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Original article

DISTINCT SUBGROUP OF PATIENTS WITH UNMUTATED IGHV1-69 CHRONIC LYMPHOCYTIC LEUKEMIA AFFECTED WITH COVID-19 INFECTION - SINGLE CENTER EXPERIENCE

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Abstract

Introduction: With the onset of infection with COVID-19, complications of this disease among vulnerable populations have been a primary alarm for health systems. Chronic lymphocytic leukemia (CLL) is characterized by variable clinical courses among patients with distinct genetic background, often leading to a compromised immune system, and, hence, these patients were seriously affected by this pandemic.

Aim of the study: Evaluation of COVID-19 infection outcome in a subgroup of patients with unmutated IGHV1-69 CLL.

Materials and methods: This was a retrospective study comprising 35 patients with CLL, diagnosed and followed in the period between January 2012 and January 2022. Traditional laboratory, clinical and biological prognostic factors were evaluated at first patient visit to the University Clinic for Hematology – Skopje, Macedonia. Mutational status and genetics were analyzed using reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing methodology.

Results: In our study, there was a male predominance, with 65.7% of patients being male. Majority of patients had Binet B stage (57.1%). According to genetic structure, the most frequently expressed D gene was 3-16 in 42.8% of patients, and J gene 3-15 and 6-15 present in 42.8% of patients. Most of the patients were treated with Obinutuzumab (Ob)-based therapy, and 68.5% of patients received Ob + chlorambucil. Infection with Covid 19 was registered in 69% of patients; 44.8% of patients were vaccinated but with fatal outcome, and the overall fatal outcome rate was 42.8%.

Conclusion: In this retrospective study on COVID-19 comprising patients with unmutated IGHV1-69 CLL, 42.8% had a fatal outcome. Risk factors associated with poorer outcomes were identified.

Keywords: chronic lymphocytic leukemia, unmutated IGHV1-69, COVID-19, mortality

Introduction

Chronic lymphocytic leukemia (CLL) is a clonal malignancy of B lymphocytes, in which the abnormal monoclonal B lymphocytes are accumulated in the peripheral blood, bone marrow,

and lymphoid tissues. One of the basic characteristics of this disease is compromised immune system by deficiencies in lymphocyte populations in quantitative and qualitative manner, vital for effective immune response and surveillance. Leukemic cells exhibit aberrant expression of surface markers and changed cytokine signaling, resulting in decreased antigen presentation, defective cytotoxicity, and compromised humoral immunity. Consequently, CLL patients show amplified predisposition to bacterial, viral, and fungal infections, with increased morbidity and mortality attributable to infectious complications. Given the vulnerability of CLL patients and their susceptibility to COVID-19-related adverse events, this study aimed to evaluate the impact of COVID-19 on the general conditions, prognosis, and clinical outcomes of patients with CLL with distinct genetic signature with unmated IGHV1-69 CLL. Since its first report in 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a global healthcare crisis. Two large cohort studies of patients with CLL and COVID-19 have reported an identical overall case fatality rate around 27%[1,2]. Immunosuppression is potentiated in patients undergoing chemoimmunotherapy[3]. The impact of chemoimmunotherapy in patients with CLL and COVID-19 infection has not been extensively elaborated in the available literature, perhaps due to differences in baseline patient characteristics.

Aim

The aim of this study was to evaluate the outcomes of COVID-19 infection in a subgroup of patients with unmutated IGHV1-69 CLL, who were known to have inferior survival due to their genetic signature.

Materials and methods

This was a retrospective study including 35 patients with unmutated IGHV1-69 CLL, diagnosed and followed in the period between January 2012 and January 2022. Traditional laboratory, clinical prognostic, and biological prognostic factors were evaluated at first patient visit to the University Clinic for Hematology in Skopje, Macedonia. CLL diagnosis was based on the International Workshop on CLL criteria[4].

