (60°C, 90 s) and extension (72°C, 90 s), and a final extension step of 72°C for 10 min. PCR products were analysed on a 3% agarose gel, visualised with ethidium bromide staining and PCR reactions that showed bands of appropriate size were identified. If several appropriate bands were present in a case, each of the positive PCR reactions was selected for sequencing. For each case, a multiplexed PCR reaction consisting of the six IGH FR1 primers and the JH consensus primer was also performed. The multiplexed PCR reaction served as an internal control for the presence of clonal rearrangements.

In preparation for sequencing, the Illustra™ ExoProStar™ 1-Step Kit (GE Healthcare, Little Chalfont Buckinghamshire, UK) was used in order to remove unincorporated primers and nucleotides from the amplification reactions. Direct sequencing was performed in both directions using the same primers as in the PCR amplification and the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). Sequencing reactions were purified by the BigDye® XTerminator™ Purification Kit and subsequently analysed on an ABI Prism 3100 Genetic Analyser (both Applied Biosystems).

Sequence data analysis was done using CLC Main Workbench software (Qiagen). DNA sequence reads were automatically trimmed based on quality scores (0.05 limit) and ambiguity characters (two residues). Manual trimming was performed in a minority of the cases (n = 4). When DNA sequence reads from both directions were obtained, they were assembled to contigs (73 sequences). Conflicts, i.e. disagreements about residues in a position, were solved by letting the read with best quality decide the nucleotide in the contig. Contigs and single DNA sequence reads were aligned to the closest IGH sequences derived from the IMGT database (http://www.imgt.org/IMGT/yquest/yquest) and the percentage of homology determined.

2.3. Statistical analysis

To compare categorical variables χ² or Fisher’s exact tests were performed. Continuous variables not following the normal distribution were compared between two or more groups using the Mann–Whitney U or Kruskal–Wallis tests. Survival curves of time to death due to lymphoma were estimated using the product-limit procedure (Kaplan–Meier method) with volume of histological diagnostic as starting point. Differences between categories were estimated by the log–rank test. Patients who died of other causes than lymphoma were treated as censored observations. Median follow-up time was estimated by the reversed Kaplan–Meier method. Univariate and multivariate analyses of survival were performed with the Cox proportional hazards method. The variables were tested by log–log plot and no non-proportionality was found. To determine their ability to be incorporated in multivariate models, the variables were tested by forward and backward stepwise selection. All results were considered significant if P ≤ 0.05. SPSS version 21.0 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses.

3. Results

3.1. Clinical and pathological characteristics

There were 106 patients included with ages ranging from 33 to 94 years (median 61 years). Sixty (56.6%) patients were initially treated with cyclophosphamide, doxorubicin, vincristine and prednisol (CHOP) or COP. In these patients, Rituximab was added in 55 and radiation therapy was applied as consolidation in 16. There were four patients with grade 3B FL, and all four were

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>%</th>
<th>HR</th>
<th>95% CI</th>
<th>P value (log–rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>64</td>
<td>60.4</td>
<td>1</td>
<td>0.326–3.662</td>
<td>0.886</td>
</tr>
<tr>
<td>Male</td>
<td>42</td>
<td>39.6</td>
<td>1.092</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>46</td>
<td>43.4</td>
<td>1</td>
<td>0.837–17.463</td>
<td>0.063</td>
</tr>
<tr>
<td>&gt;60</td>
<td>60</td>
<td>56.6</td>
<td>3.823</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grades 1/2</td>
<td>77</td>
<td>72.6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grades 3A/3B</td>
<td>29</td>
<td>27.4</td>
<td>0.678</td>
<td>0.317–1.451</td>
<td>0.305</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No involvement</td>
<td>73</td>
<td>68.9</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Involvement</td>
<td>33</td>
<td>31.1</td>
<td>1.014</td>
<td>0.304–3.385</td>
<td>0.982</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–II</td>
<td>34</td>
<td>32.1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III–IV</td>
<td>72</td>
<td>67.9</td>
<td>1.465</td>
<td>0.877–2.447</td>
<td>0.109</td>
</tr>
<tr>
<td>FLIPI score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (0–1)</td>
<td>49</td>
<td>46.2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate (2)</td>
<td>26</td>
<td>24.5</td>
<td>2.723</td>
<td>0.455–16.318</td>
<td>0.266</td>
</tr>
<tr>
<td>High (≥3)</td>
<td>31</td>
<td>29.3</td>
<td>5.492</td>
<td>1.139–26.476</td>
<td>0.016</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; FLIPI, follicular lymphoma international prognostic index.
obtained among the patients treated with CHOP or R-CHOP. Thirteen (12.3%) patients received chlorambucil. One patient (0.9%) received Rituximab monotherapy. Seventeen (16.0%) patients with stage I or II and grade 1 or 2 FL, were treated with local radiation therapy. Fifteen (14.2%) patients underwent observation only. Median follow-up period, 12 patients died as a consequence of their lymphoma disease and eight died of other causes. Clinicopathological characteristics with results from univariate analysis of survival are summarised in Table 1.

