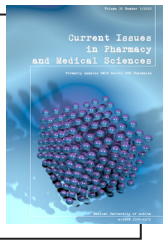
DOI: 10.2478/cipms-2024-0035 Curr. Issues Pharm. Med. Sci., Vol. 37, No. 4, Pages 216-220

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



SARS CoV-2 in tumor tissue in glioblastoma patients – preliminary study

Michal Brzozowski[1], Marcin Kolesnik[2*], Aleksander Michalski[1], Katarzyna Drop[3], Malgorzata Polz-Dacewicz[2]

[1] Neurosurgery Department, 1st Clinical Military Hospital with Outpatient Clinic, Lublin, Poland
[2] Department of Virology with Viral Diagnostics Laboratory, Medical University of Lublin, Poland
[3] Department of Language, Rhetoric and Media Law, Institute of Journalism and Management, Faculty of Social Sciences, Catholic University of Lublin John Paul II, Poland

ARTICLE INFO

Received 10 February 2024
Accepted 20 October 2024

Keywords:
SARS-CoV-2,
glioblastoma,
SARS-CoV-2 antibody,
COVID-19.

ABSTRACT

SARS-CoV-2 infection often causes neurological disorders. Experimental studies on an animal model have shown that SARS-CoV-2 is able to cross the blood-brain barrier. Researchers have also discovered that SARS-CoV-2 can infect glial cells. Gliomas are the most common type of brain tumor. Oncological patients are at high risk of infections, including SARS-CoV-2. Moreover, their weakened immunity causes the level of antibodies after infection or vaccination to be lower than in the healthy population. Therefore, the aim of our study was to evaluate the occurrence of SARS-CoV-2 RNA in tumor tissue collected during surgery. We also tested the level of anti-SARS-CoV-2 antibodies in these patients. The obtained results indicate the tropism of the virus to tumor tissue – glioblastoma. The level of anti-SARS antibodies was higher in patients with SARS-CoV-2 RNA detected in tumour tissue.

INTRODUCTION

SARS-CoV-2, a single-stranded, positive-sense RNA virus, was the cause of the global COVID-19 pandemic. Since then, many variants and subvariants have emerged, requiring continuous improvement of vaccines and treatments [1-3]. Both short- and long-lasting neurological consequences are linked to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Determining the molecular pathways driving neurological sequelae following coronavirus disease 2019 (COVID-19) is challenging due to the diversity of symptoms [4-6].

Gliomas are primary malignant tumors of the central nervous system (CNS) that affect the brain and/or spinal cord. Among the tumors of the CNS, they have the highest incidence and the worst prognosis. *Glioblastoma multiforme* is the most lethal of the clinical glioma subtypes. Due to their heterogeneity, infiltrative nature and varied response to treatment, these tumors still pose the greatest challenge in treatment. the chance of survival for patients with glioblastoma decreases with age. Moreover, patients with *Glioblastoma multiforme* are among the most susceptible to infections [7-11].

* **Corresponding author**
e-mail: marcinkolesnik@umlub.pl

Significant proof for COVID-19 neuroinvasion and neurovirulence was provided by the persistence of SARS-CoV-2, which was found in the brain at autopsy, as well as presence of SARS-CoV-2 RNA in cerebrospinal fluid [12]. As pointed out by other authors, publications examining the relationship between COVID-19 and glioblastoma are needed due to the scarcity of literature data [10,12]. Therefore, the aim of the current study was to assess the presence of SARS-CoV-2 RNA in tumor tissue as well as the level of anti-SARS-CoV-2 antibodies in the serum of the studied patients.