Medical data of all patients were implemented from the medical history of the disease. Physical examination with a two-dimensional diameter of the enlarged lymph nodes in all regions available for palpation (neck, axillar, supraclavicular, inguinal, femoral) was performed. The dimensions of the spleen and the liver were noted by physical examination and ECHO of the abdominal organs, and computerized tomography (CT) scan of the chest and abdomen were also made. Performance status was assessed by ECOG. Complete blood count with a number of leukocytes, platelets, hemoglobin value, differential blood count with percentage and absolute number of lymphocytes and reticulocytes were assessed. Clinical stratification was carried out according to the BINET system. *C-reactive protein* test (CRP) inflammatory marker with prognostic significance was examined. Either inpatient or outpatient cases of COVID-19 were included in this study. Major comorbidities were defined as Cumulative Illness Rating Scale (CIRS) score of ≥ 7[5]. D-dimer as a marker of ongoing activation of the hemostatic system was determined by using latex agglutination-based test.

Diagnosis of CLL was assessed in the Laboratory for Immunohematology of the University Clinic for Hematology using ERIC panel and standard operating protocol[6] for flow cytometry. Analysis was performed on BD FACSLyric™ (BD-Biosciensis, San Jose, CA, USA). Interpretation of the histograms was performed using a special software, BD FACSuite Software, where positive expression of antibodies was defined as > 20% of mononuclear cells.

Individual data from 35 treatments naïve CLL patients were analyzed, and mutational status and configuration of IGHV-IGHD-IGHJ rearrangements and genetics were analyzed using reverse transcriptase– polymerase chain reaction (RT-PCR) and sequencing methodology at the Center for bimolecular pharmaceutical analyses, Faculty of Pharmacy, Skopje, Republic of North Macedonia.

Mononuclear cells obtained from peripheral blood samples were analyzed by Ficoll density gradient centrifugation. Total RNA was extracted using TRIzol reagent (Ambion, Life Techlogies) and reverse-transcribed using MuLV reverse transcriptase (Applied Biosystems, Foster City, CA, USA) and random hexamer primers, according to manufacturer's instructions.

IGHV-IGHD-IGHJ gene rearrangements were amplified by RT-PCR using a mixture of 5' primers specific for leader sequences of IGHV1 to IGHV6 subgroups in conjunction with mixed 3' primers complementary to the germline IGHJ genes. Reverse transcriptase–polymerase chain reaction (RT-PCR) was carried out in a final volume of 25 μL with 10 pmol of each primer, 200 pmol of each deoxyribonucleotide, and 2.5 U Tag Gold Polymerase (Applied Biosystems, Foster City, CA, USA). Amplification consisted of an initial denaturation step of 10 minutes at 95°C, followed by 35 cycles at 95°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1.5 minute, with a final extension step of 10 minutes.

Clonal PCR products were purified using low melt agarose, and were sequenced with reverse primer with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), purified with BigDye X Terminator Purification Kit (Applied Biosystems, Foster City, CA, USA) and run on 3500 Genetic Analyzer (Applied Biosystems).

Mutational status was calculated as percentage of deviation from the closest germ line IGHV gene. Sequences with a germ line identity greater than or equal to 98% were considered unmutated and those with an identity less than 98% were considered mutated.

Statistical Analysis

Statistical analysis was performed using the SPSS software package, version 21.0. The value of $p<0.05$ was considered significant for all analyses.

Results

In this retrospective study, 35 patients with unmutated IGHV1-69 CLL were diagnosed and followed in the period between January 2012 and January 2022.

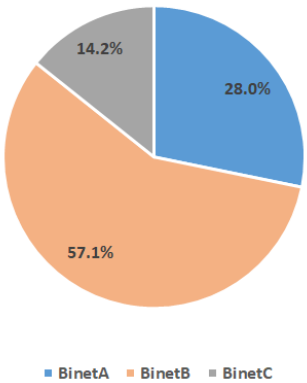

Fig. 1. Distribution of patients according to Binet staging system

The median age at the time of COVID-19 diagnosis was 58.9 years (range 33–79 years), and 65.7% were men. Majority of patients (91.2%) had ECOG score 0 and 1, and only 8.5% had ECOG 2. The median number of white blood cells was 107.7 x10 ^{3}/uL, and absolute lymphocyte count was 89 x10^{3} /uL. Distribution of patients according to Binet staging system is presented in Figure 1. Majority of patients were in Binet B stage.

All patients had unmutated IGHV1-69 CLL. Distribution of IGHD and IGHJ genes is presented in Figures 2 and 3. Most patients with unmutated IGHV1-69 CLL had IGHD gene 3-16, and an equal distribution of IGHJ genes in subgroups 3-15 and 6-15.

