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Unexpected kinetics of anti-SARS-CoV-2 total antibodies in two patients with chronic lymphocytic leukemia

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To the editor

Recently, Baumann *et al.* described the characteristics and outcomes of four patients with chronic lymphocytic leukemia (CLL) diagnosed with symptomatic COVID-19. The course of the disease was mild, and no patient required admission in an intensive care unit. The authors speculate that the CLL-related immunodeficiency might be beneficial in the outcome of the COVID-19 and deserved further investigation. No specific serological testing information was provided [1]. The American Society of Hematology (ASH) has published recommendations on the prevention of COVID-19 in patients with CLL but again not on serological investigations [2].

However, in the context of COVID-19, serological testing is a valuable strategy for the diagnosis and the characterization of the course of the disease, for identifying convalescent plasma donors, for epidemiological studies as well as for lockdown exit programs and COVID-19 vaccine development [3-5]. Our group recently reported the validation of an electrochemiluminescence immunoassay (ECLIA) for anti-SARS-CoV-2 total antibodies determination (Elecsys®, Roche Diagnostics®, Bale, Switzerland) and showed excellent analytical and clinical performances of the assay (95.1% of sensitivity and 100% of specificity), if using an optimized cut-off (i.e. > 0.165 COI) [6]. In our cohort of COVID-19 patients, two presented with CLL. Both patients required hospitalization in intensive care units, received hydroxychloroquine for 5 days, and recovered after 18 and 40 days, respectively. A control group composed of 9 patients nonsuffering from B-cells abnormalities (median age: 79) was included. Among them, 7 required hospitalization, all recovered after a mean time of 13.8 days (min-max = 6-24) and 5 received hydroxychloroquine for 5 days. In this group, total antibodies increased rapidly, were all upper the cut-off by day 14 and remain high overtime; as expected (**Figure 1)**. In comparison, the two patients with CLL showed a different kinetic profile of total antibodies.

The first patient (grey outline) was tested positive for RT-PCR 11 days after symptom onset and had COI values of 0.081, 0.100, and 0.167 at day 14, 22, and 24 since symptom onset, respectively. The latter COI value was upper the optimized cut-off (i.e. 0.165 COI) meaning that by using the cut-off of the manufacturer, this patient would not have been considered positive for SARS-CoV-2 antibodies [6]. To confirm our classification for positivity and the kinetics observed, these samples were analyzed on another platform (iFlash1800 from YHLO biotechnology co., LTD, Shenzhen, China) for specific SARS-CoV-2 IgG determination. The negative determination on day 14 was confirmed on the iFlash1800 (i.e. 0.67 AU/mL; manufacturer cut-off = 10 AU/mL). At days 22 and 24, samples become positive, with increasing antibody titers (22.65 AU/mL and 110.53 AU/mL) confirming the late antibody kinetics observed. This patient is known with CLL (Binet group A). The patient presented hypogammaglobulinemia with serum IgG (6.5 g/L) lower than the age-matched mean (11.5 g/L, reference interval (RI) 7.0-16.0 g/L). The gamma fraction of the serum electrophoresis also presented low values (values during hospitalization 5.5-7.3 g/L; RI 8.0-13.5 g/L). D-dimer (up to 2.2 µg/mL; RI <0.5 µg/mL), CRP (up to 117.8 mg/dL; RI <5

mg/dL), creatinine (up to 1.1 mg/dL; RI 0.3-0.9 mg/dL), LDH (up to 389 U/L; RI <250 U/L), and WBC (up to 11.3x10^9/L; RI 4.0-10 x10^9/L) were increased, and hemoglobin was found to be low (up to 11.0 g/dL; RI 12-16 g/dL). We observed a lymphocyte decrease from 10.7x10^9/L (pre-COVID-19) to 4.9x10^9/L (at admission) (**Figure 1**). All these features were associated with COVID-19 disease [7]. In the 9 control patients, a decrease in lymphocyte count following SARS-CoV-2 infection was also observed. The patient recovered and was discharged 18 days after admission on May 27, 2020. Unfortunately, no more follow-up samples were obtained. This case highlights the importance of performing at least two anti-SARS-CoV-2 determinations in case of COVID-19 symptoms to identify late antibody onset, an observation which strengthens the recommendations made by the Center for Disease Control and Prevention (CDC).

