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Biological and clinical characteristics of patients with chronic lymphocytic leukemia with the IGHV3-21 and IGHV1-69: analysis of data from a single center

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This study aimed at mapping the frequency of IGHV3-21 and IGHV1-69 in a group of 417 patients newly diagnosed with chronic lymphocytic leukemia (CLL) and described basic characteristics, cytogenetic abnormalities and prognosis of these patient subgroups. IGHV3-21 was found in 29 patients (7%) and IGHV1-69 in 51 patients (12.4%). The median overall survival (OS) rates were 97 months and 85 months in the IGHV3-21 and IGHV1-69 groups, respectively. In this small group of patients, the study failed to show a difference in OS of IGHV3-21 patients with mutated and unmutated IGHV status ($p<0.597$). There was also no difference in OS between IGHV3-21 patients with mutated IGHV status and all patients in the group having unmutated IGHV status ($p<0.245$). On the other hand, patients with IGHV3-21 and the presence of some other adverse prognostic factors (age ≥ 65 years, lymphocyte count ≥ 50×109/L, serum thymidine kinase ≥ 9U/L, deletion of 17p) had statistically significantly worse OS than IGHV3-21 patients without the presence of these prognostic factors. The multivariate analysis of an entire group of Binet clinical stage A patients proved that the presence of IGHV3-21 is as an independent adverse prognostic factor even though there was no statistical difference in OS between patients with IGHV3-21 and those without IGHV3-21 in the entire group ($p<0.769$). Patients with IGHV1-69 had the same probablility of OS irrespective of the presence of other adverse prognostic factors; their OS was significantly shorter as compared with the other patients from the entire group ($p<0.03$).

The study mapped the occurrence of recurrent cytogenetic changes detected by FISH in IGHV3-21 (subset #2 and non-subset #2) and IGHV1-69 and compared it with the occurrence of recurrent changes in the entire group of patients. In IGHV1-69 and in subset #2 IGHV3-21, higher proportions of deletion of 11q were found (30% and 31%, respectively), with the deletion being present in 19.2% of the entire group of patients. None of the 3 patients with IGHV3-21 and deletion of 17p had subset #2. Patients with subset #2 IGHV3-21 had higher proportions of deletion of 13 (69%) as compared with non-subset #2 IGHV3-21 patients (27%).

Key words: chronic lymphocytic leukemia, IGHV mutation status, IGHV3-21, IGHV1-69, stereotyped B-cell receptor, subset #2

Chronic lymphocytic leukemia (CLL) is a biologically heterogeneous disease with variable clinical course. It is indolent in some patients but aggressive in others. The likely course and clinical activity of the disease and probability of survival in a particular patient may be predicted using numerous prognostic factors.

Two biologically and prognostically different CLL subgroups are defined by the presence or absence of somatic hypermutations (SHMs) in the immunoglobulin heavy chain variable region (*IGHV*). The first subtype includes diseases with mutated (M) *IGHV* status (originating from post-germinal B-cells) and the other subtype is represented by diseases with unmutated (UM) *IGHV* status (originating from pregerminal B-cells). SHMs in *IGHV* genes are detected in as many as 50% to 70% of patients diagnosed with CLL [1]. UM *IGHV* status is characterized by at least 98% sequence

homology to the corresponding germline gene. It is associated with the patients' unfavorable prognosis and shorter overall survival (OS) when compared with individuals having SHMs of the gene [2, 3].