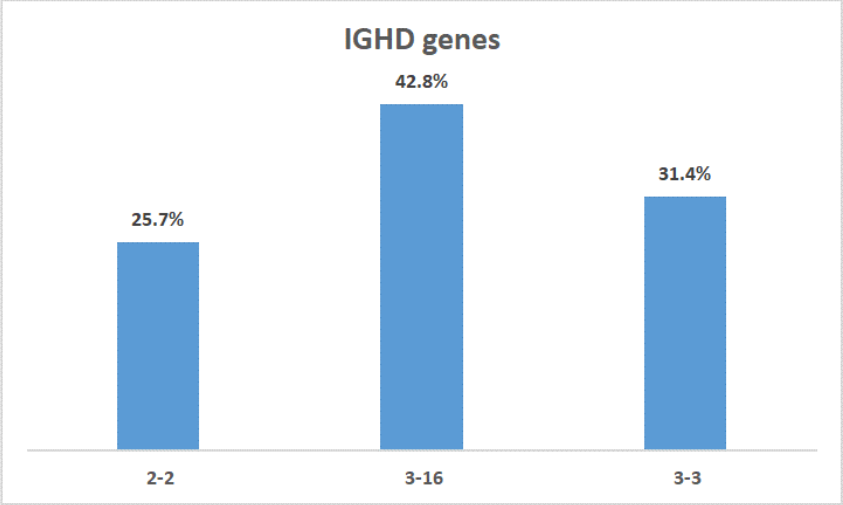

Fig. 2. Distribution of IGHD genes

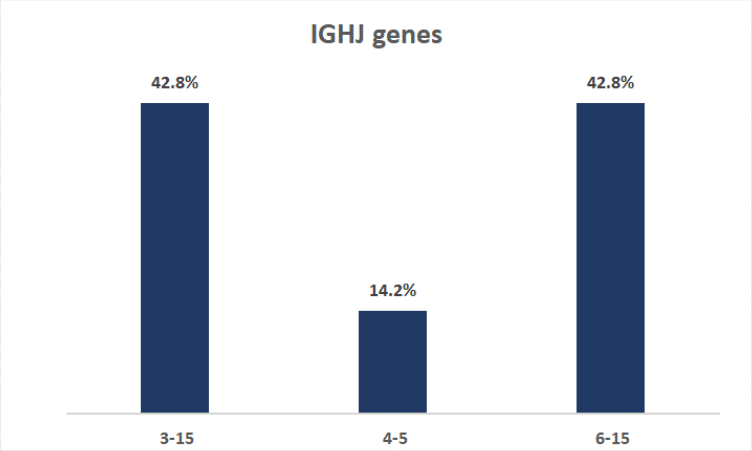

Fig. 3. Distribution of IGHJ genes

A TP 53 mutation was detected in 8.5% of patients with unmutated IGHV1-69 CLL, a subgroup of patients with an inferior prognosis, and 100% of them had COVID-19 infection. Figure 4 displays distribution of patients according to genetics. Most patients had deletion 13q (40%).

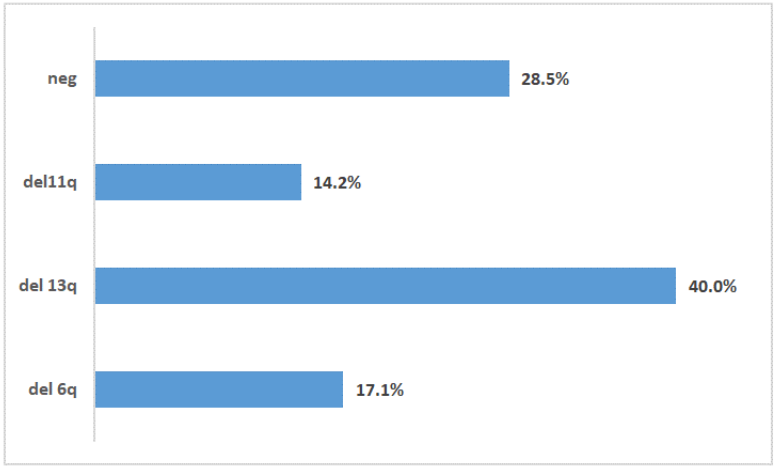

Fig. 4. Distribution of patients according to genetics
Abbreviations: neg - no detection of genetic changes,
del 11q - deletion 11q, del13q - deletion 13q, del6q - deletion 6q

B symptoms were found in 42.8% of patients. The median time to treatment failure was 40.3 months. According to number of therapy lines, patients were distributed in three subgroups (Figure 5). There was a statistically significant difference (p=0.001) between 1 and 3 lines of therapy.