MATERIALS AND METHODS

Study design

The study group included 30 patients, hospitalized at the Neurosurgery Department, 1st Clinical Military Hospital with Outpatient Clinic in Lublin, with diagnosed and histologically confirmed glioblastoma. Other types of cancer were excluded. Based on data from the medical history, it was determined that all patients with glioblastoma in whom SARS CoV-2 was detected in the tumor tissue did not suffer from COVID-19 and were not vaccinated. In contrast, a group of 24 RNA-negative patients were vaccinated, 10 of whom had experienced COVID without

hospitalisation and 14 of whom reported no history of COVID. Each of the immunized individuals were given three doses of the COVID-19 mRNA BNT162b2 vaccine (Pfizer-BioNTech). Serum from these patients was collected approximately 10-12 months from the last vaccination.

Sample collection

The clinical material used in the study was serum and 20 mg of freshly frozen tumor tissues taken from individuals with brain cancer. Venous blood samples (3-5 ml) were drawn via venipuncture in tubes without anticoagulant, in accordance with standard hospital protocol. Following a centrifugation of blood samples at room temperature for 15 minutes at 1500 × g rpm, the serum was separated. Before analysis, tumor tissue and serum were kept at -80°C. Tumor tissue collected during surgery was homogenized and the presence of SARS CoV-2 RNA was determined.

Isolation and detection of SARS-CoV-2 RNA

An Omni TH/Omni International/Kennesewa, GA, USA manual homogenizer was used to cut and homogenize the freshly frozen tumor tissues. The QIAampDNA Mini Kit (Qiagen, Hilden, Germany) was employed to extract DNA in accordance with the manufacturer's instructions. Exploiting β-globin assay, the quality of the acquired DNA (i.e., the presence of PCR inhibitors) was confirmed.

Prior to being tested for SARS-CoV-2, the material was extracted using an automated TANBead MaelstromTM 8 (TANBead Nucleic Acid Extraction Kit). SARS-CoV-2 viral RNA was found by way of application of the genesig® Real-Time PCR Coronavirus COVID-19 (CE IVD) (Primerdesign Ltd., School Lane, Chandler's Ford, Camberley, UK) detection kit. The amplification conditions and reaction system were set up in compliance with the manufacturer's instructions. When the viral gene's cycle threshold (Ct) value was 38 or less, the result was classified as positive; when it was higher than 38, it was labeled negative. With a confidence level of ≥95%, the COVID-19 CE IVD genesig® kit identifies 0.58 copies/μL of SARS-CoV-2 viral RNA.

Detection of SARS-CoV-2 variants

Samples that were positive for the RT-PCR assay were examined for SARS-CoV-2 variants. By means of a commercially available GSD NovaType IV SARS-CoV-2 RT-PCR kit (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany, cat number PCOV6191T), variants of Delta were identified.

Detection of anti-SARS-CoV-2 antibodies

The Microblot-Array COVID-19 IgG assay kit (TestLine Clinical Diagnostics s.r.o., Brno, Czech Republic) was harnessed to evaluate serum samples in order to identify specific anti-SARS-CoV-2 antibodies (NCP, RBD, S2). The results are displayed in U/mL units. The interpretation considers whether a reaction is present or absent against NCP, RBD, or S2, which is at least one antigen. Software and a Microblot-Array reader were employed for reading and interpretation: 185-210 U/mL is the borderline, <185 U/mL is negative, and >210 U/mL is positive.

Statistical Analysis

The following software was exploited in the statistical analysis: Tibco Statistica 13.3 (StatSoft, Kraków, Poland) and GraphPad Prism version 10.1.1. (San Diego, California, USA). The Shapiro-Wilk test was used to assess the normality of the distribution of continuous variables. The Mann-Whitney U test and/or the Kruskal-Wallis test were applied to assess differences in antibody levels between the study groups.

Ethics

The research was approved by the Medical University of Lublin Ethics Committee and is in accordance with the GCP regulations (no. KE-0254/194/10/2022, 6 October 2022). Written informed consent was obtained from each participant.

RESULTS

Table 1 provides specifics about the characteristics of the 30 participants in the study group. The initial purpose was to assess tumor tissue for SARS-CoV-2 RNA, which was found in 6/30 patients (20%). The Delta variant of SARS-CoV-2 was detected in all positive cases.