The extent of *IGHV* rearrangements is limited in CLL tumor cells as compared with normal B-cells. Numerous studies reported preferential presence of certain variable region (VH) gene segments in patients with this disease. This may support the theory of the role of specific antigen stimulation which may lead to a leukemogenesis [4, 5]. In this respect, some studies do not rule out the role of autoantigens in the pathogenesis of CLL [6]. The most common VH genes present in CLL patients are members of the VH3, VH4 and VH1 families [7, 8]. Somatic mutations are not uniform within various VH families. More mutations have been observed in VH3 family V subgenes as compared with V subgenes from the VH1 family. VH1 family subgenes tend to be mostly unmutated [9]. Selected V gene segments have prognostic value in CLL. These include *IGHV3-21* and *IGHV1-69* [5, 10, 11]. The presence of *IGHV3-21* in CLL is associated with poor prognosis regardless of its mutation status. Patients with this subgene tend to have shorter OS (as do patients with UM *IGHV* genes) despite the fact that two thirds of patients from this group have M *IGHV* [5, 10]. Approximately one-half of *IGHV3-21* patients have stereotyped B-cell receptor (BCR), known as subset #2, with a short and highly similar CDR3 (9 amino acids long) and usage of one particular IG lambda gene, *IGLV3-21* [5]. Survival of patients (with both stereotyped and non-stereotyped receptors) is short regardless of *IGHV* mutation status [5, 12, 13]. Patients with stereotyped BCR have a shorter progression-free survival [14, 15]. Another study compared the occurence of recurrent aberrations detected by fluorescence *in situ* hybridization (FISH). In patients with subset #2, deletions of 13q (13q-) were frequently detected (79%), as compared with non-subset #2 patients (31%). Additionally, deletion of 11q (11q-) was also more frequently (31%) detected in patients with subset #2. According to the study, deletion of 17p (17p-) was only detected in a single non-subset #2 patient [16, 17].

In the vast majority of CLL patients, presence of the *IGHV1-69* is associated with UM *IGHV* status. Due to that fact, the presence of *IGHV1-69* is related to unfavorable prognosis. Patients with this subgene appear to be a homogeneous group, with more frequent advanced clinical stage of the disease requiring early therapeutic intervention. However, their survival is not worse than that of other patients with UM *IGHV* [11]. It was found that *IGHV1-69* is expressed by 30% of unmutated CLL and rearranges to a restricted number of *IGHD* and *IGHJ* genes, often generating long and rather similar HCDR3 regions [18]. This suggests that environmental antigens play a role in the development and/or progression of CLL [19].

This retrospective study aimed at describing the basic biological and clinical characteristics, prognosis and response to treatment in a group of patients with newly diagnosed CLL and *IGHV3-21* and *IGHV1-69*. The goals were to (1) describe the proportion of VH families in the entire group of 417 patients, and map the presence of *IGHV3-21* and *IGHV1-69*; (2) characterize groups of patients with *IGHV3-21* and *IGHV1-69* with respect to *IGHV* mutation status, describe the proportion of cytogenetic changes – 11q- and 17p-, and describe the clinical picture and course of the disease – i.e. the clinical stage, clinical activity, potential need to initiate therapy and treatment response achieved; (3) compare the OS of patients with and without the presence of *IGHV3-21* as well as those with and without the presence of *IGHV1-69* and to compare the OS of *IGHV3-21* and *IGHV1-69* patients; and (4) determine the OS in *IGHV3-21* and *IGHV1-69* depending on the presence of the other predictive factors (age, gender, lymphocyte count, hemoglobin level, disease stage, beta-2-microglobulin [B2M], serum thymidine kinase [sTK], *IGHV* mutation status, presence of 11q- and 17p-) and, in *IGHV3-21* individuals, also depending on the presence of stereotyped BCR.

Materials and methods

The study group comprised 417 patients (270 males and 147 females) with newly diagnosed CLL in accordance with the National Cancer Institute (NCI) criteria who were followed or treated in our center in 2000–2011 and in whom *IGHV* mutation status was determined. The median age of the group was 61 years (range, 27–87 years).

Genetic analysis. The material for cytogenetic and molecular cytogenetic tests was peripheral blood cells. In all patients, classical cytogenetic analysis was carried out. Chromosomal aberrations 11q-, 17p-, 13q- and trisomy 12 were detected by FISH using gene and centromeric probes. To verify and precisely characterize chromosomal changes in the karyotype, whole chromosome probes were used. The comparative genomic hybridization (CGH) method was performed in selected patients with complex karyotypes or in those with unbalanced chromosomal changes that could not be detected by classical cytogenetic methods and FISH. Complex karyotypes were defined as findings of three and more clonal aberrations in a karyotype. CGH was carried out according to the manufacturer's instructions as described earlier [20].

