In this issue: Current laboratory aspects of COVID-19

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Newly diagnosed chronic lymphocytic leukemia during symptomatic COVID-19: two cases

Enikő Papp[1], Szabolcs Tasnády[1], Katalin Tisza[1], Ágnes Király[2], Gabriella Bekő[1]

[1] *Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, Central Laboratory, Budapest, Hungary*

[2] *Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, Department of Hematology and Stem Cell Transplantation, Budapest, Hungary*

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Corresponding author:
Enikő Papp, MD
E-mail: papp.eniko@dpckorhaz.hu

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ABSTRACT

Patients suffering from malignant diseases have a high risk of developing severe or critical forms of COVID-19 (Coronavirus Disease 2019). Chronic lymphocytic leukaemia (CLL) is characterized by dysregulated adaptive and innate immune responses, because both T and B cells, the function of phagocytes and the activity of the complement system may be affected. Severe SARS-CoV-2 infection also influences the immunological functions mainly via causing the depletion of CD4+ and CD8+ T cells. We present the cases of two patients, whose *de novo* CLL were observed during severe COVID-19 pneumonia. A 43-year-old man with IDDM (Insulin dependent diabetes mellitus) was sent to hospital in February 2021. He had a bilateral severe COVID-19 pneumonia. There was a suspected sign of malignancy on a thoracic vertebra in his chest CT, and haematological consultation was

requested. In parallel, a 53-year-old man was hospitalized in March of 2021 because of severe COVID-19 pneumonia. CLL was suspected based on his haematology test results (WBC: 123 G/L, lymphocytes: 91%, haemoglobin: 107 g/L). Flow cytometric analysis revealed CLL in both cases. Based on the result of the molecular genetic tests, the first patient had a good prognosis in Rai 0 stage, while the other patient suffered from Rai I stage with a worse prognosis. Both patients recovered from bilateral COVID-19 pneumonia without the need for intensive care unit treatment. The follow-up of these CLL patients that manifested during symptomatic COVID-19 disease further enriched our knowledge on such clinical conditions where the immune system is dysfunctional due to different simultaneous causes.

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INTRODUCTION

CLL is the most common type of leukaemia among adults in developed countries with an annual incidence of 3/100 000 people in Central Europe in 2019 [1]. It is characterized by the monoclonal accumulation of mature B lymphocytes of which immunophenotype and immunomodulating functions are changed resulting in the dysregulation of both the adaptive and innate immune responses. These changes affect both T and B cells, phagocytosis and the complement system leading to an immunosuppressive condition [2,3,4], thus the general risk of severe infections critically rises the morbidity and mortality [5,6]. Although 'watch-and-wait' strategy is recommended for low-risk patients (*i.e.,* Rai 0 stage), patients in higher risk category (e.g., Rai III-IV stages) require chemotherapy which includes not only conventional agents, but also new regimens, such as Bruton's tyrosine kinase inhibitors (BTKi) or B-cell lymphoma 2 (BCL-2) inhibitors. Treating patients with Rai I-II stages is feasible and highly indicated if the disease starts to progress [6,7]. CLL therapy also contributes to immunosuppression which further increases the risk of infections [5,6].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a serious risk factor for cancer patients [8]. It causes the depletion of CD4+ and CD8+ T cells, B cells and natural killer cells causing an impairment of the immune system [9]. These complications and the increased level of cytokines producing CD14+CD16+ monocytes contribute to the development of cytokine storm and related fatal outcome [10]. Moreover, initiation of treatment can induce additional immune modulation that further increases the risk for severe infections [11].

Here we present two cases where CLL was confirmed during the clinical phase of COVID-19 pneumonia. SARS-CoV-2 infection causes lymphopenia in contrast to lymphocytosis that is typical in CLL. Our aim was to investigate the effects of these comorbidities on laboratory results and to accomplish the follow-up of acute and chronic clinical conditions when the immune system is under attack from two directions simultaneously.

TWO CASES

The first patient was a 43-year-old male patient with insulin dependent diabetes mellitus and transient ischaemic attack in his medical history. He was admitted to hospital with severe respiratory symptoms in February 2021 when his COVID-19 pneumonia was treated by the current protocol including remdesivir, steroids and antibiotic therapy. His chest CT scan for COVID-19 pneumonia suggested signs of malignancy on a thoracic vertebra and he was sent to a haematology consultation. In April, his laboratory parameters were as follows: white blood cell count (WBC): 17.2 G/L with 62.9% relative lymphocyte