Table 1. Characteristics of patients with glioblastoma-vaccination and/or COVID-19 survival

	SARS -CoV-2 RNA in tumor tissue		
	Negative N=24		Positive N=6
	COVID-19 vaccinated N=10	Non COVID-19 vaccinated N=14	Non COVID-19 unvaccinated N=6
Female	4	4	2
Male	6	10	4
Age	52-65	56-67	55-66

Next, three groups of patients with glioblastoma were compared, i.e. patients in whom SARS CoV-2 RNA was detected in the tumor tissue, did not suffer from COVID-19 and were not vaccinated (RNA SARS +). The second group included vaccinated patients who suffered from COVID-19 without hospitalization (COVID +), and the third group consisted of vaccinated patients who had not had disease (COVID -). The test we employed identified specific anti-SARS-CoV-2 antibodies (NCP, RBD, S2). Due to the fact that only RBD antibodies were detected in all patients, only they were analyzed in the study.

Overall, in glioblastoma patients, higher levels of anti-SARS-CoV-2 antibodies were found in SARS-CoV-2 RNA-positive individuals than in SARS-CoV-2 RNA-negative individuals (Figure 1). Moreover, patients with glioblastoma multiforme showed higher levels of serum anti-SARS-CoV-2 antibodies compared to those who had COVID-19 and received vaccinations, as well as to those who did not have COVID-19 but received vaccinations (Table 2).

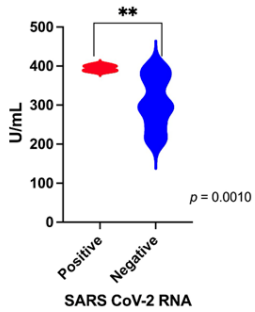


Figure 1. Anti-SARS-CoV-2 antibody levels in the serum of glioblastoma patients with detected and undetected viral RNA in tumor tissue ($p = 0.0010$)

Exact antibody levels are presented in Table 2.

Table 2. The level of anti-SARS antibodies in the serum of patients with glioblastoma in the 3 study groups

	SARS-CoV-2 RNA in tumor tissue			
	Negative N=24		Positive N=6	
	COVID-19 vaccinated	Non COVID-19 vaccinated	Non COVID-19 unvaccinated	
Mean	350.4	245.4	394.7	0.0001*
SD	40.5	33.6	7.1	
Min-max	345.2-400.5	201.4-319.1	385.2-404.6	
p	0.0154*		0.0001*	
		0.0002*		

*statistically significant

DISCUSSION

Glioblastoma is one of the most common and aggressive primary brain tumor types. Patients with this cancer tend to be the most susceptible to cancer due to a number of reasons. Initially, glioblastoma patients are often elderly and have a wide range of age-related conditions. Moreover, their regular steroid medication use exacerbates immunosuppression. Furthermore, the patient's loss of autonomy raises the risk of throm-boembolic events associated with tumors and/or treatment. An increased vulnerability to infections derives from this [10,13-17].

Infection with SARS-CoV-2 may cause neurological symptoms. Neurological symptoms including attention deficit disorder, anosmia, ageusia, ataxia, headaches, dizziness, seizures, disorientation, and in rare cases, even psychiatric illnesses, have been linked to SARS-CoV-2 infection, at least in the case of pre-omicron forms [18-20]. Our study found variants of Delta. This is understandable because at that time the delta variant was dominant in Poland. It has been observed that glioblastoma cells are susceptible to SARS-CoV-2. Research conducted on an animal model has demonstrated that SARS-CoV-2 can pass through the blood-brain barrier [21,22]. The stimulation of the MAPK pathway, NF-κB signaling and p53, which can cause inflammatory and neuro-degenerative alterations, have been suggested as contributing factors to COVID-19 neurotoxicity [23,24].

Partiot *et al*. [6] research have revealed that the accumulation of virions at synapses may be a factor in neurological disorders related to COVID-19. They discovered that SARS-CoV-2 was detectable in nerve cells from both post-mortem brain samples from COVID-19 patients and in vitro cultures of human brain grafts from people who did not have the virus. The susceptibility of nerve cells to SARS-CoV-2 virus infection is an object of research. For example, Patriot *et al*. observed that neural cells were slightly permeable to SARS-CoV-2, doing so by using brain organoids, organotypic culturing of human brain explants from persons without COVID-19, and post-mortem brain tissues from individuals with COVID-19 [6]. There are also isolated reports of GBM's susceptibility to infection with the new SARS-CoV-2 virus, limited to isolated cases [25]. Our research is among the first to investigate the connection between glioblastoma and SARS-CoV. In our study, SARS-CoV-2 RNA in tumor tissue was detected in 3 out of 12 patients with glioblastoma.

A study by Suarez-Meade *et al*. [26] showed that the SARS-CoV-2 entry factors, ACE2, TMPRSS2, and NRP1, are positively expressed in patient-derived glioblastoma tissues and matched primary cell cultures and organoids. It has also been demonstrated that the expression of cathepsins B and L is necessary for SARS-CoV-2 infection in CNS cells, particularly those originating from tumors [27]. Neuropilin 1 (NRP1) is another co-receptor for the virus [28]. Suarez-Meade *et al*. [26] point out that although ACE2 is often expressed at low protein levels in respiratory and olfactory epithelial cells, it can nonetheless make cells susceptible to SARS-CoV2 infection on its own [29]. Because of this, cofactors like TMPRSS2 and NRP1 have been found to be crucial for SARS-CoV-2 infection. Nevertheless, the list of necessary components is expanding, requiring not only input factors, but also genes involved in the virus's life cycle and replication [26].

Epidemiological research indicates that cancer stands alone as a poor predictive factor for COVID-19. Numerous people suffering from glioblastoma are impacted by COVID-19, a pandemic brought on by treatment-related immunosuppression, frequent hospitalizations and a higher frequency of the malignancy among the elderly [12,30]. Many groups of experts have already suggested treating patients with high-grade gliomas. The idea that COVID-19 is biologically vulnerable to patients with glioblastoma has also received some preliminary cross-sectional study support. Finding people who are vulnerable to SARS-CoV-2 and serious illness is essential for maximizing the use of healthcare resources [10,31].

Immunization is crucial for preventing illness, as unvaccinated populations are also seeing the emergence of new variants. Consequently, the chance of developing an infection might increase if humoral immunity declines [32-34]. For patients with compromised immune systems, such as those with cancer, this is essential. As suggested by other researchers, their ability to induce a strong enough immune response during COVID-19 or after receiving a vaccination against SARS-CoV-2 may be affected [35,36]. Individual humoral and cell-mediated immune response patterns in individuals with malignancies like glioblastoma are less well understood, despite the fact that both SARS-CoV-2 infection and vaccination induce antibody- and cell-mediated responses. This holds significant value as variable humoral immune responses within the same individuals can

nonetheless lead to reduced COVID-19 severity or disease prevention [37-40]. Gregory *et al*. point out that it is yet unknown how the COVID-19 mRNA vaccination may affect tumor biology and progression, particularly as the leading mRNA vaccines encode altered spike proteins. In our study, antibody levels in cases where viral RNA was present in tumor tissue were higher than in patients negative for SARS-CoV-2 viral RNA. Our results show higher levels of antibodies in SARS-CoV-2-RNA negative glioblastoma patients who had COVID-19 and were vaccinated than in those who did not have the disease but were also vaccinated. This may indicate that previous infections for this group of patients further increase the defense associated with the humoral response against the SARS-CoV-2 virus. In our study, only anti-RBD antibodies were detected in cases of antibodies detected. As indicated by previous studies, they are of key importance for assessing protection against SARS-CoV-2 infection due to their neutralizing activity [41].

The World Health Organization claimed on May 5, 2023, that the COVID-19 worldwide public health emergency had ended as a result of decreased mortality and less pressure on healthcare systems. However, considering the excess mortality seen during the pandemic, it is expected that the virus will continue to have an influence both directly and indirectly even with lowered surveillance efforts [24].

A limitation of the current study is the small number of patients due to the limited source of tissue from patients who had previously undergone COVID-19, which is also pointed out as a problem in the study of the relationship between SARS-CoV-2 infection and glioblastoma by other authors [12]. Our study is one of the first to examine the relationship between SARS-CoV-2 infection and glioblastoma in Polish patients. Unfortunately, cerebrospinal fluid (CSF) was not collected from patients with brain tumors. In the future, specially planned studies should take this into account. This research should be treated as preliminary; however, it constitutes an encouragement for further, in-depth research in this area.

CONCLUSIONS

The blood-brain barrier (BBB) is a structure that regulates the microenvironment enabling the proper functioning of the nervous system. Many viruses can infect the CNS. Post-mortem studies and in cell lines have shown that SARS CoV-2 also has such abilities.

The presented study indicate the tropism of the SARS CoV-2 to glial cells, even in asymptomatic infections. It appears that the glioblastoma patients we studied were asymptomatically infected with the SARS CoV-2 virus, as evidenced by the presence of antibodies in the serum. Additionally, the level of anti-SARS antibodies was higher in patients with SARS CoV-2 RNA detected in tumor tissue.

Further research is necessary on the mechanism of crossing the BBB, including the search for drugs that prevent SARS-CoV-2 from crossing this barrier, so that rapid treatment can prevent complications from the nervous system.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

ORCID iDs

Marcin Koleśnik https://orcid.org/0000-0002-0400-7416
Aleksander Michalski https://orcid.org/0000-0002-2517-8492
Katarzyna Drop https://orcid.org/0000-0001-6485-3238
Malgorzata Polz-Dacewicz https://orcid.org/0000-0002-3222-184X