### 3.2. IGHV, IGHD and IGHJ usage

One hundred and four productive IGH rearranged sequences were obtained in samples from 99 patients. Double (n = 3) or triple (n = 1) productive IGH rearranged sequences were obtained in four of these patients (Table 2).

Among the 25 functional IGHV genes used, the most frequently encountered were IGHV3–23 (12.5%), IGHV3–48 (11.5%), IGHV3–15 (8.7%), IGHV4–59 (7.7%) and IGHV5–51 (6.7%, Fig. 1). The use of these specific genes was not correlated with prognosis. The most frequently encountered IGHV genes in the cases with more than one productive sequence were IGHV3–23 (n = 3) and IGHV5–51 (n = 2, Table 2). The frequencies of IGHV subgroups in the 104 productive sequences had the following distribution: IGHV1, 19.2%; IGHV3, 57.7%; IGHV4, 11.5%; IGHV5, 7.7% and IGHV6, 3.8%. The IGHV2 subgroup was not observed in any of the sequences. Twenty three different functional IGHD genes were used. The most frequently encountered were IGHD3–10 (8.7%), IGHD3–03 (7.7%) and IGHD3–22 (6.7%). The most frequently expressed IGHJ genes were IGHJ4 (51.0%), IGHJ6 (21.2%) and IGHJ3 (17.3%).

No productive IGHV-D-J rearrangements could be obtained in the remaining seven patients. In three of these cases, we were unable to establish clonality by the BIOMED-2 framework region one, two and three primers, probably due to the technical limitations related to use of FFPE tissue. Four cases had no material (FFPE tissue or DNA) left after routine diagnostic work was completed.

### 3.3. IGHV mutation status

The percentage of homology to the closest IGHV gene ranged from 60.4% to 100.0%, with a mean of 89.4% for the 104 productive sequences.

The means for the IGHV subgroups were: IGHV1, 95.4%; IGHV3, 88.1%; IGHV4, 86.3%; IGHV5, 91.3% and IGHV6, 87.0% (Fig. 2). A Kruskal–Wallis test revealed a statistically significant difference in IGHV sequence homology levels across the different IGHV subgroups (P < 0.001). The IGHV1 subgroup recorded the highest median score (95.3%), while the IGHV4 subgroup had the lowest (86.6%). Additional testing of the median values with Mann–Whitney U test with Bonferroni adjustment showed...
significant differences between \textit{IGHV1} and \textit{IGHV3}, and between \textit{IGHV1} and \textit{IGHV4} (both $P<0.001$).

Fifteen productive sequences were unmutated, defined as $>98\%$ homology to the closest \textit{IGHV} gene. Three of these sequences showed 100\% homology. Unmutated sequences were observed in the \textit{IGHV1}, \textit{IGHV3}, \textit{IGHV5} and \textit{IGHV6} subgroups. \textit{IGHV1} subgroup exhibited the highest number of unmutated sequences. When compared with the \textit{IGHV3} and the \textit{IGHV4} subgroup, the difference was statistically significant ($P=0.004$ and $P=0.029$, Fisher’s exact test).

### 3.4. Correlation of IGHV subgroup usage with clinical outcome

The estimated survival at 5 years was 62.5\% (SE 21.3\%) for patients displaying the \textit{IGHV5} subgroup and 50.0\% (SE 25.0\%) for patients exhibiting more than one \textit{IGHV} subgroup, whereas the estimated survival for patients using the other \textit{IGHV} subgroups combined was 95.1\% (SE 2.4\%). Patients using \textit{IGHV5} or more than one \textit{IGHV} subgroup had a significantly poorer survival by log–rank test ($P=0.013$ and $P<0.001$, respectively) as compared to patients using other \textit{IGHV} subgroups. When the patients using the \textit{IGHV5} and more than one \textit{IGHV} subgroup were combined in a single group estimated hazard ratio was 7.6 (95\% CI, 2.1–26.9, Table 3) with $P<0.001$ by log–rank test (Fig. 3). Supplementary t[14;18](q32;q21) fluorescence in situ hybridisation analysis (BCL2) and PCR (BCL2 MBR and MCR) was performed in 10 cases with \textit{IGHV5} or $>1$ \textit{IGHV} subgroup expression. BCL2 translocation was detected in eight supporting the diagnosis of follicular lymphoma (data not shown).