Immunoglobulin gene rearrangement analysis. Sequence analysis of the *IGHV* genes was performed as described previously [21, 22]. *IGHV* sequences were aligned to ImMunoGeneTics directory and considered mutated if their identity to corresponding germline genes was 98%. Further details are reported in supplemental Methods.

Serum markers. The normal range of B2M was 0.8–2.34 mg/L and the normal range of sTk was 0–9U/L.

Statistical analysis. The primary end points were OS. OS was estimated using Kaplan-Meier plots and compared between groups by log-rank test. Univariate and multivariate Cox models were used to verify independent prognostic power of each parameter.

The study was performed in accordance with the 2008 revision of the Declaration of Helsinki. All patients gave informed consent to the examination and anonymous processing of data on their disease.

Results

Of the 417 patients, the largest proportions were in the VH3 (49.6%) and VH1 (25.9%) gene subfamilies (Table 1). The *IGHV3-21* subgene was present in 29 patients, that is, 7% of the entire group. The *IGHV1-69* subgene was present in 51 patients, i.e. 12.2% of the entire group. The median age of patients with the *IGHV3-21* subgene was 62.8 years (range, 35.6–78.7 years); the subgroup comprised 16 males (55%) and 13 females (45%). Binet A stage was diagnosed in 7 patients (27%), Binet B in 10 patients (38%) and Binet C in 9 patients (35%). The median lymphocyte count was 35.8×10^9/L (range, $5.2–346.0\times10^9$/L). In this group, 45% of patients had an UM *IGHV* sequence and 55% had a M *IGHV* sequence. A total of 13 patients had stereotyped *IGHV3-21* (subset #2), of whom 8 (62%) had M *IGHV* status. The mean number of *IGHV* mutations in patients with M *IGHV* status was 3.58%. The median B2M level was 3.61mg/L (range, 1.77–9.29 mg/L). Six patients were found to have a deletion of 11q (21.4% of the examined patients) and 3 patients were detected with a deletion of 17p (10.7% of the examined patients). The median follow-up of the group was 58 months (range, 10–136 months).

For active disease as defined by 1996 NCI recommendations, therapy was initiated in 26 of patients (90%) [23]. By the date of analysis, 14 patients (48%) with *IGHV3-21* had died. The median time of treatment initiation in the *IGHV3-21* group was 8.9 months (range, 4.1–63.0 months) and the

Table 1. VH gene family usage in all chronic lymphocytic leukemia patients (N=417)

VH gene family	Patients (N)	Patients (%)
VH1	108	25.9
VH2	12	2.8
VH3	207	49.6
VH4	71	17
VH5	15	3.6
VH6	1	0.2

VH – variable region

Table 2. Characteristics of patients with the *IGHV3-21* and *IGHV1-69* subgenes

	IGHV3-21 (N=29)				*IGHV1-69* (N=51)			
Characteristic	Me	No	Range	%	Me	No	range	%
Age	62.8		35.6-78.7		59.5		36.1-83.0	
males/females		16/13		57/43		39/12		77/23
lymphocytes (10^9/L)	35.8		5.2-346.0		35.5		6.4-1115.0	
Binet A/B/C stage		7/10/9		27/38/35		15/22/9		33/48/19
IGHV UM/M		13/16		45/55		50/1		98/2
B2M (mg/L)	3.61		1.77-9.29		3.19		1.31-7.97	
<2.34		5		22		8		19
≥2.34		18		78		34		81
sTk (U/L)	18.7		5.2-101.0		18.4		3.4-101.0	
<9		5		23		11		26
≥9		17		77		31		74
11q-		6		21.4		12		30
17p-		3		10.7		3		7.1
Stereotyped BCR subset #2		13		14.2				
Treated		26		90		42		82
TFS (months)	8.9		4.1-63.0		9.0		2.2-29.8	
primary therapy								
CLB/(R)FC/(R)C(H)OP		8/11/7		31/42/27		4/32/6		10/76/14
CR/PR/SD/PD		7/9/6/2		30/37/25/8		8/14/8/7		22/38/22/19
OS (months)	97		55-not reached		85		61-128	
Died		14		48		28		55
Follow-up (months)	58		10-136		50.5		7-140	