REFERENCES

1. Sievers BL, Cheng MTK, Csiba K, Meng B, Gupta RK. SARS-CoV-2 and innate immunity: the good, the bad, and the "goldilocks". *Cell Mol Immunol.* 2024;21(2):171-83.
2. Wang X, Lu L, Jiang S. SARS-CoV-2 evolution from the BA.2.86 to JN.1 variants: unexpected consequences. *Trends in Immunology.* 2024;45(2):81-4.
3. Steiner S, Kratzel A, Barut GT, Lang RM, Aguiar Moreira E, Thomann L, et al. SARS-CoV-2 biology and host interactions. *Nat Rev Microbiol.* 2024;22(4):206-25.
4. Lam ICH, Zhang R, Man KKC, Wong CKH, Chui CSL, Lai FTT, et al. Persistence in risk and effect of COVID-19 vaccination on long-term health consequences after SARS-CoV-2 infection. *Nat Commun.* 2024;15(1):1716.
5. Meinhardt J, Streit S, Dittmayer C, Manitius R, Radbruch H, Heppner FL. The neurobiology of SARS-CoV-2 infection. *Nat Rev Neurosci.* 2024;25(1):30-42.
6. Partiot E, Hirschler A, Colomb S, Lutz W, Claeys T, Delalande F, et al. Brain exposure to SARS-CoV-2 virions perturbs synaptic homeostasis. *Nat Microbiol.* 2024;9(5):1189-206.
7. Xu C, Hou P, Li X, Xiao M, Zhang Z, Li Z, et al. Comprehensive understanding of glioblastoma molecular phenotypes: classification, characteristics, and transition. *Cancer Biol Med.* 2024;21(5):363-81.
8. Richter V, Ernemann U, Bender B. Novel imaging approaches for glioma classification in the Era of the World Health Organization 2021 update: A scoping review. *Cancers.* 2024;16(10):1792.
9. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO classification of tumors of the central nervous system: A summary. *Neuro Oncol.* 2021;23(8):1231-51.
10. Dong J, Wang S, Xie H, Mou Y, Zhu H, Peng Y, et al. COVID-19 hospitalization increases the risk of developing glioblastoma: a bidirectional Mendelian-randomization study. *Front Oncol.* 2023;13:1185466.
11. Diajhete R, Cisse Y, Ba M, Sall A, Ba D, Coumé M. Glioblastoma and COVID-19 in an elderly patient about a case: Review of the literature. *Int J Neurol Res.* 2024;6(1):01-4.
12. Hu H, Wang C, Tao R, Liu B, Peng D, Chen Y, et al. Evidences of neurological injury caused by COVID-19 from glioma tissues and glioma organoids. *CNS Neurosci Ther.* 2024;30(6):e14822.
13. Mohile NA, Blakeley JO, Gatson NTN, Hottinger AF, Lassman AB, Ney DE, et al. Urgent considerations for the neuro-oncologic treatment of patients with gliomas during the COVID-19 pandemic. *Neuro Oncol.* 2020;22(7):912-7.
14. Yalamarty SSK, Filipczak N, Li X, Subhan MA, Parveen F, Ataide JA, et al. Mechanisms of resistance and current treatment options for Glioblastoma Multiforme (GBM). *Cancers.* 2023;15(7):2116.
15. Bikfalvi A, Costa CA da, Avril T, Barnier JV, Bauchet L, Brisson L, et al. Challenges in glioblastoma research: focus on the tumor microenvironment. *Trends Cancer.* 2023;9(1):9-27.
16. Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C, et al. CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016. *Neuro-Oncology.* 2019;21(Supplement_5):v1-100.
17. Gatson NTN, Barnholtz-Sloan J, Drappatz J, Henriksson R, Hottinger AF, Hinoul P, et al. Tumor treating fields for glioblastoma therapy during the COVID-19 pandemic. *Front Oncol.* 2021;11:679702.