Usage of \textit{IGHV5} was associated with age $>60$ years at diagnosis, whereas usage of more than one \textit{IGHV} subgroup was not (Supplementary Table S1). Usage of \textit{IGHV5} or more than one \textit{IGHV} subgroup was not associated with FL grade 3A/3B, clinical stage $>$III/IV or FLIPI score $>3$. Furthermore, no correlation between usage of \textit{IGHD} or \textit{IGHJ} genes and clinicopathological variables including patient’s survival was detected.

### 3.5. Correlation of \textit{IGH} mutation status with clinicopathological variables

Unmutated \textit{IGHV} sequences with $>98\%$ homology to the closest \textit{IGHV} gene was observed more frequently in patients with age $>60$

### Table 3

Univariate survival analysis of \textit{IGHV} subgroup usage and mutation status according to different homology cutoff values in the 99 patients with productive rearrangements.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>HR</th>
<th>95% CI</th>
<th>$P$ value (log–rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{IGHV} subgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHV1–4, IGHV6</td>
<td>89</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHV homology $\leq93%$</td>
<td>10</td>
<td>7.555</td>
<td>2.119–26.931</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$&gt;93%$</td>
<td>69</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHV homology $\leq96%$</td>
<td>30</td>
<td>2.589</td>
<td>0.827–8.108</td>
<td>0.090</td>
</tr>
<tr>
<td>$&gt;96%$</td>
<td>81</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHV homology $\leq98%$</td>
<td>18</td>
<td>2.198</td>
<td>0.661–7.307</td>
<td>0.187</td>
</tr>
<tr>
<td>$&gt;98%$</td>
<td>84</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHV homology $\leq99%$</td>
<td>15</td>
<td>1.824</td>
<td>0.493–6.743</td>
<td>0.360</td>
</tr>
<tr>
<td>$&gt;99%$</td>
<td>89</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHV homology $\leq99%$</td>
<td>10</td>
<td>1.617</td>
<td>0.353–7.396</td>
<td>0.532</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval.
The sequences with the highest homology score were included in patients with double or triple productive \textit{IGH} rearranged sequences.
years (13 of 58 patients; 22.4%) than in patients aged ≤60 years (two of 41 patients; 4.9%), when sequences with the highest homology scores were included for the patients with double or triple productive rearranged IGH sequences. The difference was statistically significant by Fisher’s exact test (P = 0.022). Unmutated sequences were associated with >1 IGHV subgroup usage, but not with IGHV5 subgroup usage (Supplementary Table S1). There were no significant associations between unmutated IGHV genes and the other clinicopathological variables studied.

The estimated survival at 5 years was 80.0% (SE 10.3%) for patients carrying unmutated IGHV genes and 93.6% (SE 2.8%) for patients with mutated IGHV. The difference in survival was not significant by log-rank test. Moreover, no significant impact on survival was observed according to different homology cutoff values (Table 3). The estimated hazard ratio was 2.6 (95% CI, 0.8–8.1, P = 0.090) when a cutoff of >93% homology was used.

3.6. Multivariate survival analysis

The combination of IGHV5 and >1 IGHV subgroup usage remained a significant factor in a multivariate analysis when the variables age >60 years, clinical stage >III/IV, FLIPI score ≥3 and IGHV homology >93% were included (Table 4).

4. Discussion

Our analysis showed that the usage of IGHV genes used in follicular lymphomas mostly resembled the repertoire of normal peripheral blood B cells [30,31]. Thus, the IGHV3–23 gene was the most frequently used IGHV gene. However, some discrepancies

![Graph showing survival analysis](image_url)

**Fig. 3.** Survival of follicular lymphoma patients according to IGHV subgroup usage. Kaplan–Meier survival curves of 99 patients are shown. The survival of patients using the IGHV5 subgroup (n = 6) or more than one IGHV subgroup (n = 4) were combined into a single group, and compared to the survival of patients using the IGHV1–4 or IGHV6 subgroups. Statistical difference was calculated by the log-rank test.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;60 years</td>
<td>2.830</td>
<td>0.614–13.033</td>
<td>0.182</td>
</tr>
<tr>
<td>Clinical stages III/IV</td>
<td>4.143</td>
<td>0.447–38.398</td>
<td>0.211</td>
</tr>
<tr>
<td>FLIPI score ≥3</td>
<td>1.942</td>
<td>0.535–7.042</td>
<td>0.313</td>
</tr>
<tr>
<td>IGHV5 or &gt;1 IGHV subgroup</td>
<td>5.621</td>
<td>1.484–21.290</td>
<td>0.005</td>
</tr>
<tr>
<td>IGHV homology &gt;93%</td>
<td>2.185</td>
<td>0.630–7.571</td>
<td>0.218</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; FLIPI, follicular lymphoma international prognostic index.

were observed. The IGHV3–15, IGHV3–48 and the IGHV5–51 genes, which are not among the most frequently employed IGHV genes in normal B cells, were frequently expressed in our patient cohort. Conversely, the IGHV3–30–3 and the IGHV4–39 genes that are common in normal B cells were not represented in our patients.