Me – median, B2M – beta-2-microglobulin, sTk – serum thymidine kinase, CR – complete remission, PR – partial remission, SD – stable disease, PD – progressive disease, (R)FC – fludarabine and cyclophosphamide with/without rituximab, (R)C(H)OP – cyclophosphamide, with/without adriablastin, vincristine, prednisone with/without rituximab, CLB – chlorambucil, *IGHV* UM/M – immunoglobulin heavy chain variable region unmutated(≥98% identity to germline)/mutated(<98% identity to germline), 11q- – 11q deletion, 17p- – 17p deletion, BCR – B-cell receptor, OS – overall survival, TFS – treatment-free survival, subset #2 – classified according to defined criteria [5]

median OS in this group reached 97 months (range, 55 months – not reached).

The median age of patients with the *IGHV1-69* subgene was 59.5 years (range, 36.1–83.0 years). In this subgroup, 50 cases (98%) had UM *IGHV* status and only 1 patient (2%) had M *IGHV* status. There were 39 males (77%) and 12 females (23%). Binet A stage was diagnosed in 15 patients (33%), Binet B in 22 patients (48%) and Binet C in 9 patients (19%). The median lymphocyte count was 35.5×10^9/L (range, 6.4–1115.0 $\times10^9$/L). The median B2M level was 3.19 mg/L (range, 1.31–7.97 mg/L). Twelve patients were found to have a deletion of 11q (30% of the examined patients) and 3 patients had a deletion of 17p (7.1% of the examined patients). The median follow-up of the group was 50.5 months (range, 7–140 months) For active disease therapy was initiated in 42 patients (82%) By the date of analysis, 28 patients (55%) with the *IGHV1-69* subgene had died. The median time to treatment initiation in the *IGHV1-69* group was 9.0 months (95% CI, 2.2–29.8 months) and the median OS in this group reached 85 months (95% CI, 61–128 months) (Table 2). Treatment outcomes with the overall response rate (ORR) to the administered chemotherapy in the primary therapy are shown in the Table (Table 2).

Univariate analysis (log-rank test) showed neither a difference in OS between *IGHV3-21* patients and those without *IGHV3-21* (p<0.769) (Figure 1A) nor a difference in OS between patients with M *IGHV* and those with UM *IGHV* status (p<0.597) (Figure 1B). Moreover, there was no difference in OS between *IGHV3-21* patients with M *IGHV* status and the entire group of patients with UM *IGHV* status (p<0.245) (Figure 1C). By contrast, *IGHV1-69* patients had a shorter OS than those without *IGHV1-69* (p<0.03) (Figure 1D); however, no difference in OS was shown between *IGHV3-21* patients and *IGHV1-69* patients (p<0.302) (Figure 1E).

In patients with *IGHV3-21*, the log-rank test showed a shorter OS in case of the presence of another risk factor (age ≥65 years, p<0.001; lymphocyte count $\geq50\times10^9$/L, p< 0.05; presence of deletion of 17p, p<0.05) (Table 3). The prognostic value of B2M could not be established due to a small number of observations. Patients with *IGHV1-69* had the same probability of OS irrespective of the presence of other adverse prognostic factors (age ≥65 years, p<0.850; lymphocyte count $\geq50\times10^9$/L, p< 0.119; B2M ≥2.34 mg/L, p<0.975; sTk ≥9U/L, p<0.963) (Table 3).

The log-rank test showed a shorter survival of *IGHV3-21* and *IGHV1-69* patients with a deletion of 17p than those without the deletion (p<0.05 and p<0.001, respectively) but no difference in survival of patients in both subgroups with and without deletion of 11q (p<0.986 and p<0.917, respectively) (Supplementary Figure S1 A,B,C,D).

Results of univariate analysis of selected prognostic factors in the entire group of 417 patients with respect to OS are summarized in the Table. A shorter OS was demonstrated in patients aged ≥65 years, with Binet B or C stages, lymphocyte count $\geq30\times10^9$/L, B2M ≥2.34 mg/L, sTk≥9U/L, unmutated *IGHV* status, deletion of 17p (p<0.001), deleletion of 11q (p<0.05) and in patients with *IGHV1-69* (Table 4).

Multivariate analysis of the entire group of patients revealed a shorter OS in patients aged ≥65 years (p<0.001), with lymphocyte count $\geq50\times10^9$/L (p<0.05), UM *IGHV* status (p<0.05) and deletion of 17p (p<0.05), concordance index 0.669 (Table 4). Multivariate analysis of the group of patients with Binet A stage showed a shorter OS in patients aged ≥65 years (p<0.001), with UM *IGHV* status (p<0.04) and presence of the *IGHV3-21* subgene (p<0.019), concordance index 0.736.

Additionally, the study mapped the occurrence of recurrent cytogenetic changes detected by FISH in *IGHV3-21* (subset #2 and non-subset #2) and *IGHV1-69* and compared it with the

Table 3. Univariate analysis of the other prognostic factors in relation to OS in patients with the *IGHV3-21* and *IGHV1-69* subgenes

	Univariate analysis					
	IGHV3-21			*IGHV1-69*		
	HR	95% Cl	p	HR	95% Cl	p
age ≥65 years	9.36	2.0-43.8	<0.001	1.8	0.48-2.41	<0.850
lymphocytes $\geq50\times10^9$/L	4.6	0.96-17.24	<0.05	1.93	0.83-4.48	<0.119
Binet B, C stage	2.95	0.64-13.65	<0.149	1.27	0.52-3.14	<0.600
B2M ≥2.34 (mg/L)				0.98	0.33-2.93	<0.975
sTk ≥9 (U/L)	5.74	0.74-44.6	<0.06	1.2	0.38-2.79	<0.963
IGHV M	0.75	0.26-2.16	<0.597			
17p-	4.7	0.90-24.46	<0.05	8.7	2.61-29.00	<0.001
11q-	1.1	0.21-4.79	<0.986	0.95	0.38-2.37	<0.917
Stereotyped BCR, subset #2	1.57	0.48-5.15	<0.456			

OS – overall survival, B2M – beta-2-microglobulin, sTk – serum thymidine kinase, *IGHV* M – immunoglobulin heavy chain variable region mutated(<98% identity to germline), 11q- – 11q deletion, 17p- – 17p deletion, BCR – B-cell receptor, subset #2 – classified according to defined criteria [5] HR – hazard ratio, 95% Cl – 95% confidence interval

occurrence of recurrent changes in the entire group of patients. In *IGHV1-69* and in subset #2 *IGHV3-21*, higher proportions of deletion 11q were found (30% and 31%, respectively), with the deletion being present in 19.2% of the entire group of patients. None of the 3 patients with deletion of 17p had subset #2 in *IGHV3-21*. In patients with *IGHV1-69*, deletion of 13q was less frequent (37%) than in the entire group of patients (46%). Patients with subset #2 *IGHV3-21* had a higher proportions of deletion of 13 (69%) as compared with non-subset #2 *IGHV3-21* patients (27%) (Table 5).

