Profiling of biological and environmental risk factors in immunogenetic subgroups of chronic lymphocytic leukemia - Czech national study

Kamila Stranska^{a,b,#}, Karla Plevova^{a,b,#}, Hana Skuhrova Francova^a, Hana Skabrahova^a, Magdalena von Jagwitz-Biegnitz^a, Lenka Radova^b, Anna Panovska^a, Stanislava Hrobkova^a, Yvona Brychtova^a, Renata Urbanova^c, Lukas Smolej^d, Martin Simkovic^d, Jana Zuchnicka^e, Lekaa Mohammadova^f, Martin Spacek^g, Jiri Mayer^a, Sarka Pospisilova^{a,b}, Michael Doubek^{a,b}

Aims. This is a nation-wide survey of chronic lymphocytic leukemia (CLL) patients at six large hematology centers in the Czech Republic. The aim was to identify specific populations, social, and health characteristics of CLL subgroups divided according to the immunogenetic features of their B cell receptors (BCRs) and clonality.

Patients and Methods. Questionnaires directed to specific health, social, and environmental conditions were collected in a cohort of 573 CLL patients. For these patients, immunoglobulin heavy chain gene rearrangements were also analyzed in order to gain information about their clonality, IGHV mutational status, and the presence of stereotyped BCRs. Data extracted from the questionnaires were analyzed statistically in the context of immunogenetic features of the cohort.

Results. There were no statistically significant differences in the data collected in the survey between patients with mutated and patients with unmutated IGHV. However, patients with oligoclonal CLL reported health conditions such as hypercholesterolemia, hypertension, herpes simplex, tumors, and also, separately, CLL in 1st degree relatives, more often than their monoclonal counterparts. In patients with stereotyped BCRs, we found more frequent alcohol consumption and gastric infections in subset #1 cases and frequent cholecystectomies and familial CLL in subset #2 cases.

Conclusion. To the best of our knowledge, this study is the first to investigate CLL immunogenetic features and clonality in the context of epidemiological data. We reported statistically significant associations suggesting the influence of certain health and social conditions on a number of clonal populations expanding in CLL and also on characteristic BCR features, especially stereotypy.

Key words: chronic lymphocytic leukemia, B-cell receptor, clonality, questionnaire, patient history, biological risk factor

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^aDepartment of Internal Medicine – Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

^bCentral European Institute of Technology, Masaryk University, Brno, Czech Republic

^cDepartment of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Czech Republic

^d4th Department of Internal Medicine – Hematology, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic

^eDepartment of Hematooncology, University Hospital Ostrava, Czech Republic

^fDepartment of Hematology and Oncology, University Hospital Pilsen, Czech Republic

^g1st Department of Medicine - Department of Hematology, 1st Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

[#]These authors contributed equally to this work

Corresponding author: Michael Doubek, e-mail: doubek.michael@fnbrno.cz

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by extraordinary heterogeneity in clinical presentation, disease course and molecular features of malignant B cells. Many factors influence disease onset and course, some of which are currently used as prognostic or predictive markers. These involve specific chromosomal abnormalities¹, protein expression levels²⁻⁵, gene mutations⁶⁻¹⁰, epigenetic changes¹⁰, microRNA deregulation¹¹,

or molecular features of immunoglobulin (IG) gene rearrangements^{2,12,13}. In addition to cell-intrinsic features, microenvironment also represents a prominent factor in disease development^{14,15}.

Since CLL is a clonal disease of mature B cells, each case can be described by a unique set of IG rearrangements that encode for the B-cell receptor (BCR). In a clinical setting, somatic hypermutation load in the IG heavy chain variable region (IGHV) is routinely examined as this reliably divides patients into two prognosti-

cally distinct groups^{2,13}. Antigen stimulation is generally accepted as a prerequisite for the expansion of clonal B-cell populations. The striking degree of BCR sequence similarity observed between unrelated cases in one-third of patients¹⁶ suggests promoting pressure leading to CLL clone selection caused by a shared set of antigen epitopes. In this context, various antigens/epitopes recognized by CLL BCRs have been described. Most of them are auto-antigens presented during apoptosis and inflammation¹⁷⁻¹⁹, or surface antigens of stromal cells^{20,21} and B cells²² but several exogenous bacterial, viral, and fungal antigens have been proposed as well^{19,23-26}. In addition to that, CLL BCRs have been shown to interact in BCR-binding epitopes within IG heavy and light chain, enabling autonomous BCR signaling^{27,28}. Of note, CLL BCRs with mutated IGHV and those with unmutated IGHV differ in the level of their responsiveness to stimulation, with mutated BCRs showing reduced activation of the signaling response, and also in interactions with antigens, with unmutated BCRs being more polyreactive and more frequently binding autoantigens^{15,29,30}. Furthermore, despite the generally monoclonal nature of the disease, CLL comprising several leukemic clones with different BCRs can be observed in up to a quarter of CLL cases, depending on the detection method used³¹⁻³³. Expansion of multiple clones in such cases raises the question of whether these clones might proliferate upon chronic stimulation by antigens present in a case.

In line with molecular observations, epidemiological studies showed that prior to diagnosis, CLL patients experience some common infections more frequently than controls³⁴⁻³⁷. Increased risk of CLL development was also documented for several autoimmune and chronic inflammatory diseases^{35,38}. Notably, chronic stimulation in general (including both autoimmune diseases and chronic infections) in CLL is associated with unfavorable cytogenetics and unmutated IGHV (ref.³⁹). CLL occurrence has also been connected with several occupational and environmental exposures^{37,40}. Among first-degree relatives of CLL patients, there is an increased risk of the same malignancy or other lymphoproliferative diseases^{37,41-44}. To our best knowledge, however, there has been only one epidemiologic study published to date that took into consideration immunogenetic features of the investigated CLL cases³⁹ and no study taking the CLL clonality into account.

In this work, we employed a carefully designed questionnaires in a nation-wide survey among CLL patients of six large Czech hematological centers to reveal specific population, social, and health characteristics of subgroups of CLL patients distinguished primarily by their immunogenetic features and clonality. Our aim was to gain insight into antigen stimuli and other influences in individual CLL patients, that could be reflected in the selection and character of IG rearrangement of a leukemic clone.

METHODS

CLL patients

The study was performed on 573 consecutive CLL patients treated or monitored at one of the six large collaborating Czech hematological centers (University Hospital Brno, University Hospital Hradec Králové, General University Hospital Prague, University Hospital Olomouc, University Hospital Pilsen and University Hospital Ostrava) between years 2014–2018. All patients fulfilled the iwCLL/NCI diagnostic criteria for CLL (ref.⁴⁵), and completed questionnaires and the results of IGH analysis were obtained from all of them. All blood samples and questionnaires were taken after written informed patient's consent in accordance with the Declaration of Helsinki, under protocols approved by the Ethical Committees of all involved institutions. Standard CLL-related clinical and laboratory parameters (age at diagnosis, sex, time to first treatment, overall survival, clinical stage at diagnosis, cytogenetic aberrations, *TP53* mutation status) obtained from the Czech national CLL database CLLEAR, where all involved hematological centers contributed, were paired with respective questionnaires.

Analysis of clonal IGH rearrangements

Negative B lymphocyte separation, total RNA isolation, cDNA synthesis, and IGHV mutational status analysis by direct Sanger sequencing were performed for all patients involved in the study as previously described^{31,32}. PCR/Sanger analysis was complemented by IGH fragment analysis and also by subcloning when necessary. Stereotyped BCRs were identified using ARResT/AssignSubsets tool⁴⁶, enabling assignment to the 19 major stereotyped CLL subsets¹⁶.

Analysis of cytogenetic aberrations and *TP53* mutations

The presence *TP53* mutation was analyzed by Sanger sequencing⁴⁷ and/or next-generation sequencing⁴⁸. The hierarchical classification of four common cytogenetic aberrations¹ was analyzed by fluorescence in situ hybridization as described elsewhere^{49,50}.

