Incidence and Immunomic Features of Apyretic COVID-19 in Patients Affected by Solid Tumors: a Prospective Cohort Study.

Francesco Ravera

Università degli Studi di Genova

Roberto Borea

IRCCS Ospedale Policlinico San Martino

Gabriella Cirmena

IRCCS Ospedale Policlinico San Martino

Martina Dameri

Università degli Studi di Genova

Lorenzo Ferrando

IRCCS Ospedale Policlinico San Martino

Maurizio Gallo

Università degli Studi di Genova

Cecilia Casini

Università degli Studi di Genova

Neri Fallani

Università degli Studi di Genova

Valentina Barbero

IRCCS Ospedale Policlinico San Martino

Roberto Murialdo

IRCCS Ospedale Policlinico San Martino

Lucia Tixi

IRCCS Ospedale Policlinico San Martino

Margherita Cappuccio

IRCCS Ospedale Policlinico San Martino

Andrea Cuboni

IRCCS Ospedale Policlinico San Martino

Irene Sivieri

Università degli Studi di Genova

Giuseppe Fomarini

IRCCS Ospedale Policlinico San Martino

Andrea De Maria

Università degli Studi di Genova

Alberto Ballestrero

Università degli Studi di Genova

Gabriele Zoppoli (✉ gabriele.zoppoli@unige.it)

Università degli Studi di Genova <https://orcid.org/0000-0002-1619-1708>

Research Article

Keywords: COVID-19, Asymptomatic infection, Serology, Cancer, Transcriptome sequencing

Posted Date: February 1st, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-864803/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background and rationale: Little is known about SARS-CoV-2 seroconversion in asymptomatic patients affected by solid cancer, and whether it is associated with specific transcriptomics changes in peripheral blood mononuclear cells (PBMC).

Methods: patients affected by solid cancer treated in a top comprehensive cancer center in Italy during the first COVID-19 pandemic wave, and negative for COVID-19-symptoms since the first detection of COVID-19 in Italy, were prospectively evaluated by SARS-CoV-2 serology in the period between April 14th and June 23rd 2020. Follow-up serologies were performed, every 21-28 days, until August 23rd, 2020. All SARS-CoV-2 IgM+ patients underwent confirmatory nasopharyngeal swab (NPS). PBMCs from a subset of SARS-CoV-2 IgM+ patients were collected at baseline, at 2 months, and at 7 months for transcriptome sequencing.

Results: SARS-CoV-2 serology was performed on 446 of the 466 recruited patients. A total of 14 patients (3.14 %) tested positive for at least one SARS-CoV-2 immunoglobulin in the period between April 14th and August 23rd 2020. Viral RNA could not be detected in any of the NPS. PBMC serial transcriptomic analysis showed progressive downregulation of interleukin 6 upregulated signatures, chemokine-mediated signaling and chemokine-chemokine receptor KEGG pathways. B- and T-cell receptor pathways (p-values = 0.0002 and 0.017 respectively) were progressively upregulated.

Conclusions: SARS-CoV-2 seroconversion rate in asymptomatic patients affected by solid cancer is consistent with that of asymptomatic COVID-19 assessed in the general population through NPS at the peak of the first wave. Transcriptomic features over time in IgM+ asymptomatic cases are strongly indicative of previous viral exposure.

Introduction

The global emergency caused by Coronavirus Disease 2019 (COVID-19) brought substantial changes in the approach to infective and non-infective diseases. Massive testing coupled with contact tracing performed on individuals presenting a high risk of contracting the infection or suffering of serious complications from it, such as health care workers or nursing homes residents respectively, is one of the main strategies carried out to limit the spread of the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). Asymptomatic infections represent one of the main challenges for the pursuit of this intent, given the difficulties typically encountered in performing extensive and effective screening tests in order to identify and isolate asymptomatic carriers, preventing the spread of SARS-CoV-2 to susceptible individuals.

The study of the infection dynamics and the immune reaction in asymptomatic COVID-19 patients is of great interest. Asymptomatic carriers identified through real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assay performed on nasopharyngeal swab (NPS) seem to yield a viral load comparable to symptomatic patients [1], with a relevant potential of transmission which must be considered in order to perform effective public health strategies [2]. Moreover, even though most asymptomatic COVID-19 patients develop neutralizing antibodies, an average lower rate of high antibody titer production is reported in comparison to severe infections [3]. Several works suggest that NPS alone presents low sensitivity in detecting asymptomatic infections, being outperformed by the association of NPS and serology [4]. COVID-19 has had a major impact on the management of chronically ill patients, with patients affected by neoplastic disorders being amongst the most negatively impacted categories by the pandemic. In particular, delays or cancellation of surgery and early interruption of palliative treatment are widely reported, with substantial consequences on patients' prognosis and quality of life [5].