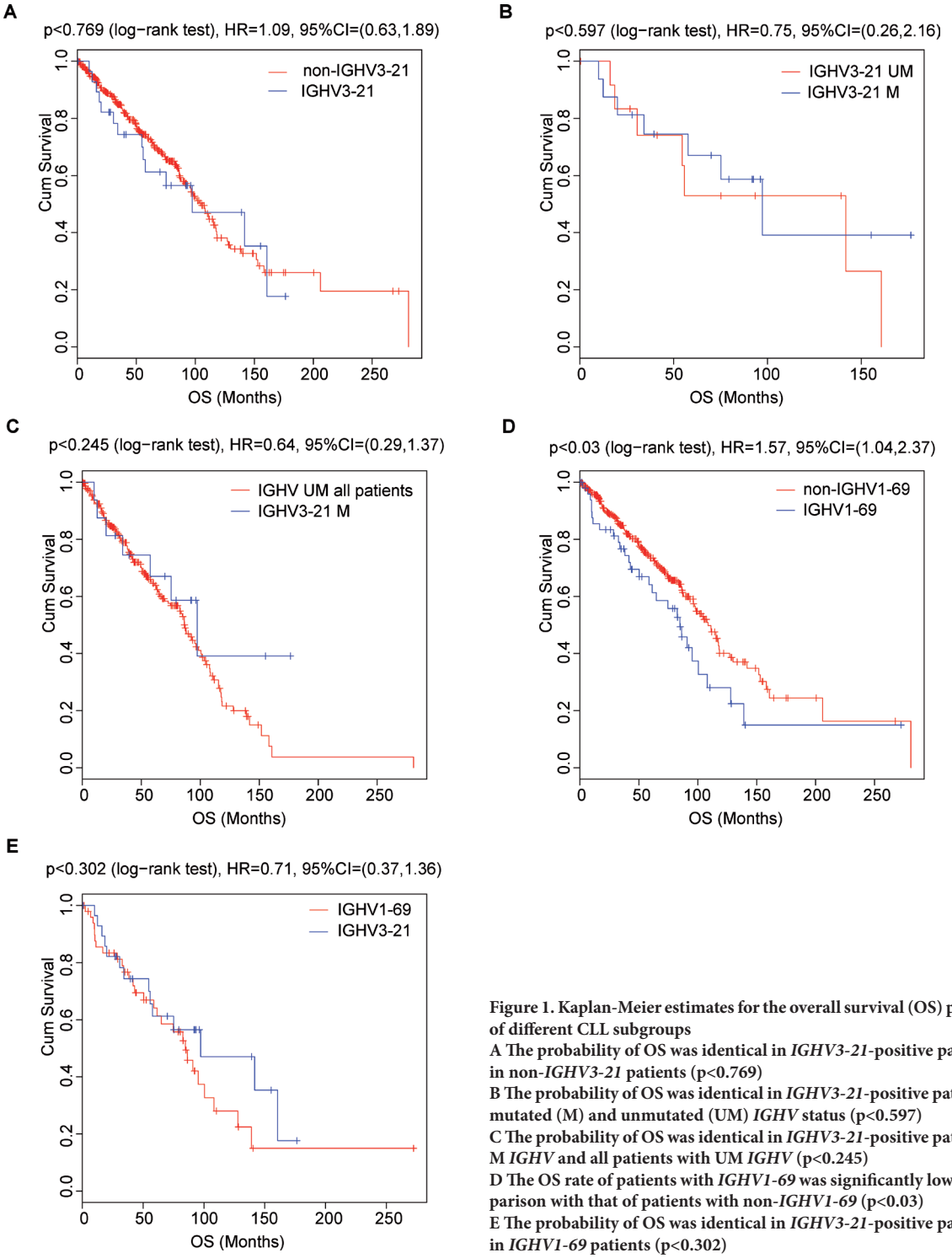

Figure 1. Kaplan-Meier estimates for the overall survival (OS) probability of different CLL subgroups
A The probability of OS was identical in *IGHV3-21*-positive patients and in non-*IGHV3-21* patients (p<0.769)
B The probability of OS was identical in *IGHV3-21*-positive patients with mutated (M) and unmutated (UM) *IGHV* status (p<0.597)
C The probability of OS was identical in *IGHV3-21*-positive patients with M *IGHV* and all patients with UM *IGHV* (p<0.245)
D The OS rate of patients with *IGHV1-69* was significantly lower in comparison with that of patients with non-*IGHV1-69* (p<0.03)
E The probability of OS was identical in *IGHV3-21*-positive patients and in *IGHV1-69* patients (p<0.302)

Excluding recurrent aberration in this group, a total of 4 cases displayed complex cytogenetic aberrations. The complex aberrations were not only heterogeneous but unique in each patient. The losses were detected on chromosomes 3p, 6p, 7p, 9q, 14q, 18p and 18q, and gains involved chromosomes 2p, 3q and 18q. Two patients had unbalanced translocations, one of them a jumping translocation with donor chromosome 2p.

In the *IGHV1-69* subgene, a total of 51 patients were included and also 4 patients displayed complex karyotypes. As in subgene *IGHV3-21*, the complex rearrangements were high heterogeneous. Losses in chromosomes 6q, 9p and gains on 2p and 12q were detected. An unbalanced translocation involved chromosomes 13 and 18. Composite karyotypes with 55–94 chromosomes were detected in one patient. The frequency of gains and losses was evaluated, showing that losses were more common than gains in both subset.

Discussion

Numerous studies suggest that in CLL, individual subgenes are not evenly distributed. Certain subgenes are significantly more commonly present, namely the VH1, VH3 and VH4 genes [7, 24]. Also in the present group of 417 patients, the most frequent genes were VH3 (49.6%) and VH1 (25.9%).

In addition to preferential usage of specific *IGHV* genes, the BCRs of approximately 30% of CLLs cases are clustered into stereotyped subsets, each of which is characterized by a high degree of homology of the complementarity determining regions (CDRs). Since it is extremely unlikely that two individuals would share identical BCRs, this indicates that CLL development and its natural history is not a random and stochastic event [25]. Importantly, normal B cells carrying stereotyped BCRs similar to those of CLL (and from which CLL may develop) have been identified recently [19].

The presence of certain subgenes is associated with a patient's adverse prognosis, as previously described in the analyzed *IGHV3-21* and *IGHV1-69* subgenes [10-12, 26]. In the aforementioned group, the *IGHV1-69* and *IGHV3-21* subgenes were present in 51 (12.2%) and 29 (7%) patients, respectively. The proportion of *IGHV3-21* is consistent with geographical distribution of the subgene, being lower than the reported prevalence in Scandinavia (10%) and slightly higher than that in Southern European countries (5%) [10].

Table 4. Univariate analysis and multivariate analysis of prognostic factors in relation to OS in all patients

N=417	Univariate analysis			Multivariate analysis		
	HR	95% Cl	p	HR	95% CI	P
age ≥65 years	2.31	1.65-3.24	<0.001	2.2	1.48-3.28	<0.001
sex (male)	1.19	0.85-1.67	<0.314			
Lymphocyte count ≥50 (10^9/L)	2.04	1.38-3.02	<0.001	1.82	1.18-2.80	<0.05
Lymphocyte count ≥30 (10^9/L)	1.9	1.33-2.73	<0.001			
Binet B, C stage	2.05	1.45-2.90	<0.001			
B2M ≥2.34 (mg/L)	2.99	1.81-4.94	<0.001			
sTk ≥9 (U/L)	2.6	1.72-3.94	<0.001			
IGHV M	0.35	0.24-0.51	<0.001	0.49	0.31-0.76	<0.05
17p-	2.39	1.43-3.98	<0.001	2.13	1.17-3.87	<0.05
11q-	1.56	1.04-2.33	<0.05			
IGHV1-69	1.57	1.04-2.37	<0.05			
IGHV3-21	1.09	0.63-1.89	<0.769			

OS – overall survival, B2M – beta-2-microglobulin, sTk – serum thymidine kinase, *IGHV* M – immunoglobulin heavy chain variable region mutated(<98% identity to germline), 11q- – 11q deletion, 17p- – 17p deletion, HR – hazard ratio, 95% Cl – 95% confidence interval

Table 5. Recurrent aberrations in *IGHV3-21* (subset #2, non-subset #2), *IGHV1-69* and all patients

Subgroup			*IGHV3-21* (N=29)	*IGHV1-69* (N=51)	All CLL (N=417)
Subsets	Subset #2 (N=13)	Non-subset #2 (N=16)			
13q-	9 (69%)	4 (27%)	13 (46%)	15 (37%)	46.2%
11q-	4 (31%)	2 (13%)	6 (21%)	12 (30%)	19.2%
Trisomy 12	0 (0%)	1 (7%)	1 (8%)	6 (14%)	12.0%
17p-	0 (0%)	3 (20%)	3 (10%)	3 (7%)	7.7%
Complex cytogenetic abnormalities			4 (14%)	4 (10%)	

13q- – 13q deletion, 11q- – 11q deletion, 17p- – 17p deletion, subset #2 and non-subset #2 – classified according to defined criteria [5], complex cytogenetic abnormalities – three and more clonal aberrations

The presence of *IGHV1-69* in our group corresponds with the reported occurrence of 10–20% [27].