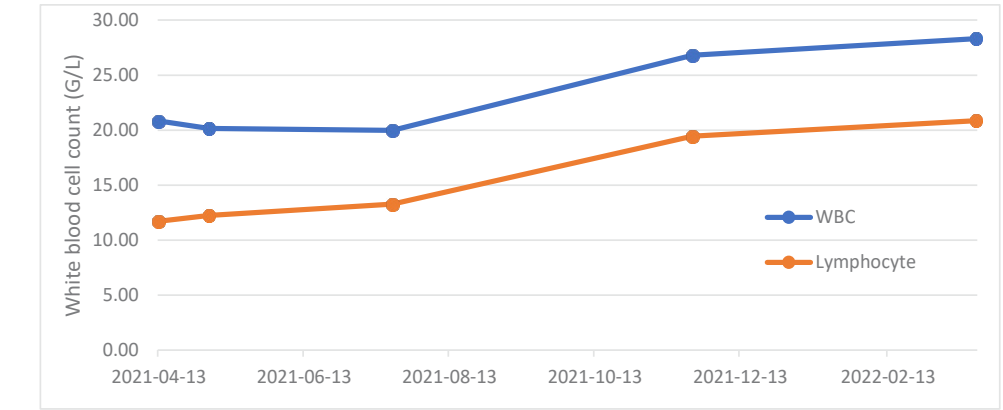
ratio, haemoglobin was 144 g/L, thrombocyte count was 214 G/L. In the peripheral blood smear, there were lymphocytes in 45% and their atypical forms in 6%. The result of the flow cytometric analysis in the peripheral blood found CD19 positive pathological B cells in 33% which were divided into two subclones (CD38+ and CD38-). FISH (fluorescence in situ hybridization) analysis proved the presence of del(13)(q14) deletion. The final diagnosis was CLL. The bone scintigraphy did not prove any solid tumour. Three months after the onset of SARS-CoV-2 infection, WBC count was elevated (20-21 G/L) with higher absolute lymphocyte count (11-12 G/L), but there was no anaemia or thrombocytopenia. The patient had neither hypogammaglobulinaemia nor paraproteinaemia, and the level of β2 microglobulin was 1.73 µg/ml. No lymph nodes or the spleen were palpable, however, the liver could be reached. After three months of these analyses, anti-SARS-CoV-2 IgG antibody test was positive (Table 1). Further genetic tests were performed as IgH gene rearrangement could not be detected, and IgHV somatic hypermutation status was uninterpretable. In November, WBC and absolute lymphocyte count began to rise to 20 G/L, but other laboratory parameters remained stable (Figure 1). CLL in this patient was determined in Rai 0 stage and 'watch and wait' strategy was suggested under his follow-up.

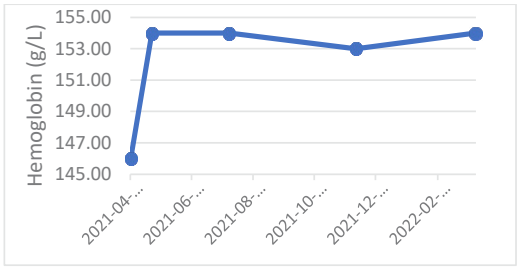
The other patient at the age of 53-years was treated in hospital with bilateral SARS-CoV-2 pneumonia in the end of March in 2021. He did not receive remdesivir or steroid therapy. The suspicion of CLL arose this time due to his haematology parameters (WBC: 123 G/L, lymphocytes: 91%, haemoglobin: 107 g/L), with enlargement of mediastinal and axillar lymph nodes. His peripheric blood smear showed lymphocytes in 93% and several smudge cells. The result of his flow cytometric analysis showed 82% pathologic

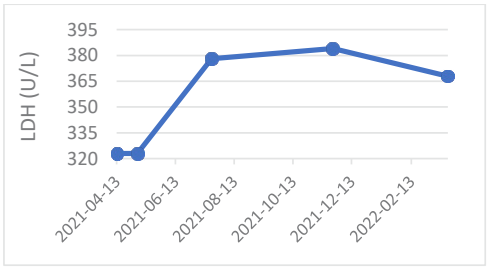
Table 1 Three months after SARS-CoV-2 infection, the test for anti-SARS-CoV-2 antibodies (IgM and IgG) was positive and they were neutralizing in both patients. First patient received Moderna vaccinations without any complications in May and June 2021. The other patient was vaccinated with Pfizer/BioNTech in December 2021 after his steroid therapy was ended.