The study of the incidence of asymptomatic infection along with the immune response and clinical features of patients affected by solid tumors is particularly relevant in a pandemic setting, given the necessity for these patients to prosecute treatments typically administered in dedicated facilities.

The aim of the present study is to estimate the incidence of asymptomatic infections, together with the prevalence of SARS-CoV-2 immunoglobulins, in patients affected by solid tumors who had access to the Cancer Center of a large tertiary care hospital during the first wave of COVID-19 in North-West Italy. Infection incidence was investigated by serological testing. Infected patients were identified as SARS-CoV-2 IgM and/or IgM and IgG positive and underwent confirmatory NPS. Serology was repeated every 21 days for each patient, in order to detect new seroconversions occurring after the first test and monitor variations in the immunologic setting of detected positive patients.

Moreover, in order to study transcriptomic changes associated with the seroconversion of SARS-CoV-2 immunoglobulins, peripheral blood mononuclear cells (PBMC) signatures were evaluated through sequential RNA sequencing on RNA extracted from blood samples collected at diagnosis and in the follow-up period from two patients testing positive for SARS-CoV-2 serology.

Patients And Methods

Ethics approval and consent to participate

This study was approved by the local Ethics Committee (Comitato Etico Regione Liguria, reference number: 10575). Written informed consents were obtained from all study participants, and all methods were carried out in accordance with relevant guidelines and regulations.

Study population

Patients included in this study were selected upon the following inclusion and exclusion criteria. Inclusion criteria were: informed written consent; diagnosis of solid tumor in any stage, undergoing active treatment; outpatient access for an active, scheduled treatment to U01H and H04 units of the Cancer Center of Ospedale Policlinico San Martino; corporeal temperature < 37 °C when accessing the Cancer Center facility.

Exclusion criteria were: diagnosis of hematologic malignancy in the five years preceding the study; previously confirmed SARS-CoV-2 infection; CT elevation > 37 °C when accessing the Cancer Center facility; COVID-19 suggestive symptoms in the period following the first confirmed case in Italy, i.e. February 21st 2020. Suggestive COVID-19 symptoms included fatigue, dry cough, anorexia, myalgias, dyspnea, sputum production, fever (> 38 °C) and low-grade fever (37-38 °C), anosmia, dysgeusia, headache, sore throat, rhinorrhea, suggestive gastrointestinal symptoms, suggestive dermatologic findings.

As per hospital protocol, outpatients were allowed access to the Cancer Center facilities only after CT measurement < 37° C and the fulfillment of a questionnaire which investigated possible contacts with infected individuals and suggestive COVID-19 symptoms over the previous two weeks. Surgical or FFP2 masks were mandatory for both patients and health care workers in the Cancer Center during the entire period of the study. Type and stage of cancer, along with ongoing therapy and comorbidities were recorded for all enrolled patients.

Study design: Patients' accrual and follow-up

Patients were recruited in the period from April 14th to June 23rd 2020. Extensive serology was performed until August 23rd 2020. Follow-up serologies were performed for each patient at a distance of at least 21 days from each other (Figure 1). Confirmatory NPS was performed at the first positivity for SARS-CoV-2 IgM. In case of SARS-CoV-2 IgM and/or IgG detection after the first serology, patients were investigated for COVID-19 suggestive symptoms over the previous months.

SARS-CoV-2 serology

SARS-CoV-2 IgM and IgG were assessed through chemiluminescence immunoassay (CLIA) kits, supplied by Shenzhen YHLO Biotech Co., Ltd (China). Magnetic beads of these CLIA assays are coated with two antigens of SARS-CoV-2 (nucleocapsid protein or N protein, spike protein or S protein). All serum antibody tests were performed with an iFlash 1800 fully automated CLIA analyzer from Shenzhen YHLO Biotech Co., Ltd (China).