18. Bhola S, Trisal J, Thakur V, Kaur P, Kulshrestha S, Bhatia SK, et al. Neurological toll of COVID-19. *Neurol Sci*. 2022;43(4):2171-86.
19. Belopasov VV, Yachou Y, Samoilova EM, Baklaushev VP. The nervous system damage in COVID-19. *J Clin Pract*. 2020;11(2):60-80.
20. Granholm AC. Long-term effects of SARS-CoV-2 in the brain: Clinical consequences and molecular mechanisms. *J Clin Med*. 2023;12(9):3190.
21. Zhang L, Zhou L, Bao L, Liu J, Zhu H, Lv Q, et al. SARS-CoV-2 crosses the blood-brain barrier accompanied with basement membrane disruption without tight junctions alteration. *Signal Transduct Target Ther*. 2021;6(1):337.
22. Smirnova OA, Ivanova ON, Fedyakina IT, Yusubalieva GM, Baklaushev VP, Yanvarev DV, et al. SARS-CoV-2 establishes a productive infection in hepatoma and glioblastoma multiforme cell lines. *Cancers*. 2023;15(3):632.
23. Kyriakopoulos AM, Nigh G, McCullough PA, Seneff S. Mitogen Activated Protein Kinase (MAPK) activation, p53, and autophagy inhibition characterize the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike protein induced neurotoxicity. *Cureus*. 14(12):e32361.
24. Gregory TA, Knight SR, Aaroe AE, Highsmith KN, Janatpour ZC, O'Brien BJ, et al. Accelerated tumor progression after COVID-19 infection in patients with glioblastoma: A retrospective case – control study. *Neurooncol Pract*. 2024;11(4):475-83.
25. Lei J, Liu Y, Xie T, Yao G, Wang G, Diao B, et al. Evidence for residual SARS-CoV-2 in glioblastoma tissue of a convalescent patient. *Neuroreport*. 2021;32(9):771-5.
26. Suarez-Meade P, Watanabe F, Ruiz-Garcia H, Rafferty SB, Moniz-Garcia D, Schiapparelli PV, et al. SARS-CoV2 entry factors are expressed in primary human glioblastoma and recapitulated in cerebral organoid models. *J Neurooncol*. 2023;161(1):67-76.
27. Keyhanian K, Umeton RP, Mohit B, Davoudi V, Hajighasemi F, Ghasemi M. SARS-CoV-2 and nervous system: From pathogenesis to clinical manifestation. *J Neuroimmunol*. 2021;350:577436.
28. Crunfli F, Carregari VC, Veras FP, Silva LS, Nogueira MH, Antunes ASLM, et al. Morphological, cellular, and molecular basis of brain infection in COVID-19 patients. *Proc Natl Acad Sci*. 2022;119(35):e2200960119.
29. Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M, Lindskog C. The protein expression profile of ACE2 in human tissues. *Mol Syst Biol*. 2020;16(7):e9610.
30. Dai M, Liu D, Liu M, Zhou F, Li G, Chen Z, et al. Patients with cancer appear more vulnerable to SARS-CoV-2: A multicenter study during the COVID-19 outbreak. *Cancer Discovery*. 2020;10(6):783-91.
31. Li Z, Wei Y, Zhu G, Wang M, Zhang L. Cancers and COVID-19 risk: A mendelian randomization study. *Cancers* (Basel). 2022;14(9):2086.
32. Jiang Y, Wu Q, Song P, You C. The variation of SARS-CoV-2 and advanced research on current vaccines. *Front Med* (Lausanne). 2021;8:806641.
33. Kannan S, Shaik Syed Ali P, Sheeza A. Omicron (B.1.1.529) – variant of concern –molecular profile and epidemiology: a mini review. *Eur Rev Med Pharmacol Sci*. 2021;25(24):8019-22.
34. Boehm E, Kronig I, Neher RA, Eckerle I, Vetter P, Kaiser L. Novel SARS-CoV-2 variants: the pandemics within the pandemic. *Clin Microbiol Infect*. 2021;27(8):1109-17.
35. Greenberger LM, Saltzman LA, Senefeld JW, Johnson PW, DeGennaro LJ, Nichols GL. Antibody response to SARS-CoV-2 vaccines in patients with hematologic malignancies. *Cancer Cell*. 2021;39(8):1031-3.
36. Thakkar A, Pradhan K, Jindal S, Cui Z, Rockwell B, Shah AP, et al. Patterns of seroconversion for SARS-CoV2-IgG in patients with malignant disease and association with anticancer therapy. *Nat Cancer*. 2021;2(4):392-9.
37. Sahin U, Muik A, Vogler I, Derhovanessian E, Kranz LM, Vormehr M, et al. BNT162b2 vaccine induces neutralizing antibodies and poly-specific T cells in humans. *Nature*. 2021;595(7868):572-7.
38. Ehmsen S, Asmussen A, Jeppesen SS, Nilsson AC, Østerlev S, Vestergaard H, et al. Antibody and T cell immune responses following mRNA COVID-19 vaccination in patients with cancer. *Cancer Cell*. 2021;39(8):1034-6.
39. Bange EM, Han NA, Wileyto P, Kim JY, Gouma S, Robinson J, et al. CD8+ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nat Med*. 2021;27(7):1280-9.
40. Mairhofer M, Kausche L, Kaltenbrunner S, Ghanem R, Stegemann M, Klein K, et al. Humoral and cellular immune responses in SARS-CoV-2 mRNA-vaccinated patients with cancer. *Cancer Cell*. 2021;39(9):1171-2.
41. Lo Sasso B, Giglio RV, Vidali M, Scazzone C, Bivona G, Gambino CM, et al. Evaluation of Anti-SARS-Cov-2 S-RBD IgG Antibodies after COVID-19 mRNA BNT162b2 Vaccine. *Diagnostics*. 2021; 11(7):1135.