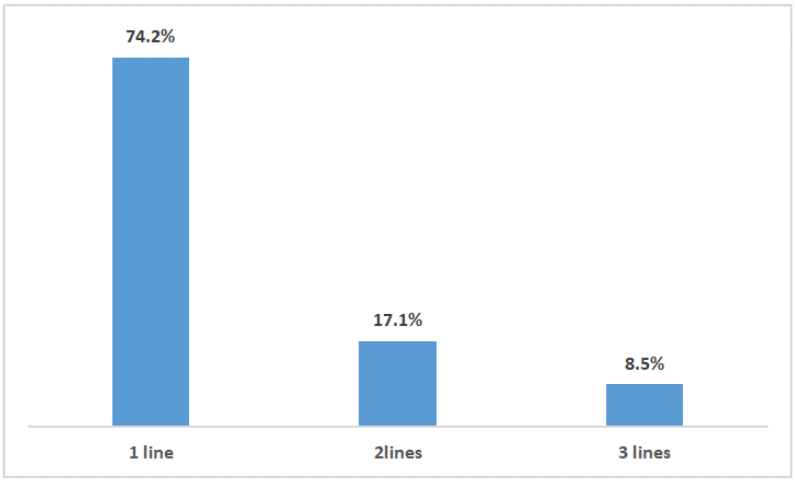

Fig. 5. Distribution of patients according to number of therapy lines

In Figure 6 illustrates distribution of COVID-19 diagnosed patients according to chemoimmunotherapy regimen. Majority of patients (68.5%) were treated with Obinutuzumab-(Gazyiva) based therapy, and 31.4% of patients were treated with GFC-protocol (Gazyva, Fludarabine, Cyclophosphamide).

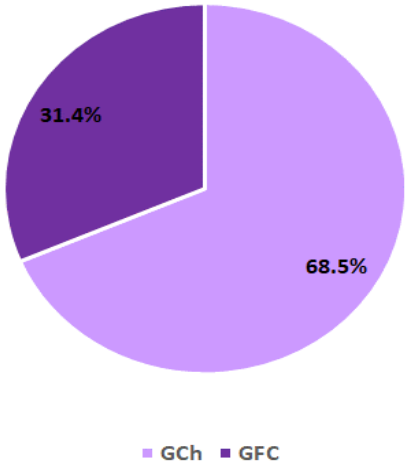

Fig. 6. Distribution of patients according to therapy administered at the time of COVID-19 infection
Abbreviations: GCh - Gazyva – Chlorambucil, G
FC- Gazyva – Fludarabine, Cyclophosphamide

The most frequently adverse events from chemoimmunotherapy based on Obinutuzumab (Gazyiva) was grade 2 neutropenia registered in 42.8% of patients. Hypogammaglobinemia was another adverse event from therapy, with the median level of IgG 2.54g/l. This led to an increased rate of infections, including COVID-19.

Response to therapy is shown in Figure7. A complete response was achieved by 37.1% of patients, but the fatal outcome rate was 42.8%.

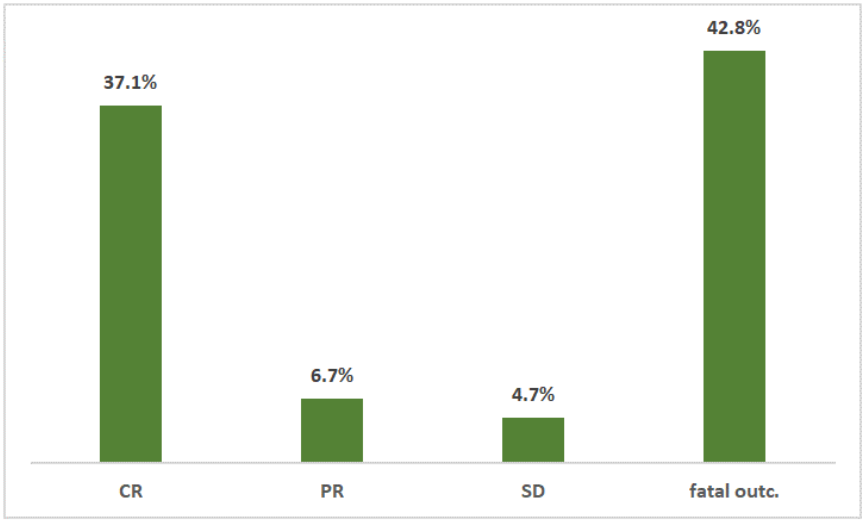

Fig. 7. Distribution of patients according to response to therapy
Abbreviations: CR- complete response, PR - partial response,
SD - stable disease, fatal outc. -fatal outcome