Questionnaires

Questionnaires were filled in with the assistance of a clinician. They were designed to obtain information on basic characteristics of a patient (sex, age, date of birth), social and work history (marital status, number of children, living alone, profession), alcohol consumption and cigarette smoking, personal medical history (metabolic diseases, cardiovascular and respiratory diseases, chronic infections, autoimmune diseases and rheumatic disorders, allergies, surgical history, oncological diseases), and family history (oncological and autoimmune diseases). Most questions on basic patient characteristics and social and work history were open-ended. The rest of the questions had standardized closed-ended answers, either dichotomous (yes/no) with space for specification, or polytomous (alcohol drinking: never, occasionally, regularly; smoking: yes, no, stop-smoker). The questionnaire form is provided in the Supplementary material.

Statistics

Clinical characteristics and data from collected questionnaires were compared between the cohorts of monoclonal CLL and oligoclonal CLL, and their subgroups based on the IGHV mutational status; the most numerous subsets of stereotyped BCRs were compared to a respective monoclonal subgroup (with either mutated, or unmutated IGHV; the exception was the subset #2, which we compared to both monoclonal subgroups separately and also to overall monoclonal cohort, as it contains both mutated and unmutated cases): The two-tailed Mann-Whitney U test and the Kruskal-Wallis test followed by the Dunn post hoc test were used to compare age at diagnosis; log-rank (Mantel-Cox) test and the Wald test were used for time to first treatment (TTFT) and overall survival (OS) comparisons; the two-tailed Mann-Whitney U test and the Kruskal-Wallis test were used for comparing the number of children; all other categories were tested by two-tailed Fisher's exact test. Value $P < 0.05$ was considered as a threshold for statistical significance in all comparisons. All analyses were performed in R.

RESULTS

Composition of the cohort and immunogenetic findings

In total, 573 CLL patients, from which both filled questionnaires and IGHV analysis results were collected, were included in the study. The cohort was composed of 343 men and 230 women, with median age at diagnosis 63.6 years (range 33.2–90.3 years). Median TTFT of the cohort was 47.4 months, median OS was 269.6 months. Basic characteristics of the cohort are summarized in Table 1. Three hundred and twenty-two (322) patients filled the questionnaire before first treatment, 229 patients after it.

Analysis of the clonal IGH rearrangements divided patients into several subgroups: whereas 518 patients (90.4%) had a monoclonal form of the disease, in 55 patients (9.6%) more than one clone was detected. Two hundred thirty-nine (239) monoclonal patients had their IGHV mutated (monoM), and 279 unmutated (monoU). In the oligoclonal group of patients, 18 had concordantly mutated clones (oligoM), 16 concordantly unmutated (oligoU), and the remaining 21 patients had coexisting clones with discordant IGHV mutational status (oligoD). Seventy-five (75) patients of the whole cohort had at least one clone belonging into one of the 19 major CLL subsets with stereotyped BCR. However, only subsets #1 and #2 were sufficiently numerous (19 and 18 cases, respectively) to be suitable for most comparisons – for several other subsets (#202: 5 cases; #3, #14, and #99: 4 cases each) only the most striking results had the chance to come out as significant.

Detailed information on the cohort composition and results extracted from the questionnaires are summarized in Supplementary Table 1.

Social and health conditions do not influence IGHV mutational status in monoclonal CLL

MonoM and monoU groups showed statistically significant differences in all compared clinical and laboratory categories (female/male ratio, age and Rai stage at diagnosis, FISH aberrations, *TP53* mutations, TTFT, OS) in concordance with published data, with adverse parameters being more frequent in the monoU group (Table 2). This observation illustrates that our cohort was representative and suitable for further analyses.

With regards to information collected from the questionnaires, there were no statistically significant differences in external factors or family disease burden between monoM and monoU cases (Supplementary Table 2). Only two nearly significant tendencies were encountered – in monoU cases autoimmune diseases were more frequent in either patients themselves ($P = 0.0786$) or in their 1st degree family relatives ($P = 0.0615$).