SARS-CoV-2 RNA assessment on NPS

Confirmed infection was defined as RT-PCR positive from a nasal and/or throat swab or BALF according to World Health Organization interim guidance [6]. (WHO. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. 2020)

SARS-CoV-2 RNA detection on NPS was performed using RT-PCR targeted at open reading frame 1ab (ORF 1ab) and nucleocapsid protein genes. RNA was extracted from the samples and then underwent RT-PCR with SARS-CoV-2-specific primers and probes. A cycle threshold (Ct) value of less than 37 was defined as a positive test, and a Ct value of 40 or more was considered as a negative test. An equivocal result, defined as a Ct value between 37 and 40, required confirmation by retesting. If the repeated Ct value was less than 40 and an obvious peak was observed, or if the repeated Ct value was less than 37, the result was deemed positive.

Exploratory aim: Transcriptome sequencing

Blood samples (3 mL each) were collected at 3 different time points from two patients who tested positive for SARS-CoV-2 IgM, remaining asymptomatic for all the course of seven months and repeatedly negative for molecular testing, in order to perform PBMC transcriptome sequencing. Blood was collected in Applied Biosystems Tempus Tubes, prefilled with 6 ml of preserving and virus-inactivating buffer, and stored at - 80 °C. RNA was isolated by using Tempus™ Spin RNA Isolation Kit (Thermo Fisher) following the manufacturer's recommended protocol.

We determined RNA yield and purity based on 260/280 and 260/230 absorbance ratios with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher). RNA integrity number was assessed using a TapeStation 2200 System (Agilent Technologies). Concerning the library preparation, RNA was reverse-transcribed by using the SuperScript® VILO™ cDNA Synthesis kit (Thermo Fisher) on 50 ng of total RNA. Complementary DNA was then amplified by using Ion AmpliSeq™ Transcriptome Human Gene Expression Chef-Ready Kit (Cat. No. A31446), which allows simultaneous gene expression measurement of over 20,000 human Refseq genes. This panel contains 41,604 primers, approximately 150 bases in length, in a single pool. The barcoded libraries were prepared on the Ion Chef™ system (Cat. No. A27759) (ThermoFisher Scientific). Library size was checked using High Sensitivity DNA ScreenTape (Agilent) on a TapeStation 2200 device, while library concentration was evaluated with a Qubit® 2.0 Fluorometer using Qubit™ dsDNA HS Assay Kit. Each barcoded library was diluted into 50 pM concentrations for template preparation and sequenced on an Ion 540™ chip (Cat. No. A27766) on the Ion S5 System, according to the manufacturer's protocol [7].

Gene expression analysis was performed in the R environment. Raw counts were imported using the edgeR package. Scaling of raw library size was used to calculate normalization factors. The normalization method used was upper quantile [8], in which the scale factors are calculated from 75% quantile of the counts for each library. The Gene set enrichment analysis was carried out using the GSVA R package, with the gsva method and Poisson distribution. The C7 (immunologic signatures) gene set was obtained from MSigDB (v.7) [9].

Only pathways displaying an increasing/decreasing monotonic profile across all time points were selected for the transcriptional analysis. Finally, we selected the top 5 enriched/depleted immune pathways with the highest variance across samples. KEGG and Gene Ontology pathway enrichment analyses were performed using DAVID v6.8 [10,11]. Uninominal p-values and not false discovery rates were reported, due to the very low number of transcriptome samples and the exploratory nature of the analysis.

Results

Demographics

A total of 466 patients accessing the U01H and H04 of Ospedale Policlinico San Martino Cancer Center were screened in the period between April 14th and June 23rd 2020 with the inclusion and exclusion criteria outlined above before undergoing the first SARS-CoV-2 serology, which was performed on 446 individuals. Of these 146 were affected by breast cancer, 103 by colorectal cancer, 42 by prostate cancer, 28 by pancreatic cancer, 25 by ovarian cancer, 18 by renal cancer, 17 by GIST, 14 by gastric cancer, 12 by hepatobiliary cancer, 11 by lung cancer, 8 by sarcoma, 5 by endometrial cancer, 4 by cervical cancer, 3 by esophageal cancer, 3 by urothelial cancer, and 7 by other neoplasms. Twenty patients were ruled out (6 because of COVID-19 suggestive symptoms occurred in the period preceding the date of enrollment, 6 because of hematologic malignancy, 1 because of previous NPS-confirmed SARS-CoV-2 detection, 7 because of antineoplastic therapy interruption or prosecution in domiciliary regimen).

Patients' age ranged between 27 and 90 (9 patients under 40, 30 patients between 40 and 50, 92 patients between 50 and 60, 139 between 60 and 70, 135 between 70 and 80, and 40 between 80 and 90)

Of the recruited patients, 397 underwent at least two serologies, 312 underwent at least three serologies, and 122 underwent four serologies. Patients detected as IgM and/or IgG positive were excluded from the total count of the following tests.

SARS-CoV-2 seroprevalence decreases after lockdown measures in oncologic outpatients.

A total of 14 patients (3.14 %) tested positive for at least one SARS-CoV-2 immunoglobulin in the period between April 14th and 23th August 2020. See Tab.1 for serology results in the accrual and follow-up phases.

In the first month of the accrual, from April 14th to May 17th, SARS-CoV-2 serology was performed on 270 patients, of which 48 underwent a second serology in the same period. SARS-CoV-2 immunoglobulins were detected in 7 patients (2.59 % of patients, 2.20 % of serologies), of which 2 positive for IgM (1 weakly positive), 3 for IgG (1 weakly positive), 2 for both IgM and IgG.

In the second month of the accrual, from May 18th to June 23rd, SARS-CoV-2 serology was performed on 390 patients, of which 134 underwent a second serology in the same period. SARS-CoV-2 immunoglobulins were detected in 6 patients (1.54 % of patients, 1.14% of serologies), of which 3 positive for IgM (1 weakly positive) and 3 for IgG (of which 1 weakly positive).

In the third month of the study, from June 24th to July 23rd, SARS-CoV-2 serology was performed on 261 patients, of which 18 underwent a second serology in the same period. SARS-CoV-2 IgGs were detected in 1 patient (0.38 % of patients, 0.36% of serologies).

In the fourth month of the study, from July 24th to August 23rd, SARS-CoV-2 serology was performed on 97 patients, of which 1 underwent a second serology in the same period. No SARS-CoV-2 immunoglobulins were detected in this period.

NPS was performed after serology on all 7 patients detected as positive for SARS-CoV-2 IgM. None of the NPS detected viral RNA.

COVID-19 elicits a multiform serological response in patients affected by solid tumors

Of the 11 patients detected as positive for SARS-CoV-2 immunoglobulins at the first serology two underwent three follow-up serologies, six underwent two follow-up serologies, and two underwent one follow-up serology (Tab.1). Two patients did not undergo follow-up serologies.

UPN8 maintained the positivity for both antibodies across all tests, while UPN5 lost the IgM positivity at the fourth serology. Two patients (UPN4, UPN11) detected as IgG positive at the first serology maintained the IgG positivity at the third test. Four IgM + patients (UPN1, UPN2, UPN10, UPN12) did not undergo IgG seroconversion. Among those patients who resulted negative at the first serology, UPN3 tested weakly positive for SARS-CoV-2 IgM only at the second serology, presumably a false positive result, while UPN13 tested positive for SARS-CoV-2 IgG at the second and third serology.

PBMCs transcriptomic features assessed in asymptomatic patients affected by solid tumor with serological evidence of SARS-Cov2 are consistent with a previous viral infection.

Blood samples (3 ml each) were collected from two SARS-CoV-2 IgM positive patients (UPN 1 and UPN 8) affected by advanced breast cancer (stage IV and IIIB, respectively) at three different time points (Baseline, at 2 months, at 7 months). Both patients underwent a further serology at the moment of the last blood collection. One patient showed IgM seroconversion at baseline, and never developed IgG, albeit maintaining IgM positivity at all time points, whereas the other showed seroconversion to IgG with disappearance of IgM at the last blood draw. Neither tested positive for SARS-CoV-2 NPS at any time point. Both were consistently asymptomatic throughout the collection period.

The evaluation of PBMC transcriptomic signatures over time showed a pattern consistent with immune dysregulation. Of particular interest, we observed in both patients a decreasing monotonic profile in the GSE13485 transcriptomic signature, reported as downregulated in PBMCs of subjects vaccinated with the Yellow Fever vaccine YF-17D in PBMCs at three days from vaccination compared to 21 days (See Figure 2A). While apparently contradictory with our findings, this behavior is consistent with an initial perturbation of the signature at an unspecified time before the initial finding, which may have resulted in high levels for YF-17D at baseline assessment, followed by its decrease, as time from putative viral infection passed by. YF-17D signature includes fibroblast growth factor 23 (FGF23) and prostaglandin I2 synthase (PTGIS), genes upregulated by interleukin-6 (IL6) at the onset of COVID-19 [12]. IL6 has indeed been recently shown to be highly overexpressed in such disease, possibly acting as a therapeutic target to prevent severe symptoms [12]. Likewise, genes pertaining to chemokine-mediated signaling pathways such as CCL20, CXCL12, and CXCR1, were found overexpressed at the first blood draw and gradually decreased over time, as did several genes of the chemokine-chemokine receptor KEGG pathway (p-value = 0.018, see Supplementary Figure 1), such as CXCR6, IL4, LIF, OSM, IL6ST, IL12RB2 – part of the Interleukin 6/12-like signaling. All these transcripts are part of other top decreasing profiles in transcriptomic signatures shared by both patients (pre-B cells development – GSE24814 signature, dendritic cells – GSE29949 signature, CD4 differentiation versus CD8 – GSE8835 signature,

and IL-10 mediated anti-inflammatory pathway in macrophages – GSE9509 signature) (Figure 2A). Overall, the observed behavior is consistent with an initial early upregulation of immune-related transcripts – likely induced by viral infections – followed by their normalization over time during the course of a few months.

On the other hand, the top five enriched transcriptomic pathways (see Figure 2B) included genes which are part of the B-cell receptor pathway (p-value = 0.0002, see Figure 3) and T-cell receptor pathway (p-value = 0.017, see Supplementary Figure 2), as highlighted by KEGG enrichment analysis. Genes included in such pathways were part of the signatures regulated by IL4 pathway versus Interferon pathway (GSE16385 signature), genes regulating myelo-monocytic differentiation and immunoregulatory activity of tumor-induced myeloid suppressor cells (GSE21927 signature), and interferon induced factors in endothelial cells (GSE3920 signature). Taken together, such findings highlight the progressive development of B- and T-cell mediated immune response towards the likely initial infectious stimulus.

Discussion

Neoplastic patients represent a peculiar population in the panorama of COVID-19 pandemic, as they are exposed to both the immunosuppressive and immunostimulating effects of the neoplasm itself and the systemic treatment, with a higher risk of developing a severe SARS-CoV-2 infection compared to healthy individuals [13]. At the same time, however, immunosuppressive agents and ACE-inhibitors, often administered concurrently with the antineoplastic therapy, are proved to interact with the so-called cytokine and bradykinin storm, which are imputed to be at least partially responsible for severe infections, altering the clinical response to the virus and resulting in milder or absent symptoms compared to non-treated patients in particular sub-settings [13]. All these elements make the detection of asymptomatic carriers of SARS-CoV-2 in this population particularly challenging.

The Cancer Center of Ospedale Policlinico San Martino is one of the major Italian facilities for cancer treatment. Over the initial few weeks of COVID-19 outbreak, periodical serologic testing for SARS-CoV-2 specific class IgM and IgG was performed by practicing oncologists, although without a fixed or agreed upon schedule. A preliminary series of 21 cases referring to the Cancer Center, obtained between April 14th and April 24th, was able to identify one apyretic case of a patient affected by a solid tumor with SARS-CoV-2 specific positive serology (both IgM and IgG).

In order to have a realistic estimate of the incidence rate of asymptomatic infections in oncologic patients during the first wave of COVID-19, serological tests were systematically performed on asymptomatic neoplastic patients who had access to two of the clinical units of the Cancer Center, screening for the occurrence of COVID-19 suggestive symptoms in the period following the first diagnosis of COVID-19 in Italy (i.e. February 21st 2020). At the time of this study an extensive NPS-based testing was hardly applicable for screening procedures on a large population, this being mainly reserved to confirm suspected cases of infection or to monitor health care workers exposed to relevant risks of infection on daily bases. NPS was therefore reserved to those patients who resulted positive for SARS-CoV-2 IgM, the latter undergoing seroconversion before IgG and declining after about a month from the infection [14], being typically reported as more suitable for the detection of acute or recent infections.

Of note, none of the NPS performed on SARS-CoV-2 IgM + patients was able to detect viral RNA. This is indeed consistent with the substantial reduction of SARS-CoV-2 viral load typically occurring after seroconversion [15]. The persistence of IgM positivity across the follow-up serologies, without IgG seroconversion and observed in four patients, may reflect a peculiar immune response to epithelium-dwelling nidovirales, that entices an isolated IgM response with absent isotypic switch, in line with data shown by Zhao *et al.* [16]. These observations may also imply an impaired immune reaction to the virus in asymptomatic neoplastic patients undergoing systemic treatment, and are apparently in contrast with data shown by Thakkar *et al.*, who report a 92% IgG seroconversion rate after infection [17]. The latter study, however, mostly involved patients affected by cancer diagnosed with symptomatic COVID-19, all with previous PCR-confirmed detection of SARS-CoV-2.