Percentage of COVID-19 infection, vaccination and fatal outcome distribution of patients is presented in Figure 8. In 69% of patients with unmutated IGHV1-69 CLL COVID-19 infection

was present, 82.8% were vaccinated prior to COVID-19 infection, but 44.8% of them had fatal outcome.

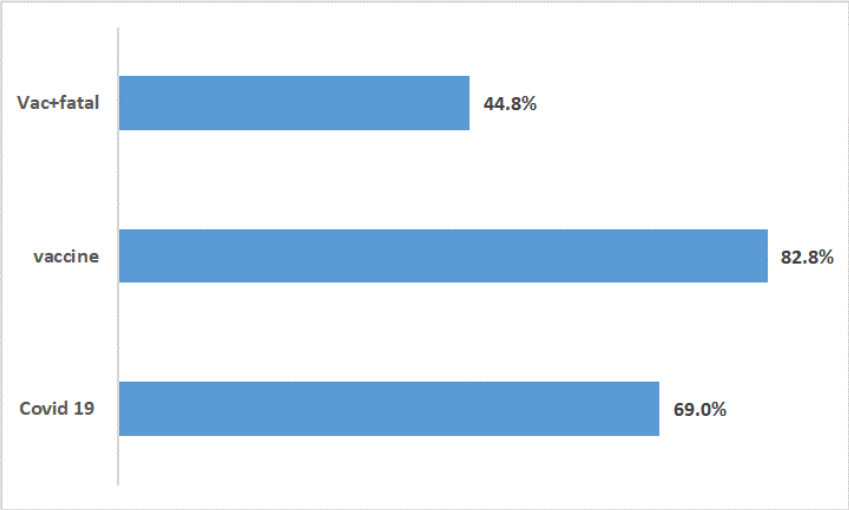

Fig. 8. Distribution of patients according to COVID-19 infection, vaccination and fatal outcome
Abbreviations: Vac + fatal - vaccination and fatal outcome

Distribution of patients according to comorbidities is displayed in Figure 9. The most frequently present comorbidity was high blood pressure (HBP), with a statistically significant difference (p=0.001) between HBP and other comorbidities (hypothyroidism, obesity, malignancy).

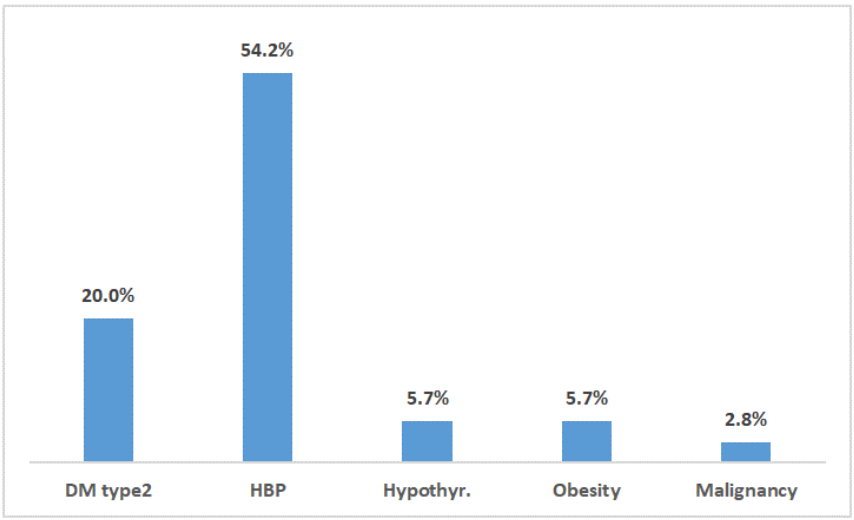

Fig. 9. Distribution of patients according to comorbidities
Abbreviations: DM tip 2 - diabetes mellitus type 2,
HBP - high blood pressure, hypothyr. - hypothyroidism

The median level of D-Dimers was 4055 μg/mL, and the median level of the inflammatory marker CRP was 104.5 mg/dL.