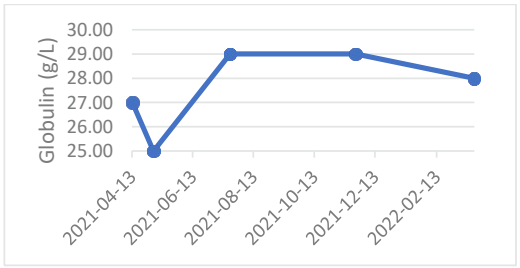
Antibody (AB) test	Results of 1st patient	Evaluation	Results of 2nd patient	Evaluation
SARS-CoV-2 IgM Architect (S/C)	33.56	Positive	6.61	Positive
SARS-CoV-2 IgG (AU/mL)	113	Positive	108	Positive
SARS-CoV-2 IgG Architect (S/C)	5.18	Positive	4.52	Positive
SARS-CoV-2 AB Neutralizing (%)	96.0	Positive	59.4	Positive

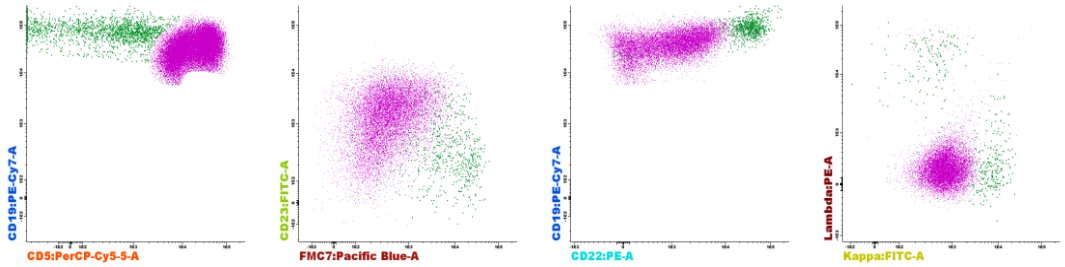
Figure 1 **Kinetics of different routine laboratory parameters during the follow-up and the main results of flow cytometric analysis in patient (No 1) with CLL (Rai 0 stage) depicted with dot plots***