Specific health conditions are frequent in oligoclonal CLL

In the next step, we compared questionnaire findings between monoclonal and oligoclonal CLL cases (Table 2, Supplementary Table 2). No statistically significant differences in clinical parameters of the groups were noted with the only exception of patient age at diagnosis – oligoclonal CLL cases were typically older (median age at diagnosis 65.7 vs 63.3 years, $P = 0.03577$). Interestingly, several health conditions were more frequent in oligoclonal cases. Patients with oligoclonal CLL more often reported hypercholesterolemia ($P = 0.0047$), hypertension ($P = 0.01442$), herpes simplex ($P = 0.33195$), and tumors in 1st degree relatives ($P = 0.03278$) – the latter retained statistical significance even when limited to CLL only ($P = 0.02104$).

When categorized according to the IGHV mutational status, as expected, oligoM and oligoU cases showed similar clinico-biological behavior to their monoclonal counterparts. Considering the questionnaire data, oligoM cases featured increased frequency of non-smokers compared to oligoU group ($P = 0.0209$), and compared to both monoU and monoM groups they frequently reported hypertension ($P = 0.025$; $P = 0.0249$) and hematological diseases in family ($P = 0.0347$; $P = 0.0402$; when limited to CLL: $P = 0.0312$; $P = 0.079$). In oligoU group there were no significant differences compared to both monoU and monoM. Patients from oligoD group reported hypercholesterolemia the most often among all other groups tested (vs monoM $P = 0.00084$; vs monoU $P = 0.00007$; vs oligoM $P = 0.0549$; vs oligoU $P = 0.0233$).

Stereotyped CLL subsets are associated with specific medical conditions

We also investigated whether the presence of stereotyped BCRs associates with specific biological features in the tested cohort. Subset #1 patients more often reported occasional alcohol consumption ($P = 0.02749$) and less abstinence ($P = 0.01379$) compared to the rest of monoU CLL. Interestingly, this observation was accompanied by more chronic infections of the digestive system, in-

Table 1. Basic clinico-biological characteristics of the cohort.

	all		mono		oligo		monoU		monoM		oligoU		oligoM		oligoD		s1		s2	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Cases	573		518		55		279		239		16		18		21		19		18	
Age at dg [y]	63.6		63.3		65.7		64.0		62.7		66.3		63.7		68.2		63.5		68.0	
Male/female ratio	1.49		1.50		1.39		1.79		1.23		1.29		1.57		1.33		5.33		0.80	
TTFT [mo]	47.4		47.2		62.3		23.0		97.0		15.2		86.2		48.9		21.9		28.0	
Stage at dg (Rai):																				
low risk	181	44.1 %	161	43.3 %	20	53 %	69	33.5 %	92	55.4 %	6	55 %	9	60 %	5	42 %	5	36 %	5	56 %
intermediate risk	185	45.1 %	170	45.7 %	15	39 %	105	51.0 %	65	39.2 %	4	36 %	5	33 %	6	50 %	6	43 %	2	22 %
high risk	44	10.7 %	41	11.0 %	3	8 %	32	15.5 %	9	5.4 %	1	9 %	1	7 %	1	8 %	3	21 %	2	22 %
Hierarchical FISH:																				
del(13q)	218	39.6 %	198	39.7 %	20	39 %	66	24.8 %	132	56.7 %	4	25 %	10	63 %	6	32 %	7	37 %	9	50 %
normal	134	24.4 %	121	24.2 %	13	25 %	61	22.9 %	60	25.8 %	4	25 %	5	31 %	4	21 %	4	21 %	6	33 %
trisomy 12	70	12.7 %	63	12.6 %	7	14 %	40	15.0 %	23	9.9 %	4	25 %	1	6 %	2	11 %	0	0 %	0	0 %
del(11q)	94	17.1 %	85	17.0 %	9	18 %	74	27.8 %	11	4.7 %	4	25 %	0	0 %	5	26 %	5	26 %	3	17 %
del(17p)	34	6.2 %	32	6.4 %	2	4 %	25	9.4 %	7	3.0 %	0	0 %	0	0 %	2	11 %	3	16 %	0	0 %
TP53 mutations at dg	31	6.6 %	28	6.5 %	3	8 %	20	8.3 %	8	4.3 %	1	8 %	1	8 %	1	7 %	3	19 %	1	7 %
(>10%)																				
TP53 mutations overall	94	24.7 %	87	25.4 %	7	19 %	67	33.7 %	20	13.9 %	4	33 %	2	15 %	1	8 %	5	36 %	3	25 %
(>0.2%)																				

mono - monoclonal CLL; oligo - oligoclonal CLL; U - unmutated IGHV; M - mutated IGHV; D - discordant hypermutation status between coexisting clones; s1 - stereotyped BCR subset #1; s2 - stereotyped BCR subset #2; N - number of cases in a category; dg - time of diagnosis.