Regarding hospitalization and ICU admission due to COVID-19 infection, the distribution of patients is presented in Figure 10.

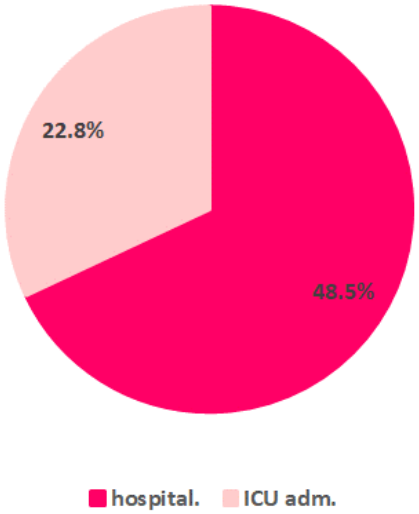

Fig. 10. Distribution of patients according to need for hospitalization and ICU admission because of COVID-19 infection.
Abbreviations: hospital.- hospitalization, ICU adm.- Intensive care unit admission

A high percentage of patients (48.5%) were hospitalized because of COVID-19 infection, and half of them were admitted to intensive care unit treated with oxygen therapy, corticosteroids, antibiotics, anticoagulant therapy. Some patients (9.4%) were treated with cocktail of two monoclonal antibodies - REGEN-COV (casirivimab and imdevimab).

Discussion

This study presented the outcomes of 35 patients with CLL and COVID-19 reported from a single center for hematology in the Republic of North Macedonia. Fatal outcome rate was 42.8%, which is in agreement with findings from two extensive reports on COVID-19 in patients with CLL. Roeker *et al.* conducted an international analysis of 374 cases[7]. They reported a higher rate of hospitalization than that in our study; 85% were hospitalized, and 32% required ICU admission. The case fatality rate (CFR) of the entire cohort was 28%, and 36% of those were admitted to the hospital. In the largest cohort to date, similar results were reported by the European Research Initiative on CLL (ERIC) and Campus CLL. They analyzed the outcome of 941 cases (2); total 75% of patients were admitted to hospital, 26% required ICU stay, and the overall CFR was 27% for all patients. Similar results were published in a review article by Akbarzadeh *et al.* [8], who presented a large number of studies on patients with COVID-19 infection in patients with CLL.

The high mortality rate for COVID-19 is not surprising as CLL primarily affects older adults with a median age of 70 years. In our study a lower median age was present (58.8), and this population is characterized by a wide range of comorbidities. Age and comorbidities are some of the predispositions for COVID-19 infection and its more severe clinical manifestation. The most frequent comorbidity in our patients was HBP, which reflects the level of culture of life and level of primary health care. The severity of the clinical picture is also influenced by other factors that are part of the pathogenesis of CLL. Factors include hypogammaglobulinemia (also observed in

our study), T cell abnormalities, impaired phagocytosis and complement systems[9]. Reduced production of type 1 interferon is one of the reasons for the severe clinical picture of COVID-19 infection in patients with CLL[10]. The severity of the clinical picture of COVID -19 is also influenced by the impact of chemoimmunotherapy, which causes immunocompromised condition in this subgroup of patients. Therapy with new generations of immunochemotherapy and anti-CD20 monoclonal antibodies contributes to the potentiation of hypogammaglobinemia and a reduced resistance to infections. Our study has some limitations. Asymptomatic or mildly symptomatic patients were underrepresented, given the retrospective study design. Our study showed a higher prevalence of COVID-19 infection in this subgroup of patients with a specific genetic marker. Additionally, the study demonstrated that this subgroup of patients had a greater need for hospitalization and intensive care treatment compared to other patients with CLL. Particularly striking was the high percentage of vaccinated patients within this subgroup who experienced fatal outcomes. Perhaps the specific genetic pattern contributes to this, which requires more clinical studies on this topic, to crystallize this hypothesis.

Conclusion

In this single-center retrospective study comprising COVID-19 patients with unmutated IGHV1-69 CLL, the fatal outcome rate was 42.8%. Risk factors for an inferior outcome were identified.

Conflict of interest statement. None declared.

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