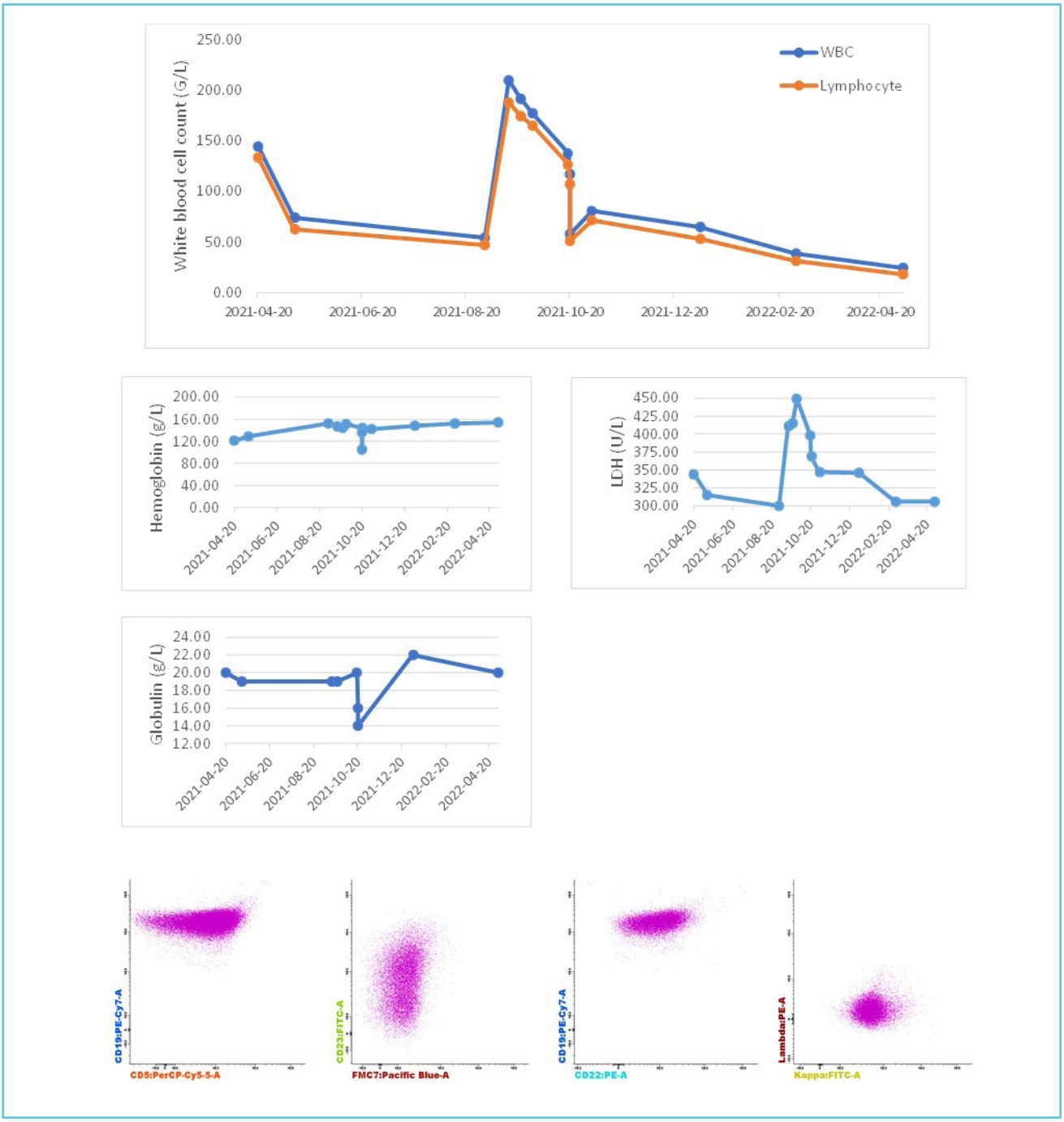






**Laboratory findings in accordance with the clinical conditions did not display a significant difference in the observed period (13 April 2021 – 05 May 2022). Cell counts were moderately elevated (WBC 20.8-28.36 G/L, lymphocyte 11.7-20.31 G/L, globulin 27-29 g/L, IgG 11.5 g/L, IgA 2.7 g/L, IgM 1.2g/L, with no sign of paraproteins). The infection was eliminated and the CLL did not show a progression.*

Figure 2 **Changes in different routine laboratory parameters during the study period and the characterization of pathological B-cell population by flow cytometry in COVID-19 positive patient (No 2) diagnosed with CLL (Rai I stage)***



**The extremely high WBC and lymphocyte counts were halved in the first four months (WBC 144→74 G/L, lymphocyte 133 → 62 G/L). Mild hypogammaglobulinaemia (IgG 6.1 g/L, IgA 2.6 g/L, IgM 1.3 g/L) with the presence of monoclonal IgG (1.2 g/L) was observed. Severe thrombocytopenia occurred at the end of August 2021. This state proved to be a secondary immune thrombocytopenia that responded well to the treatment. In September, WBC was increased to an extremely high level again, therefore urgent leukapheresis and ibrutinib therapy (280 mg/day) was required for a year.*

and CD38- B cells supporting the diagnosis of $CD38^-$ CLL. FISH analysis proved the presence of del(13)(q14) and *ATM* gene deletion. Molecular genetic test detected the monoclonal *IGH* gene rearrangement, while *TP53* gene mutation and IgHV somatic hypermutation status were negative (UM-CLL status). The patient had anti-CMV IgG and anti-EBV IgG titers in association with a mild hypogammaglobulinaemia and slightly elevated β2 microglobulin level (2.77 μg/ml). The immunofixation showed the presence of 1.2 g/L monoclonal IgG κ paraprotein in the gamma fraction. In May, WBC was 144 G/L, lymphocyte count was 133.4 G/L, and haemoglobin was 121 g/L. He did not have palpable lymph nodes, and the spleen was not enlarged either. The test for anti-SARS-CoV-2 antibodies were positive (Table 1). This patient had Rai I stage CLL, and he had no post-COVID-19 symptoms. In August, severe autoimmune thrombocytopenia developed with a platelet count of 35 G/L, which was treated successfully by steroid administration. One month later WBC and lymphocyte count were increased permanently, his disease showed a rapid progression with an extremely short (one-week long) lymphocyte doubling time. These results indicated the initiation of CLL-related treatment. In October, leukapheresis was required and BTKi (ibrutinib) was administrated to the patient with UM-CLL. In a couple of days, his clinical status and laboratory parameters gradually improved (Figure 2).

DISCUSSION

Both COVID-19 patients recovered from bilateral COVID-pneumonia uneventfully. They had a sufficient level of anti-SARS-CoV-2 antibody in the observed period. Their chronic lymphocytic leukaemia was diagnosed during SARS-CoV-2 infection. The stage of CLL and the clinical symptoms did not change in the case of the first patient. The second patient had CLL with a poor prognosis. The progress of their diseases was probably independent from the subsequent infection. The long-term follow-up of patients with CLL that manifested during symptomatic COVID-19 could further enrich our knowledge on such conditions where the immune system is attacked from multiple sides.

Our data potentially suggests a protective role of the complex immune dysfunction caused by CLL; this effect needs to be further investigated in case of severe SARS-CoV-2 infection that might cause an excessive inflammatory response.

CONCLUSION

The observation of these CLL patients with different case history implies that simultaneous manifestation of COVID-19 with a newly emerging CLL does not automatically cause difficulties in laboratory data interpretation neither during the diagnostic procedures nor under the follow-up period.

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