Table 2. Results of the statistical analysis – *P* values; only categories with statistically significant results and only statistically significant *P* values are shown. Grey shading shows the comparisons where a group B has shorter TTFT / OS, higher age at dg, or higher incidence of a tested feature. For detailed information about all categories tested see Supplementary Tables 1 and 2.

Group A:		mono	oligo	monoM	oligoM	oligoU	oligoM	oligoM	oligoM	oligoU	oligoD	oligoD	oligoD	s1	s2	s2	s2	s1	s3	s14	s99	s202
Group B:		mono	oligo	monoM	oligoM	oligoU	oligoM	oligoM	oligoM	oligoU	oligoD	oligoD	oligoD	monoU	monoM	monoU	monoU	s2	s1	monoU	monoM	monoU
TTFT		n.s.	n.s.	<0.00001	0.0035	0.00025	n.s.	n.s.	n.s.	0.00002	n.s.	0.00627	0.0455	n.s.	<0.00001	n.s.	0.02977	n.s.	n.s.	n.s.	n.s.	n.s.
OS		n.s.	n.s.	<0.00001	n.s.	n.s.	n.s.	n.s.	n.s.	0.0288	n.s.	0.00047	n.s.	n.s.	0.02265	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Age at dg		0.03577	0.00888	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.00515	n.s.	n.s.	0.04081	0.01351	n.s.	n.s.	n.s.	n.s.	0.04035	n.s.	0.03105
Male sex		n.s.	0.0477	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.01704	n.s.	n.s.	0.01588	n.s.
Low risk Rai stage		n.s.	0.00003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Intermediate risk Rai stage		n.s.	0.0278	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
High risk Rai stage		n.s.	0.00239	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
del(17p)		n.s.	0.00524	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.04501
del(11q)		n.s.	<0.00001	n.s.	0.00336	n.s.	0.0102	n.s.	0.00365	n.s.	0.0493	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Trisomy 12		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.04958	n.s.	n.s.
del(13q)		n.s.	<0.00001	n.s.	0.00229	n.s.	0.0183	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.02417	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.03502
TP53 mut at dg (>10%)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TP53 mut at dg (>0.2%)		n.s.	0.02	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TP53 mut overall (>10%)		n.s.	0.00296	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TP53 mut overall (>0.2%)		n.s.	0.00003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Living alone		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.00523
Children born - women only		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.04503	n.s.	n.s.
Non-smokers		n.s.	n.s.	n.s.	0.0209	n.s.	0.0375	0.0112	n.s.	0.01379	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Alcohol abstinence		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Alcohol occasionally		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.03278	n.s.	n.s.	n.s.	n.s.
High blood pressure		0.01442	n.s.	n.s.	0.025	0.0249	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.04052	n.s.
Low blood pressure		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0296	n.s.
Hypercholesterolemia		0.0047	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.00084	0.00007	0.0233	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.03671
Herpes simplex		0.03195	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0266	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Gastritis, stomach ulcers		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.01047	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cholecystectomy		n.s.	n.s.	n.s.	0.0427	n.s.	n.s.	n.s.	n.s.	n.s.	0.04371	0.0423	n.s.	0.00799	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Tonsillectomy/appendectomy		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Parents/siblings with tumor		0.04803	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0308	n.s.	n.s.
Tumor in 1 st degree relatives		0.03278	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hematology disease in 1 st degree relatives		n.s.	n.s.	n.s.	0.0347	0.0402	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CLL in 1 st degree relatives		0.02104	n.s.	n.s.	0.0312	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.02804	0.02019	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. – not significant; n.a. – not assessed due to zero number of positive cases.

cluding gastritis and stomach ulcers in subset #1 patients ($P=0.01047$).

Subset #2 patients tended to undergo cholecystectomies more often than other CLL patients (vs all mono $P=0.04371$; vs monoU $P=0.0423$; vs monoM $P=0.0905$). They had also a high prevalence of CLL among their relatives (vs all mono $P=0.02803$; vs monoU $P=0.2018$; vs monoM $P=0.06327$).

In less abundant CLL stereotyped subsets in our cohort, we identified the following differences: Four of 5 subset #202 patients reported living alone ($P=0.00523$), the same number reported hypercholesterolemia ($P=0.03671$), and albeit non-significant, all cases of this subset reported hypertension ($P=0.06651$). All patients of subset #99 were women ($P=0.01588$). Three out of the 4 reported rheumatic disorders (not significant: $P=0.05002$); significant differences were observed in high blood pressure with 2 out of the 4 patients reporting hypotension ($P=0.0296$) and none of them hypertension ($P=0.04052$). All cases of subset #14 reported tonsilectomies and/or appendix surgery ($P=0.0308$). No statistically significant difference in patient history data was observed for stereotyped subset #3.

DISCUSSION

In the present study, we aimed to elucidate which environmental, lifestyle, health and other factors might influence CLL clonal selection with respect to specific features of CLL BCRs. For this purpose, we surveyed 573 CLL patients monitored in six large hematology centers across the Czech Republic. The distributed questionnaire contained mostly questions with dichotomic answers to facilitate easy responding, and afterwards, straightforward processing of the data. Nevertheless, during the extraction of data from the questionnaires, we faced issues caused by question misunderstanding or inadequate knowledge of patients' and their family members' health conditions, resulting in a potential burden of subjective responses which we tried to reduce by further investigation of patients' clinical records and direct surveying the patients and their accompanying relatives. Furthermore, some questions were eventually excluded from the final analysis as we did not collect enough valid data. This issue mainly concerned patient vaccination and childhood illnesses as these were the categories which patients mostly did not remember. Still we believe that our study has provided valuable insight into the real world CLL population and their native environment.

In the published data it is widely appreciated that specific environmental stimuli, social habits, and health conditions are present in CLL patients with increased occurrence compared to healthy age-matched controls³⁵⁻³⁷. On the other hand, to our best knowledge, no study has been published that would investigate the correlation of patient CLL immunogenetic features of their clonotypic BCR with patient history data. There are a handful of studies which touched upon a comparison of IGHV mutated and unmutated CLL subgroups^{39,51,52}. A similar study

focusing on CLL clonality and the presence of stereotyped BCR subsets has not been published to date. In this respect our study attempts to fill the gap in the current knowledge about CLL clonal selection and onset.

The whole collected cohort was representative of the general CLL population. In monoclonal CLL cases surveyed in this study we observed all differences in clinical and laboratory parameters that are expected between IGHV mutated and unmutated subgroups^{2,13,53}. Considering questionnaire data obtained for the subgroups we did not find any statistically significant differences. We observed only a weak, statistically insignificant association between unmutated IGHV and the occurrence of autoimmune diseases. This association is partly in agreement with observation by Vanura et al. (ref.³⁹) who found increased frequency of autoimmune diseases in female CLL patients with unmutated IGHV. Analysis of larger cohort would be needed to confirm the association. Nevertheless, overall, our findings indicate that extrinsic stimuli likely do not affect to a large extent whether IGHV mutated or unmutated clone would expand.

Further, we focused on clonality in our CLL cohort. We collected 55 CLL cases with oligoclonal disease (9.6% of the cohort) which was sufficient for statistical analysis of patient history data. In published studies, oligoclonal CLL has been observed in 2 to 24.4% of cases, depending on the detection method used^{31-33,54}. Prognostication of these oligoclonal cases may be challenging, especially in cases of coexisting clones with discordant IGHV mutational status⁵⁴, and in this area, only limited work has so far been reported^{31-33,55}. For the IGH rearrangement analysis within the current study, we used Sanger sequencing which has typically lower detection rate of multiple rearrangements. From this point of view, capturing nearly 10% of oligoclonal cases in our cohort is quite high but still within an acceptable range. We did not find any differences concerning clinical and laboratory status between oligoclonal and monoclonal cases with the exception of age at diagnosis. This observation confirms the results of our previous studies on oligoclonal CLL (ref.^{31,32}). Admittedly there was a partial overlap between the cohorts but the difference in age stayed significant even after adding new cases from other workplaces included in the present cohort.

In agreement with other studies^{31,33,55}, we observed concordant IGHV mutational status of coexisting clones in almost two-thirds of oligoclonal cases (34/55; 62%). The remarkably high occurrence of concordant IGHV mutational status in oligoclonal cases suggests that coexisting clones experienced the same or similar influences affecting their development. According to the recent evidence, shared epitope reactivity⁵⁶ or origin from a common cellular progenitor^{57,58} might be such influences. Here we found health conditions, such as hypercholesterolemia, hypertension, herpes simplex, and tumors in 1st degree relatives, more frequent in oligoclonal cases than the monoclonal ones. Out of these only hypercholesterolemia was reported to precede a CLL diagnosis with a higher prevalence compared with control subjects⁵⁹. Particularly, the higher prevalence of CLL in the families

of our oligoclonal CLL cases poses an interesting basis for further investigation of shared antigenic stimuli and possible genetic background in CLL families. An observation indicative of similar findings was also gained in monoclonal B lymphocytosis⁶⁰ where a high occurrence of multiclonal cases was noted⁶¹.

Finally, stereotyped BCR subsets were reported to share many clinico-biological features, for example, the presence of prognostically relevant gene mutations and cytogenetic aberrations, or clinical aggressiveness of the disease^{12,51,62-65}. Thus, we were interested in whether this clinical similarity might be reflected in common extrinsic influences. The number of stereotyped BCR subsets in our cohort precluded extensive analyses of this phenomenon, however, still for subsets #1 and #2 we collected enough cases and obtained results worth further interest. Namely, subset #1 patients more often reported occasional alcohol consumption compared to the rest of monoU CLL and also more chronic infections of the digestive system which can create an antigenic pressure due to chronic inflammation known mainly from other lymphoid malignancies⁶⁶. In subset #2 patients, we observed frequent cholecystectomies more often than in other CLL patients and high prevalence of CLL in their families. All these observations have not been reported previously and represent novel findings of our study. The last subset worth mentioning is subset #99 with unmutated IGHV1 rearrangements consisting exclusively of women wherein the majority of subjects reported rheumatoid diseases, markedly reminiscent of female patients with unmutated IGHV and autoimmune conditions reported elsewhere³⁹.

CONCLUSIONS

Thorough analysis of both biological features and clinical course in each CLL case facilitates a more personalized approach in patient care and enables the formation of homogeneous patients' groups for answering many important clinical and research questions. To our knowledge, our study is the first to investigate CLL immunogenetic features and clonality in the context of patient history data. We reported several statistically significant associations suggesting the influence of certain health and social conditions on a number of clonal populations expanded in CLL. Specifically, patients with oligoclonal CLL reported hypercholesterolemia, hypertension, herpes simplex, and tumors (and separately also CLL) in 1st degree relatives more often than patients with monoclonal disease. With regards to stereotyped BCR, subset #1 patients often reported occasional alcohol consumption and chronic infections of the digestive system, and subset #2 patients underwent cholecystectomies more often than other CLL patients. Our study only refers to potential relationships of the given conditions to CLL immunogenetic subgroups, which needs to be validated further. However, it may form the basis for future epidemiological studies that would clarify conclusively the observed associations.

ABBREVIATIONS

CLL, Chronic lymphocytic leukemia; BCR, B cell receptor; mono, monoclonal; oligo, oligoclonal; IG, Immunoglobulin; IGHV, IG heavy chain variable region; U, Unmutated; M, Mutated; D, Discordant; TTFT, Time to first treatment; OS, Overall survival.

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SUPPLEMENTAL MATERIAL

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