The IGHV3–23 was the most frequently used gene also in a previous study of 30 FL samples [12]. In that study, however, IGHV3–15, IGHV3–48 and IGHV5–51 were not commonly expressed, contrasting our results. In another study of 26 FLs, the IGHV1–18 and the IGHV3–48 were the most frequently encountered genes [32]. Although relative frequencies of individual IGHV genes diverge among series, the frequencies of IGHV subgroups tend to be more similar for FL [8,12,26,32,33]. In agreement with previous studies, IGHV3 was the most frequently used subgroup, followed by the IGHV1 and IGHV4 subgroups.

A novel finding in our study was that usage of IGHV subgroups was related to survival. Unfavourable prognosis was observed for two patient groups; the patients using the IGHV5 subgroup and
the patients using more than one IGHV subgroup. Whether these patient groups were compared separately or combined, survival was significantly different from that of patients using other IGHV subgroups. The poor survival associated with subgroup usage was also of independent prognostic importance in a multivariate model. Similarly, the IGHV–23 gene was correlated with poor clinical outcome in studies of CLL [21,22]. The IGHV–23 gene was expressed in only two of our FL patients, and an association with prognosis could; therefore, not be evaluated. Other IGHV genes or subgroups have, to our knowledge, not been associated with adverse prognosis, neither in CLL nor in other B cell neoplasms.

The IGHV genes were commonly mutated in our study, as also observed in previous studies of FL [10,12,32,34]. Unexpectedly, however, unmutated IGHV genes were detected in 15.2% (15/99) of the patients from whom productive rearrangements were obtained. Three of these patients even had truly unmutated IGHV genes showing 100% homology. Unmutated IGHV genes have previously been observed in a minority of other germinal or post-germinal centre-associated lymphomas, such as DLBCL and Burkitt lymphoma [35–39], but to our knowledge not in FL. Due to the unexpected high frequency of unmutated IGHV genes in our patients, the morphology of these cases were re-evaluated. Morphological and immunophenotypic characteristics of FL were confirmed in all cases. Nine showed FL grades one and two, five grades 3A and one grade 3B.

We were unable to show any significant difference in survival for patients carrying unmutated IGHV genes as compared with patients carrying mutated sequences, despite the fact that unmutated IGHV genes were seen more frequently in patients with age >60 years. The reason for the association with age is unclear. The 98% homology cutoff value for assigning cases to the unmutated or mutated subgroup has been widely used for prognostication in CLL. Recently, the best cutoff identity of IGHV genes to predict survival was shown to be 97% in MCL [40]. Cutoffs of 98 or 97% may not necessarily be appropriate for FL. We were, however, not able to detect any significant differences in survival according to different homology cutoff values in our FL patients (Table 3).

Although no differences in outcome were detected, the observation of unmutated and truly unmutated IGHV genes indicates that a subset of FL cases could be derived from pre-germinal centre B cells. A more naive cell of origin has previously also been suggested for the unmutated cases of CLL and MCL. [15,40] On the other hand, mutual status appeared to be related to the use of different IGHV subgroups with the IGHV1 subgroup showing highest number of unmutated sequences. Such biased usage could also reflect selection and activation by a restricted set of antigens, superantigens, or both [41]. Accordingly, the definition of a cell of origin for a subset of FL cases may be facing the same challenges already encountered for CLL [42].

We also observed a somewhat higher frequency of IGHV5 subgroup usage in our FLs (7.7%) than that reported for non-B peripheral blood B cells, i.e. 1.4% for CD5+ and 2.9% for CD5– B cells [30]. These differences suggest an overrepresentation of IGHV5 genes in FL. Given the poorer survival of IGHV5–positive patients, it is tempting to speculate that specific antigens may be responsible for a more aggressive clinical course in this subset of FL patients.

In summary, the IGHV3 subgroup and in particular the IGHV3–23 were the most frequently used in FL. Usage of the IGHV5 and more than one IGHV subgroup were associated with shorter survival, and was an independent risk factor in multivariate analysis. Although validation in larger series is warranted, the survival data indicate that IGHV sequence analysis could aid in predicting prognosis of FL patients. Finally, heterogeneity in mutational status was noted as also unmutated IGHV genes were present. This could raise the possibility that a subset of FLs might be derived from pre-germinal centre B cells.

Conflicts of interest statement

There are no conflicts of interest to declare.

Acknowledgements

The study has been funded by a fellowship from the University of Bergen, and grants provided by the Western Norway Regional Health Authority and Dr. Med. F. Gades Legat.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2015.03.003.

References