On the one side the above studies also suggest that mutations are not uniform within the V families. Two-thirds of *IGHV3-21* patients have M *IGHV* status, with a relatively low percentage of mutations; on the other side, patients with *IGHV1-69* mostly have UM *IGHV* status [10, 11, 26]. In the present group, M *IGHV* status was found in 55% of *IGHV3-21* patients, with a mean number of mutations reaching 3.58%. As many as 98% of *IGHV1-69* patients had UM *IGHV* status. Interestingly, the proportion of males was higher in *IGHV1-69* than in *IGHV3-21* (76.5% vs 58.6%), but the difference was not shown to be significant (p= 0.08).

In the present study, the median OS rates in *IGHV3-21* and *IGHV1-69* patients were 97 and 85 months, respectively. The rates were not statistically different from the median OS of patients with UM *IGHV* status which was 86 months. The median survival of patients with M *IGHV* status was significantly different, reaching 206 months. The differences in median OS rates between patients with M and those with UM *IGHV* status are consistent with data published in previous studies [2].

The present detailed observations regarding the predictive value of the other adverse prognostic factors in patients with *IGHV3-21* (age ≥65 years, lymphocyte count $\geq 50\times10^9$/L and deletion of 17p) evidenced the group heterogeneity, as reported by some authors admitting varied prognosis within this group of patients [12, 14, 16]. In *IGHV1-69* patients, however, the OS was not shown to be influenced by the presence of other adverse prognostic factors, the only exception being deletion of 17p. This demonstrated a relatively high homogeneity of the group, undoubtedly due to almost exclusive proportion of patients with UM *IGHV*. At the same time, this is consistent with the published data clearly showing that deletion of 17p significantly worsens the prognosis of patients with UM *IGHV* status [28, 29]. Unlike these published data, the present *IGHV3-21* and *IGHV1-69* groups were not found to differ in the OS of patients with deletion of 11q.

Multivariate analysis of the entire present group of patients did not show an independent prognostic impact of the presence of the *IGHV3-21* and *IGHV1-69* subgenes on the patients' OS. When analyzing Binet A stage patients, however, the prognostic role of *IGHV3-21* with respect to OS was clearly confirmed.

In a proportion of CLL patients, surface BCR is similar, i.e. stereotyped, suggesting a potential role played by the antigen in leukemogenesis [4-6]. Currently, more than 200 subsets are defined having the stereotyped BCR structure, most of them being subsets 1-8 [30].

Whereas the *IGHV* mutation status defines various clinical and biological CLL subgroups, determination of stereotyped BCRs does not play the same role [17]. Yet many recent studies were concerned with a preferential link between stereotyped BCRs and some genetic changes [16, 31]. It was shown that only some stereotyped BCRs predict the development of genetic and molecular changes subsequently influencing the course of disease. One of them is the stereotyped BCR in patients with *IGHV3-21*, subset #2 [12, 16, 17, 32]. A recent study on subset #2 was concerned with not only assessment of recurrent aberrations but also analysis of mutations, showing a significantly specific genetic and molecular pattern with a high occurrence of the *SF3B1* mutation, more frequent deletions of 13q and 11q and, conversely, the absence of trisomy 12 and deletion of 17p [17]. The present group of patients with subset #2 was characterized by a high frequency of deletion of 11q (31%) and a higher occurrence of deletion of 13q (69%) as compared with non-subset #2 patients (13% and 27%, respectively) and the entire group (19.2% and 46.2%, respectively). None of 3 patients with deletion of 17p in *IGHV3-21* had subset #2. The same observations were reported by other authors [16, 17]. In the *IGHV3-21* and *IGHV1-69* groups, we detected recurrent gains of 2p in two patients, as published earlier. This aberration was associated with UM *IGHV* status and an advanced stage of the disease [33].

To conclude with, CLL patients with the *IGHV3-21* and *IGHV1-69* subgenes have as adverse prognosis as the other patients with UM *IGHV* status. The presence of deletion of 17p makes OS in both groups even shorter. In *IGHV3-21* patients, OS is influenced by other predictive factors and the subgene itself is an independent prognostic factor in patients with Binet A stage CLL. The data presented in the study show that the patients constitute a biologically rather interesting heterogeneous CLL subset that should be intensively investigated.

Supplementary information is available in the online version of the paper.

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