According to our findings, an isolated IgM positivity cannot be considered as an accurate indicator of acute or recent infection. Nevertheless the rate of SARS-CoV-2 IgM detection (1.48 % of patients) in the first month of accrual in asymptomatic neoplastic patients is consistent with the rate of asymptomatic infections diagnosed after massive testing via NPS in particular settings such as Vo' Euganeo during the peak of the first wave [18]. The end of lockdown measures occurred in Italy on May 18th did not cause an increase of asymptomatic COVID-19, as proved by the progressive decrease of SARS-CoV-2 IgM detection rate (0.77 % over the second month, 0% over the third and fourth month of the study).

Despite the inability of detecting SARS-CoV-2 RNA on any NPS, the serial analysis of PBMC transcriptomic signatures conducted on two asymptomatic patients positive for SARS-CoV-2 IgM revealed, in both IgG positive and negative patients, a trend consistent with an initial immune perturbation that occurred before the first serology. The analysis of the top depleted immunologic signatures, with particular concern to the GSE13485 signature, showed indeed a downregulation of genes typically upregulated by IL6, reported as overexpressed in COVID-19 with implications concerning the severity of the disease [12]. Also, a progressive decrease in the expression of genes belonging to chemokine-mediated signaling pathways, such as CCL20, CXCL12, and CXCR1, together with genes of the chemokine-chemokine receptor KEGG pathway was observed. On the other hand, the analysis of the top enriched immunologic signatures showed an upregulation of genes implied in both B-cell receptor and T-cell receptor pathways. Taken together, these observations point toward an immunostimulating event that occurred before the first test, followed by a subsequent normalization in the expression of genes related to a likely infection-mediated inflammation, in parallel with the development of B cells and T cells mediated immunity toward the infective agent.

The present study is burdened by several limitations. The detection of SARS-CoV-2 IgM in patients anamnestically screened as asymptomatic since the first detection of a COVID-19 case in Italy does not allow to exclude the occurrence of a symptomatic infection before that period, especially if no NPS detected viral RNA at the time of the first positive serology. Confirmatory NPS were performed in some cases after several days, reducing the possibility of detecting viral RNA even in those patients presenting a detectable viral load after seroconversion. Serological tests performed through CLIA present suboptimal sensitivity (around 70%) but high specificity (above 90%) [19]. The IgM weak positivity in UPN3 is reasonably the only false positive result. PBMC

transcriptional analysis was performed on an exploratory cohort of patients. Finally, the approach outlined above is hardly replicable in a global setting where the rate of vaccinated people is increasing.

Conclusions

Even though the effective rate of asymptomatic COVID-19 in patients affected by solid tumors is suboptimally assessed through SARS-CoV-2 serology, as no NPS actually detected viral RNA in any patient, the seroprevalence of SARS-CoV-2 IgM in this population reflects the incidence of asymptomatic COVID-19 in the general population during the first wave of COVID-19 pandemic. The assessment of PBMC transcriptomic profile supports the effective occurrence of a viral infection, even in case of complete absence of symptoms, and may be exploited in future for clinical purposes, such as the development of predictive transcriptional signatures from peripheral blood of *de novo* diagnosed patients [20].

Abbreviations

Coronavirus Disease 2019: COVID-19; Severe acute respiratory syndrome-coronavirus 2: SARS-CoV-2. Reverse-transcriptase polymerase chain reaction: RT-PCR; Nasopharyngeal swab: NPS; Peripheral blood mononuclear cells: PBMC; Chemiluminescence immunoassay: CLIA; cycle threshold: Ct; Fibroblast growth factor 23: FGF23; Prostaglandin I2 synthase: PTGIS; Interleukin-6: IL6.

Declarations

Ethics approval and consent to participate

This study was approved by the local Ethics Committee (Comitato Etico Regione Liguria, reference number: 10575). Written informed consents were obtained from all study participants, and all methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not Applicable.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

GZ received consulting fees from Novartis, travel grants from Novartis, Pfizer, and Roche, and research reagents from Cytiva and ThermoFisher Scientific. The other Authors report no conflict of interest.

Funding

GZ is in part funded by an IG AIRC (ID: 21761), by an IRCCS 5x1000 (Ospedale Policlinico San Martino), and by an intramural Curiosity grant (Università degli Studi di Genova).

Authors' contributions

FR and RB were in chief of data curation and interpretation. FR the manuscript with support from GZ. GC and MD carried out the experiments. LF performed the bioinformatic analyses and prepared the figures. MG supervised patients' recruitment. CC, NF, VB, RM, LT, GF recruited all patients and collected blood samples for serology and transcriptomic analyses. MC, AC gave critical support in samples collection. IS gave support in data curation. AD critically revised the manuscript. AB and GZ conceived the study and were in charge of overall direction and planning.

Acknowledgements

Not Applicable.

References

1. Zhou R, Li F, Chen F, Liu H, Zheng J, Lei C, et al. Viral dynamics in asymptomatic patients with COVID-19. *Int J Infect Dis.* 2020;96: 288–290. doi:10.1016/j.ijid.2020.05.030
2. Huff HV, Singh A. Asymptomatic Transmission During the Coronavirus Disease 2019 Pandemic and Implications for Public Health Strategies. *Clin Infect Dis.* 2020;71: 2752–2756. doi:10.1093/cid/ciaa654
3. Ko J-H, Joo E-J, Park S-J, Baek JY, Kim WD, Jee J, et al. Neutralizing Antibody Production in Asymptomatic and Mild COVID-19 Patients, in Comparison with Pneumonic COVID-19 Patients. *J Clin Med Res.* 2020;9. doi:10.3390/jcm9072268
4. Lei Q, Li Y, Hou H-Y, Wang F, Ouyang Z-Q, Zhang Y, et al. Antibody dynamics to SARS-CoV-2 in asymptomatic COVID-19 infections. *Allergy.* 2021;76: 551–561. doi:10.1111/all.14622
5. Onesti CE, Tagliamento M, Curigliano G, Harbeck N, Bartsch R, Wildiers H, et al. Expected Medium- and Long-Term Impact of the COVID-19 Outbreak in Oncology. *JCO Glob Oncol.* 2021;7: 162–172. doi:10.1200/GO.20.00589

6. WHO. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. 2020
7. Thermo Fisher Scientific (2019) Application note: Simple, scalable transcriptome-level gene expression analysis with next-generation sequencing. Pub. No. COL330760619
8. Bullard JH, Purdom E, Hansen KD, Dudoit S. Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. *BMC Bioinformatics*. 2010;11: 1–13. doi:10.1186/1471-2105-11-94
9. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005;102: 15545–15550. doi:10.1073/pnas.0506580102
10. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4: 44–57. doi:10.1038/nprot.2008.211
11. Jiao X, Sherman BT, Huang DW, Stephens R, Baseler MW, Lane HC, et al. DAVID-WS: a stateful web service to facilitate gene/protein list analysis. *Bioinformatics*. 2012;28: 1805–1806. doi:10.1093/bioinformatics/bts251
12. Rubin EJ, Longo DL, Baden LR. Interleukin-6 Receptor Inhibition in Covid-19 – Cooling the Inflammatory Soup. *N Engl J Med*. 2021;384: 1564–1565. doi:10.1056/NEJMe2103108
13. Bakouny Z, Hawley JE, Choueiri TK, Peters S, Rini BI, Warner JL, et al. COVID-19 and Cancer: Current Challenges and Perspectives. *Cancer Cell*. 2020;38: 629–646. doi:10.1016/j.ccell.2020.09.018
14. Arkhipova-Jenkins I, Helfand M, Armstrong C, Gean E, Anderson J, Paynter RA, et al. Antibody Response After SARS-CoV-2 Infection and Implications for Immunity. *Ann Intern Med*. 2021. doi:10.7326/M20-7547
15. Fu Y, Li Y, Guo E, He L, Liu J, Yang B, et al. Dynamics and Correlation Among Viral Positivity, Seroconversion, and Disease Severity in COVID-19 : A Retrospective Study. *Ann Intern Med*. 2021;174: 453–461. doi:10.7326/M20-3337
16. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. *Clin Infect Dis*. 2020;71: 2027–2034. doi:10.1093/cid/ciaa344
17. Thakkar A, Pradhan K, Jindal S, Cui Z, Rockwell B, Shah AP, et al. Patterns of seroconversion for SARS-CoV-2 IgG in patients with malignant disease and association with anticancer therapy. *Nature Cancer*. 2021;2: 392–399. doi:10.1038/s43018-021-00191-y
18. Lavezzo E, Franchin E, Ciavarella C, Cuomo-Dannenburg G, Barzon L, Del Vecchio C, et al. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. *Nature*. 2020;584: 425–429. doi:10.1038/s41586-020-2488-1
19. Wang H, Ai J, Loeffelholz MJ, Tang Y-W, Zhang W. Meta-analysis of diagnostic performance of serology tests for COVID-19: impact of assay design and post-symptom-onset intervals. *Emerg Microbes Infect*. 2020;9: 2200–2211. doi:10.1080/22221751.2020.1826362
20. Tang B, Shojaei M, Wang Y, Nalos M, Mclean A, Afrasiabi A, et al. Prospective validation study of prognostic biomarkers to predict adverse outcomes in patients with COVID-19: a study protocol. *BMJ Open*. 2021;11: e044497. doi:10.1136/bmjopen-2020-044497

Table

Tab. 1. Serological profile of patients detected as positive for SARS-CoV-2 immunoglobulins in the period between April 14th and August 23rd. Both accrual and follow-up serologies are reported, along with NPS date and result, type and stage of cancer, and administration of steroid-based immunosuppressive therapy.

UPN	Cancer	Stage	Steroids	Date First Serology	IgM	IgG	Date Second Serology	IgM	IgG	Date Third Serology	IgM	IgG	Date Fourth Serology	I
1	Breast	IV	No	22/04/2020	+	-	20/05/2020	+	-	17/06/2020	+	-	16/07/2020	-
2	Pancreatic	IV	Premedication	23/04/2020	W ₊ *	-	18/06/2020	+	-					
3	Breast	IV	No	29/04/2020	-	-	22/05/2020	W ₊ *	-	19/06/2020	-	-		
4	Breast	IV	No	02/05/2020	-	+	27/05/2020	-	+	26/06/2020	-	+		
5	Breast	I	No	13/05/2020	+	+	03/06/2020	+	+	25/06/2020	ND*	+	16/07/2020	-
6	Ovarian	IV	Premedication	14/05/2020	-	+	08/06/2020	-	+					
7	Breast	IV	No	14/05/2020	-	W ₊ *								
8	Breast	IIIB	Premedication	15/05/2020	+	+	05/06/2020	+	+	17/07/2020	+	+		
9	Ovarian	IV	No	22/05/2020	-	W ₊ *	19/06/2020	-	+	17/07/2020	-	ND*		
10	Ovarian	IV	No	22/05/2020	+	-	17/07/2020	+	-	14/08/2020	+	-		
11	Ovarian	IV	No	25/05/2020	-	+	22/06/2020	-	+	20/07/2020	-	+		
12	Renal	IV	No	27/05/2020	+	-	26/06/2020	+	-	27/07/2020	+	-		
13	Lung	IV	No	01/06/2020	-	-	29/06/2020	-	+	27/07/2020	-	+		
14	Breast	IV	No	04/06/2020	-	+								

*W+: weakly positive; ND: undetermined

Figures

Figure 1

Study diagram. Patients were recruited in the period between April 14th and June 23rd. May 18th corresponds to the end of lockdown measures in Italy. Follow-up serologies were performed until August 23rd at a distance of at least 21 days from each other.

Figure 2

PBMCs transcriptomic profile assessed on peripheral blood samples collected from two positive patients for SARS-CoV-2 IgM at baseline (t₀), 2 months (t₁), and 7 months (t₂) from the first serology. The top 5 deplete (panel A) and enriched (panel B) immunologic signatures with the highest variance across samples were selected for the transcriptomic analysis.

Figure 3

KEGG enrichment analysis of genes pertaining to the B-cell receptor signaling pathway in the RNA extracted from PBMCs collected from two positive patients for SARS-CoV-2 IgM. Genes coding for the star-marked elements, including LYN, SHIP, CD81, PI3K, Rac, CaN, Ras, BCL-10, MEK 1/2, IKK α , IP1, and NF κ B, and implied in the B-cell receptor signaling pathway, were found to be increasingly upregulated in these patients over time (p-value = 0.000)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure1.png](#)
- [SupplementaryFigure2.png](#)
- [SupplementaryFigureslegends.docx](#)