The profile of the second patient (black outline) was also peculiar by presenting a rise of antibody until 18 days after symptom onset followed by a continuous drop of total antibodies until day 55. The patient was tested positive for RT-PCR 11 days after symptom onset and the RT-PCR turned out negative at day 41. At days ≥ 38 and based on the optimized cut-off (i.e. > 0.165 COI), the patient was still considered positive. Four samples of this patient were also analysed on the iFlash1800. Results were consistent with the rise and fall pattern observed on the Elecsys platform with IgG/M values of 4.90/1.10 AU/mL, 10.16/3.69 AU/mL, 21.76/7.00 AU/mL and 14.68/6.25 AU/mL at days 11, 15, 17 and 28, respectively. Tang *et al.* also observed a tendency towards a decrease in antibody signals in 5 patients and speculated about the presence of lower affinity IgM antibody binding [8]. However, IgM, as analyzed on the iFlash1800 platform, remained negative in our study (manufacturer cut-off: 10.00 AU/mL). This second patient is also known with CLL (Binet group A). The patient had a serum IgG (4.9 g/L) lower than the age-matched mean (11.5 g/L, reference interval (RI) 7-16 g/L). The gamma fraction of the serum electrophoresis also presented low values (values during hospitalization 5.0-7.1 g/L; reference interval 8.0-13.5 g/L). As observed for the first patient, D-dimer (up to 3.8 µg/mL), CRP (up to 335 mg/dL), creatinine (up to 1.3 mg/dL), LDH (up to 639 U/L), and WBC (up to 76 x10^9/L) were increased, and Hb was low (up to 7.4 g/dL). We observed a massive lymphocyte increase up to 98.7x10^9/L at day 24 (**Figure 1**). Lymphocyte counts then decreased to 9.6, 8.7, and 11.2x10^9/L at day 38, 40 and 42, respectively. Pre-COVID-19 lymphocyte count (i.e. before December 2019) was 17.6x10^9/L. No more samples were available for this patient to assess if further COI values will pass below the cut-off (i.e. becoming negative). The patient recovered and was discharged on May 16, 2020.

Chronic lymphocytic leukemia is a disease related to a profound immunodeficiency [9] and characterized by hypogammaglobulinemia in up to 85% of CLL patients [10, 11]. These particular antibody kinetics in patients suffering from CLL remains unclear. It could be speculated that the related immunodeficiency is part of the explanation. This immunodeficiency is caused by a combination of increased numbers of regulatory T cells and nurse-like cells, and impaired natural killer cell and T-cell function [9]. However, the

mechanisms by which immunodeficiency develops and evolves in B-cells abnormalities are still not clear [10, 11] but it is known that CLL patients are more susceptible to infection [11, 12].

Several studies aiming at studying the prevalence of COVID-19 in CLL, its clinical characteristics, optimal therapy, and outcome are also needed. These cases also show that interpretation of anti-SARS-CoV-2 antibodies data can be challenging and that further serological investigations are needed in more powered studies as well in patients with hematological abnormalities. Such information will be of particular interest in the development of COVID-19 vaccines since vaccination plan may need to consider the seropositivity status in the general population but also in some specific patient groups.

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Disclosures

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Authorship

FJ, CE, ME, JD, JMD performed the research, FJ, CE, ME, JD, JMD designed the research study, Roche Diagnostics contributed essential reagents or tools, FJ, CE, ME, CG, KL, PG, FJ, JD, and JMD analysed the data, FJ, JD, and JMD wrote the paper.

References

1. Baumann T, Delgado J, Montserrat E. CLL and COVID-19 at the Hospital Clinic of Barcelona: an interim report. Leukemia 2020.
2. https://www.hematology.org/covid-19/covid-19-and-cll assessed on June 4, 2020.
3. Winter AK, Hegde ST. The important role of serology for COVID-19 control. Lancet Infect Dis 2020.
4. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020.
5. Farnsworth CW, Anderson NW. SARS-CoV-2 Serology: Much Hype, Little Data. Clin Chem 2020.
6. Favresse J, Eucher C, Elsen M, Marie TH, Dogne JM, Douxfils J. Clinical performance of the Elecsys electrochemiluminescent immunoassay for the detection of SARS-CoV-2 total antibodies. Clin Chem 2020.
7. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med 2020.
8. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical Performance of the Roche SARS-CoV-2 Serologic Assay. Clin Chem 2020.
9. Riches JC, Gribben JG. Understanding the immunodeficiency in chronic lymphocytic leukemia: potential clinical implications. Hematol Oncol Clin North Am 2013;27:207-35.
10. Dhalla F, Lucas M, Schuh A, Bhole M, Jain R, Patel SY, et al. Antibody deficiency secondary to chronic lymphocytic leukemia: Should patients be treated with prophylactic replacement immunoglobulin? J Clin Immunol 2014;34:277-82.
11. Levinson AI. The Impact of Malignancy on Adaptive Immunity. Stiehm's Immune Deficiencies: Elsevier, 2014:875-887.
12. Kalpadakis C, Pangalis GA, Sachanas S, Vassilakopoulos TP, Kyriakaki S, Korkolopoulou P, et al. New insights into monoclonal B-cell lymphocytosis. Biomed Res Int 2014;2014:258917.

Figure legend

Figure 1: (a) seroconversion in 11 patients with serial samples (one color per patient). Optimized cut-off (i.e. > 0.165) and the LOQ of the assay (i.e. > 0.151) are represented with black and grey dotted lines, respectively. **(b) Lymphocyte kinetics in 11 patients with serial samples (one color per patient) from RT-PCR positivity**. Reference interval is represented with black dotted lines.

Figure 1: