




# COVID-19 in people living with HIV: Clinical implications of dynamics of the immune response to SARS-CoV-2

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## Abstract

Little evidence on coronavirus disease 2019 (COVID-19) in people living with HIV (PLWH) is currently available. We reported clinical and viroimmunological data of all HIV-positive patients admitted to our center with COVID-19 from March 1 to May 12, 2020. Overall, five patients were included: all were virologically-suppressed on antiretroviral therapy and CD4+ count was greater than 350 cell/mm<sup>3</sup> in all but two patients. Although all patients had evidence of pneumonia on admission, only one developed respiratory failure. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA was never detected from nasopharyngeal swabs in two patients, whereas in the others, viral clearance occurred within a maximum of 43 days. Immunoglobulin G production was elicited in all patients and neutralizing antibodies in all but one patient. Specific-T-cell response developed in all patients but was stronger in those with the more severe presentations. Similarly, the highest level of proinflammatory cytokines was found in the only patient experiencing respiratory failure. Despite a mild presentation, patients with more pronounced immunosuppression showed high degrees of both cytokines production and immune activation. Our study did not find an increased risk and severity of COVID-19 in PLWH. Adaptative cellular immune response to SARS-CoV-2 appeared to correlate to disease severity. The mild clinical picture showed in advanced HIV patients, despite a significant T-cell activation and inflammatory profile, suggests a potential role of HIV-driven immunological dysregulation in avoiding immune-pathogenetic processes. However, other possible explanations, as a protective role of certain antiretroviral drugs, should be considered. Further larger studies are needed to better clarify the impact of HIV infection on COVID-19.

## KEYWORDS

COVID-19, HIV infection, immune response, SARS-CoV-2

## 1 | INTRODUCTION

Currently, the limited available data on coronavirus disease 2019 (COVID-19) in people living with HIV (PLWH) do not clearly suggest a higher infection rate or a more severe course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection compared to the general population,<sup>1–5</sup> as it might be expected given the immunosuppression and the high prevalence of classic risk factors for severe COVID-19 manifestations (older age and comorbidities) in this population. Particularly, the role of HIV-related immunodepression on the COVID-19 course is still a matter of debate. Indeed, a low CD4 T-cell count has already been associated with an increased risk of mortality in several infections. However, in light of the central role of the host immune response in the pathogenesis of severe manifestations of SARS-CoV-2 infection, it has been postulated that a defective cellular immunity could paradoxically prevent COVID-19 complications.<sup>6</sup> Despite this uncertainty and the growing evidence on immune response to SARS-CoV-2 infection in the general population, data on immunological dynamics in HIV-positive subjects are still lacking.

Here, we describe the experience of an Italian reference HIV/AIDS center in one of the national referral hospitals for COVID-19 with particular insights into immunologic response to SARS-CoV-2 infection.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population and setting

We included all subjects with a diagnosis of HIV infection and a laboratory-confirmed SARS-CoV-2 infection admitted to the National Institute for the Infectious Diseases (INMI) L. Spallanzani, IRCCS between March 1 and May 12, 2020. Demographic and clinical data were collected through a review of medical records. Laboratory and radiologic assessments during hospitalization and after discharge were performed according to treating physician judgment and hospital internal protocols. All patients gave informed consent for using their data for research purposes.

### 2.2 | Definitions

A confirmed case of COVID-19 was defined by a positive real-time reverse-transcription PCR (RT-PCR) assay for SARS-CoV-2 on the nasopharyngeal swab and/or positive serology for SARS-CoV-2 (positivity of immunoglobulin [Ig] G or M or A).

Severe disease was defined as clinical signs of pneumonia plus one of the following: respiratory rate greater than 30 breaths/min, severe respiratory distress, or oxygen saturation less than 90% on room air.<sup>7</sup>

Viral clearance was defined as two consecutive negative RT-PCR for SARS-CoV-2 on a nasopharyngeal swab.

### 2.3 | Virological and immunological assessment

All included patients underwent follow-up nasopharyngeal swabs to assess the viral clearance. RT-PCR targeting the E and RNA-dependent RNA polymerase viral genes was used to assess the presence of SARS-CoV-2 RNA.<sup>8</sup>

All patients included in the study underwent a complete immunological assessment. Humoral immunity (SARS-CoV-2-specific Igs) was evaluated by quantifying IgA, IgG, and IgM by immunofluorescence, and by dosing the neutralizing antibodies (nAb) in a biosafety level-3 facility.

Detection of specific Ig for SARS-CoV-2 was performed using indirect immunofluorescence assay on slides prepared in-house with Vero E6 cells infected with SARS-CoV-2 isolate, as described elsewhere.<sup>9</sup> Each sample was tested using 1:20 screening dilution; positively screened samples were titrated by limiting dilution. FITC-conjugated rabbit anti-human IgM, IgA, and IgG antibodies (Euroimmun) were used as secondary antibody and Evans Blue as cell counterstain.

For the microneutralization test, patients' sera were heat-inactivated, diluted 1:10 in serum-free medium, and titrated in duplicate in twofold dilutions. Equal volumes of 100 50% tissue culture infectious dose/well SARS-CoV-2 (2019 novel coronavirus/INMI) and serum dilutions were mixed and incubated at 37°C for 30 min. Virus-serum mixtures were added to subconfluent Vero E6 cells and incubated at 37°C and 5% CO<sub>2</sub> for 2 days. nAb titers were calculated as the last serum dilution not presenting cytopathic effect.

Innate immunity was evaluated in three of five patients (Patients 1, 2, and 4) for whom follow-up whole blood samples were available. The gating strategy was used to identify the natural killer (NK) cell population. NK cells were gated as CD45+CD3–CD56+ cells. In addition, the expression of activatory (NKG2C) and inhibitory (NKG2A) receptors was longitudinally quantified by flow cytometry at three different time points (approximately at 1, 2, and 3 weeks from symptom onset or hospital admission in Patient 1 and Patient 4, respectively, and at 7, 11, and 14 days from symptoms onset in Patient 2). Moreover, the content of granzyme and perforin in NK cells was quantified by the flow cytometry.

Specific cellular immune response was evaluated by quantifying SARS-CoV-2 specific T-cells (specific for spike and nucleocapsid proteins) by Elispot assay. As a positive control (the evaluation of the immunocompetence) peripheral blood mononuclear cells (PBMCs) were stimulated with phytohemagglutinin (data not shown) and with cytomegalovirus-specific antigen. As a negative control (spontaneous interferon- $\gamma$  release), PBMCs were maintained in a culture medium without specific stimulation. The inflammatory profile in plasma samples was quantified by enzyme-linked immunosorbent assay for interleukin (IL)-6, IL-8, and IL-1 $\beta$ .

## 3 | RESULTS

Over the observation period, 604 subjects with confirmed SARS-CoV-2 infection were admitted to our center. Among them, five HIV-positive patients were found (crude prevalence 0.8%).

TABLE 1 Baseline characteristics and outcomes

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Demographic/medical history					
Age	61	46	31	55	55
Gender	Male	Male	Transgender Woman	Male	Male
Nationality	Argentina	Italy	Colombia	Ethiopia	Italy
Relevant comorbidities <sup>a</sup>	Asthma	None	CNS cryptococcosis	Cardiopathy, chronic cerebral vasculopathy, chronic HBV, <i>Mycobacterium chimeria</i> meningitis	None
HIV status					
Year of HIV diagnosis	NA	1998	2017	2019	2000
Year of ART start	NA	2009	2019	2019	2000
Months of virological suppression	NA	2 years	5 months	1 month	>10 years
Previous virological failure	Yes	Yes	No	No	No
Previous/current AIDS event	No	No	Yes	Yes	No
CD4 cell nadir, cell/mm <sup>3</sup>	NA	350	<200	59	156
BL <sup>b</sup> ART regimen	DTG + DRV/r	DTG + DRV/c	TDF/FTC + EFV	TDF/FTC + DTG	TAF/FTC/RPV
BL <sup>b</sup> HIV RNA, copies/ml	<30	<30	<30	<30	<30
BL <sup>b</sup> CD4+ cell count, cell/mm <sup>3</sup> (%)	438 (43%)	1127 (37%)	219 (10%)	127 (18%)	352 (36%)
CD4/CD8 ratio at baseline <sup>b</sup>	1.2	0.7	0.1	0.3	1.2
Clinical presentation at BL <sup>b</sup>					
Days from symptoms start to admission	1	5	Asymptomatic	Asymptomatic	7
Symptoms	Dry cough, shortness of breath, mild fever	Dry cough, fever, myalgia	Asymptomatic	Asymptomatic	Dry cough, mild fever
SO <sub>2</sub> in ambient air—PaO <sub>2</sub> /FIO <sub>2</sub> ratio	95%—376	98%—NA	97%—376	98%—414	97%—500
Radiological imaging	Bilateral ground glass	Bilateral ground glass	Bilateral ground glass	Focal lung consolidation	Unilateral ground glass
Inflammatory index					
lymphocytes, cell x10 <sup>6</sup> /L	1252	2514	2306	644	1443
LDH, U/L	207	227	128	154	167
D-dimer, ng/ml	413	243	752	292	163
Fibrinogen, mg/dl	570	556	383	666	388
Ferritin, ng/ml	241	NA	NA	265	245
NP swab for SARS-Cov-2	Negative	Positive	Negative	Positive	Positive

TABLE 1 (Continued)

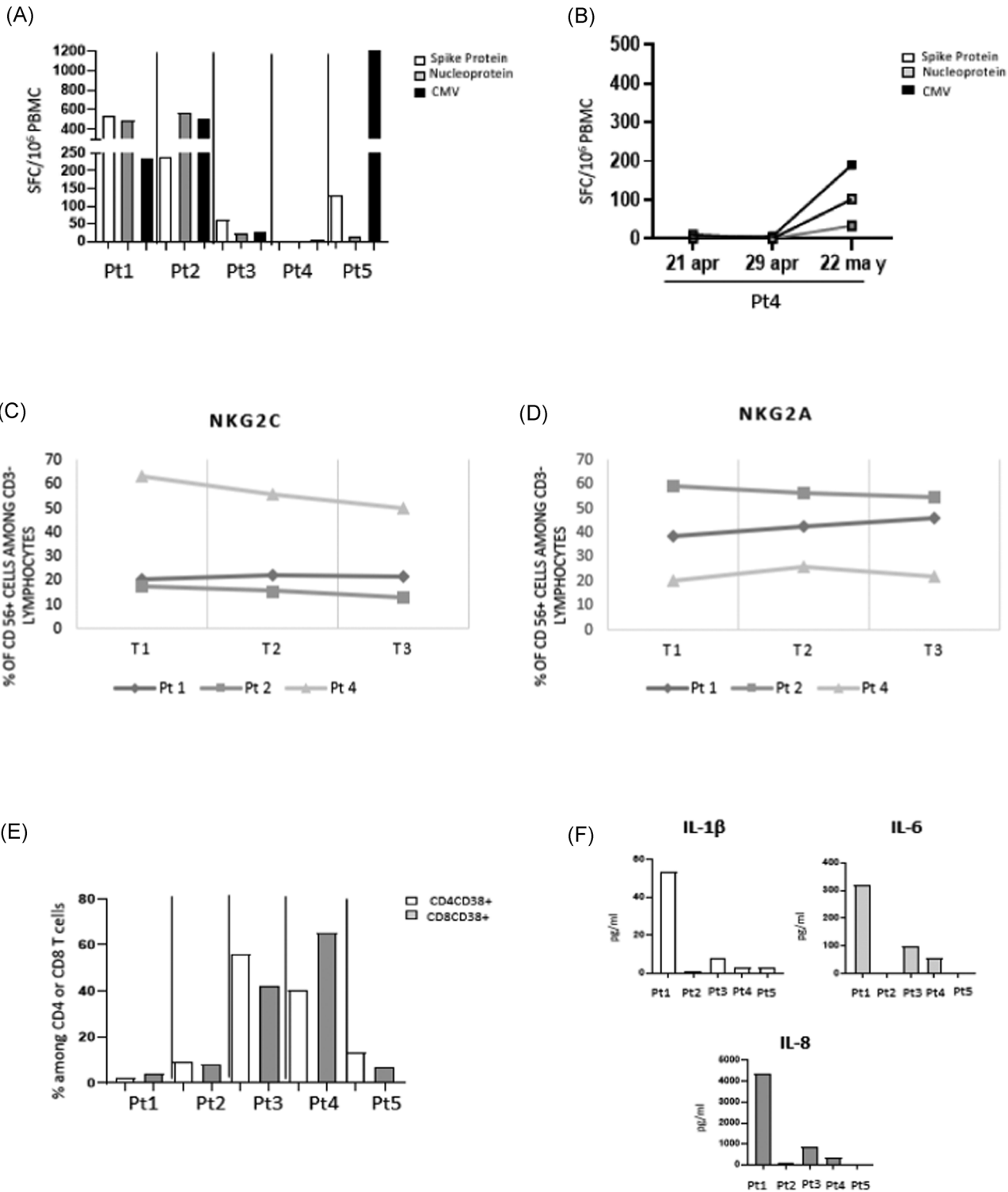
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Treatment during hospitalization					
ART change	None	None	None	None	TDF/FTC+ LPV/r (14 days)
Other antiviral therapy (days of therapy)	HCQ (10 days)	HCQ (10 days)	HCQ (10 days)	HCQ (10 days)	None
Immunomodulatory agents	Tocilizumab 8 mg/kg for 2 doses + methylprednisolone 20 mg/die for 6 days	None	None	None	None
Clinical outcomes					
Lowest PaO <sub>2</sub> /FIO <sub>2</sub>	225	NA, lowest SO <sub>2</sub> 91% in AA	376	433	457
Need for oxygen supplement	Yes (VM 40%)	Yes (VM 31%)	No	No	No
Radiologic evolution	Improvement after 10 days	Improvement after 40 days	No change after 20 days	No change after 16 days	Resolution after 60 days
Length of hospital stay	17 days	12 days	12 days	19 days	6 days
Clearance of NP swab (time for clearance from symptoms onset or hospital admission <sup>c</sup> )	NA	Yes (43 days)	NA	Yes (6 days <sup>c</sup> )	Yes (29 days)
Serological response					
Serology for SARS-CoV-2 (days from symptoms onset or hospital admission <sup>c</sup> )	IgG, IgM, IgA pos (2 days)	IgG, IgA pos; IgM neg (43 days)	IgG pos; IgM, IgA neg (4 days <sup>c</sup> )	IgG, IgA pos; IgM neg (1 day <sup>c</sup> )	IgG, IgA pos, IgM borderline (59 days)
Neutralizing antibodies (titer)	Yes (1:80)	Yes (1:40)	Yes (1:40)	No (< 1:10)	Yes (1:40)

Abbreviations: AA, ambient air; ART, antiretroviral therapy; BL, baseline; CNS, central nervous system; DRV/c, darunavir/cobicistat; DRV/r, darunavir/ritonavir; DTG, dolutegravir; EFV, efavirenz; HBV, hepatitis B virus; HCQ, hydroxychloroquine; Ig, immunoglobulin; LPV/r, lopinavir/ritonavir; NA, not available; neg, negative; NP, nasopharyngeal; pos, positive; RPV, rilpivirine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SO<sub>2</sub>, oxygen saturation; TDF/FTC, tenofovir disoproxil fumarate/emtricitabine; VM, venturi mask.

<sup>a</sup>Other from HIV.

<sup>b</sup>Baseline: At hospital admission.

<sup>c</sup>For asymptomatic patients.



**FIGURE 1** Specific T-cell response to SARS-CoV-2 antigens in total population (A) and in Patient 4 (B). Percentage of natural killer (NK) cells (CD56+) expressing activatory (NKG2C) (C) or inhibitory (NKG2A) (D) receptors in three of five patients (Patients 1, 2, and 4). Percentage of activated TCD4 and CD8 cell (E) and levels of proinflammatory cytokines (F) in the total population. Apr, April; CMV, cytomegalovirus; IL-6, interleukin-6; PBMC, peripheral blood mononuclear cells; pt, patient; SFC, spot forming cells

### 3.1 | Clinical characteristics and outcomes

The main demographic and clinical characteristics and outcomes are summarized in Table 1. Briefly, four patients were male with an age

range between 46 and 61 years and one was a 31-year-old transgender woman. At the time of COVID-19 diagnosis, all five patients were aware of HIV diagnosis and virologically suppressed on antiretroviral therapy (ART): two patients on a dolutegravir plus

boosted darunavir (DRV/b) dual therapy and three patients on a tenofovir-based standard triple ART with a nonnucleoside reverse transcriptase inhibitor or an integrase inhibitor as anchor drug. Two patients had a baseline CD4 count below 350 cell/mm<sup>3</sup> and reported a history of AIDS-defining illness for which they were on treatment at the time of hospitalization. Non-HIV-related comorbidities were observed in two patients.

At hospital admission, COVID-19 presentation was mild-moderate in three patients, whereas the other two patients were asymptomatic. Of note, this latter had the most severe HIV-related immunodeficiency. At the time of admission to the hospital, none of the patients showed either clinical signs of severity or markers of hyperinflammation. A high-resolution computed tomography (HRCT) scan showed lung involvement in all patients with an interstitial pattern in four of them and a focal lung consolidation in the other one. Concerning antiviral therapy, four patients received hydroxychloroquine for 10 days without changing baseline ART whereas one patient underwent a transitional change of initial ART to a boosted protease inhibitor (PI/b)-based regimen for 14 days. During hospitalization, only one patient (Patient 1) developed acute respiratory failure that successfully responded to immunomodulatory therapy with steroids and intravenous tocilizumab, an IL-6 inhibitor. Only two patients (Patients 1 and 2) temporarily required oxygen supplement with a Venturi mask and both weaned from oxygen before the discharge. Three patients showed improvement or resolution of the lung involvement at the follow-up HRCT scan, whereas in the other two (Patients 3 and 4), no significant changes were observed. All patients were discharged within 20 days. Two of them were discharged with positive RT-PCR for SARS-CoV-2 on the nasopharyngeal swab and viral clearance was assessed by the territorial health service.

### 3.2 | Virological and immunological response

In two of five patients (Patients 1 and 4), SARS-CoV-2 RNA was never detected in nasopharyngeal swabs and COVID-19 diagnosis was made by means of positive serology. In the other three patients, viral clearance occurred within a maximum time of 43 days from illness onset or hospital admission.

Concerning immune response, SARS-CoV-2 infection elicited IgG response in all patients, whereas IgA was positive in four patients and IgM just in one patient. The range between symptoms onset (or hospital admission for asymptomatic patients) and serology collection varied widely among patients (Table 1). All but one patient (Patient 4) displayed nAb production. Patients 1 and 4 underwent a second serology determination after 7 and 32 days, respectively, which showed a fourfold increase of IgG and IgA and a twofold increase of IgM titers in the former and no change in the Ig titers in the latter.

A greater T-cell response against SARS-CoV-2 antigens was observed in patients with a more severe COVID-19 presentation and/or with a more prolonged viral shedding (Patients 1 and 2) whereas was

less pronounced in the other ones (Figure 1A). Particularly, Patient 4, who was the most severely immunocompromised subject, lacked both specific T-cell response and nAb production. Of note, this patient showed a significant increase in the cellular immune response after 32 days from admission (Figure 1B) whereas the production of nAb persistently lacked.

With regard to the innate immune response, as shown in Figure 1C,D, NK cells from Patient 4 expressed a higher level of activatory receptors (NKG2C) than the other two patients. In addition, in the same patient, a parallel lower expression of inhibitory receptors (NKG2A) compared to the others was observed, suggesting the presence of highly engaged/activated NK cells. These cells were activated (90% expressed CD38) and enriched in perforin/granzyme cytotoxic molecules (76.4% were granzyme positive and perforin positive).

As expected, the proportion of CD4 and CD8 T lymphocytes expressing activation markers (CD38) was high in patients with advanced HIV-related immunosuppression (Patients 3 and 4). On the contrary, a low degree of immune activation was observed in the subjects with better control of HIV infection regardless of COVID-19 severity (Figure 1E). Finally, the highest levels of proinflammatory cytokines were observed in Patient 1 showing the most severe COVID-19 presentation. Significant production of IL-6 and IL-8 was also observed in the two patients with less controlled HIV-infection (Patients 3 and 4), although to a lesser extent than Patient 1 (Figure 1F).

## 4 | DISCUSSION

This study describes clinical characteristics and outcomes of HIV-positive patients admitted with a COVID-19 diagnosis to our hospital, a reference national HIV/AIDS center, regularly following approximately 7000 outpatients, and one of the Italian reference hospitals for the COVID-19 pandemic.

Our findings did not seem to support the hypothesis of increased risk and greater severity of COVID-19 in PLWH. Indeed, consistently with previous data,<sup>1,5,10</sup> in this study, HIV-positive subjects accounted for less than 1% of patients admitted to our hospital with a diagnosis of SARS-CoV-2-infection. Unfortunately, due to the lack of updated information about clinical and serological data of our HIV-positive outpatient cohort, we could not draw any conclusion about the COVID-19 incidence rate in our center. However, recent results from other cohorts reported very low rates of SARS-CoV-2 infection in PLWH, approximately 1% or less.<sup>2,4,11</sup>

Concerning clinical severity, in our series, although all patients had evidence of lung involvement on admission with radiological patterns similar to those described for the HIV-negative population,<sup>12</sup> none of them had a critical course of COVID-19 and all were discharged from the hospital within 20 days. The only subject experiencing a severe course of infection with acute respiratory failure successfully responded to immunotherapy. These findings are in line with recent reports showing that HIV infection did not seem to impact on clinical severity and mortality of COVID-19.<sup>1-5,10</sup>

Despite these optimistic findings, the lack of higher risk for severe COVID-19 in PLWH has been recently questioned by several studies reporting worse outcomes in HIV-positive subjects compared to the general population, and particularly in frailer subgroups as elderly,<sup>4,13,14</sup> patients with underlying comorbidities,<sup>14,15</sup> more severely immunosuppressed patients,<sup>13,14</sup> and black people.<sup>16</sup> Indeed, older age and comorbidities, already identified as risk factors for severe disease in the general population, have been clearly related to a greater risk of both acquiring and having a severe clinical course of COVID-19 also in PLWH.<sup>4,13-15</sup> Notably, several studies have reported a significant correlation between age and disease severity,<sup>14</sup> with a greater risk of morbidity and mortality for COVID-19 in the older age strata of the HIV-positive population compared to the general population.<sup>4,13</sup> The premature aging and the premature burden of age-related diseases due to the chronic immune activation, which characterizes HIV-positive subjects, might probably explain these results.<sup>17</sup> Of note, in this study, the only subject experiencing a severe course of COVID-19 was the oldest among our patients.

Conversely, the association between the classic HIV-related parameters of immunosuppression and the severity of disease appeared to be less defined. Indeed, in two large cohort studies on HIV-positive patients with SARS-CoV-2 infection, although subjects with a more severe course of infection had lower CD4 cell count, a significant correlation between immunological status and adverse outcomes was not found.<sup>12,13</sup> In addition, in a recent case series describing four SARS-CoV-2/HIV-coinfected patients, despite a worse evolution of COVID-19 in patients with advanced HIV stage, a direct association between immunosuppression and disease course could not be assumed.<sup>18</sup> In fact, the overlapping of opportunistic infections and the lack of ART and virologic control in severely immunocompromised patients might partially account for the worse evolution.

In this study, consistently to how previously reported in both SARS-CoV-2 infection<sup>1-5</sup> and previous coronavirus diseases,<sup>19,20</sup> we did not find an association between the severity of HIV-related immunodeficiency and a worse clinical course of COVID-19. Indeed, Patient 1, the only experiencing a severe course of SARS-CoV-2 infection, was a Caucasian man with an optimal immunological profile (baseline CD4 count: 438 cell/mm<sup>3</sup>). Conversely, the two asymptomatic patients (Patients 3 and 4) were the more severely immunocompromised. Of note, one of them was black and had several comorbidities.

As already suggested,<sup>2,6</sup> a possible explanation is that a highly defective cellular immunity in HIV-positive patients may reduce the immune-mediated pathogenesis of COVID-19. Indeed, in seronegative subjects, the severity of COVID-19 was associated with a huge inflammatory response, to a stronger T-cell activation and to a dramatic lymphopenia.<sup>21-23</sup> Accordingly, Patient 1 showed the most significant proinflammatory response. Conversely, the significant immune activation and inflammatory profile observed in the most severely immunocompromised patients did not seem to worsen the course of COVID-19. These data confirm the role of inflammation in COVID-19 severity in PLWH and suggest that the low and impaired immune reactivity in patients with advanced HIV disease may possibly play a role in reducing the COVID-19 evolution towards severity. However, further well-design studies with larger sample sizes

are mandatory to confirm this hypothesis and to identify immunological players of COVID-19 severity.

The degree of immunosuppression is not the only potential explanation for the different courses of SARS-CoV-2 infection in our cohort. In this regard, a possible impact of the different antiretroviral regimens should be considered. Indeed, given the effect of some antiretroviral drugs on the SARS-CoV-2 life cycle, a potential role of chronic ART in preventing incidence and complications of COVID-19 has been postulated.<sup>24</sup> Particularly the PI/b lopinavir/ritonavir and DRV/b and the nucleoside reverse transcriptase inhibitor tenofovir have been proposed as potentially protective drugs for SARS-CoV-2, based on contradictory results of randomized clinical trials and observational studies for lopinavir/ritonavir and in vitro activity or molecular docking studies for the others.<sup>25</sup> It is worth noting that in our cohort, patients on tenofovir-based regimens (Patients 3, 4, and 5) seemed to have milder clinical manifestations compared to patients on DRV/b-based dual ART who experienced severe disease course (Patient 1) or prolonged viral shedding (Patient 2). Our finding is consistent with a recent large cohort study reporting a lower risk for both acquiring and having complications of SARS-CoV-2 infection in patients assuming tenofovir disoproxil fumarate (TDF)/emtricitabine<sup>4</sup> but contrasts with most reports which did not find any significant association between the use of either ART or specific antiretroviral drugs and clinical outcomes. The results of an ongoing trial on TDF as pre-exposure prophylaxis<sup>26</sup> and of larger studies on HIV-positive cohorts may better clarify this aspect.

To better understand the potential role of HIV-driven immunological dysregulation on the SARS-CoV-2 specific response, we evaluated both humoral and cellular (innate and adaptive) arms of the immune system. Patients with moderate/severe disease showed a higher T-cell response to the viral antigens, suggesting a stronger and prolonged host/pathogen interaction able to boost SARS-CoV-specific T-cell clones. In contrast, the cellular immune response was low/absent in asymptomatic patients suggesting a rapid clearance of virus mainly induced by innate immune cells. This hypothesis is supported by the finding of a significantly higher number of activated NK cells in Patient 4 compared to patients with more severe disease evolution (Patient 1) or longer shedding (Patient 2). The relevance of innate immune response in the SARS-CoV-2 infection course has been pointed out by several studies suggesting a correlation between NK cell inhibitory phenotype and worse course of COVID-19. Particularly, Zheng et al.<sup>23</sup> reported, in patients with severe COVID-19, a hyperexpression of inhibitory receptors (NKG2A) compared to mild cases, potentially responsible for the functional exhaustion of these cytotoxic immune cells and disease progression.<sup>23</sup> Another recent report showed an association between the development of critical illness and the presence of NK2GC receptor deletion/genetic variants of its ligand (human leukocyte antigen) characterized by lower surface expression.<sup>27</sup> In addition, on the basis of these findings, some authors have proposed the use of anti-NKG2A monoclonal antibody monalizumab as a weapon for severe COVID-19 cases.<sup>28</sup> According to these reports, in our study, the activatory profile of NK cells, with a high level of NKG2C and low level of NKG2D, expressed by Patient 4 might have contributed to the rapid viral clearance and to the lack of disease progression.

Concerning humoral immunity, all patients showed a good specific antibody response, and, interestingly, in Patient 4, the low and delayed T-cell response was associated with a lack of nAb production. The significance of low T-cell response and the absence of nAb on long-lasting protection represents a critical issue to be addressed in both HIV-positive and negative subjects.

Finally, regarding viral kinetic, a recent study reported in PLWH a median time to viral clearance of 18 days with a higher risk of prolonged shedding in individuals with a more severe course, similarly to how described in the general population,<sup>29</sup> and in subjects with a low CD4 nadir.<sup>13</sup> In our cohort, viral shedding was not evaluable for three patients (two in whom viral RNA was never detected on nasopharyngeal swabs and one, asymptomatic, in whom time of illness onset was not defined). The other two patients had prolonged viral shedding (more than 20 days) which was not associated with a severe clinical course or a history of a serious immunodeficiency.

Our study has several limitations. First, the exiguous sample size that, making it difficult to distinguish real effects from random variations, prevents us to draw any definitive conclusions. Second, the observational and uncontrolled nature of the study which does not allow us to completely rule out residual or unmeasured confounders. Furthermore, the retrospective collection of medical records may have introduced bias due to the potential inaccurate reporting of data and missing data. Finally, the limited duration of follow-up prevented us from follow-up the evolution of immune response. However, this study has also the strength to provide, for the first time to the best of our knowledge, insights into the dynamics of the immune response to SARS-CoV-2 infection in PLWH and the potential clinical significance of these pathogenic features.

In conclusion, our findings seem to confirm that HIV-positive patients are not at increased risk of both acquiring and having a more severe clinical course of COVID-19. Advanced HIV-positive patients, despite a significant immune-activation and a moderate inflammatory profile, showed a mild/moderate clinical presentation suggesting that the lower and impaired immune reactivity in chronic HIV infection could contribute to avoiding immune–pathogenetic processes. However, other possible explanations, as a potential protective role of certain antiretroviral regimens, should be considered. Concerning immunological dynamics, the specific cellular and humoral response developed in all patients but was stronger in those with a more severe clinical course.

Given the abovementioned limitations of this study and the contradictory available evidence, often based on small and non-controlled case series,<sup>30</sup> the impact of HIV infection on SARS-CoV-2 clinical presentation, outcomes, and immune response deserves to be investigated in larger cohort studies and metaanalysis to draw more solid and realistic conclusions.

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#### CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

#### AUTHOR CONTRIBUTIONS

Annalisa Mondì designed the study, collected the data, wrote the first draft of the manuscript, and performed the appropriate literature. Andrea Antinori conceived and supervised the study and finalized the draft of the manuscript. Chiara Agrati and Concetta Castilletti contributed to study design, data interpretation, and article drafting. Concetta Castilletti, Francesca Colavita, Giulia Matusali, and Maria Rosaria Capobianchi provided for virological assay and revised the manuscript. Chiara Agrati, Eleonora Cimini, Rita Casetti, and Markus Maeurer provided for immunological assay and revised the manuscript. Stefania Cicalini, Carmela Pinnetti, Alessandra Vergori, Valentina Mazzotta, Roberta Gagliardini, and Federico De Zottis followed the patients during the diagnostic and therapeutic path and discussed the results of the study. Enrico Girardi, Vincenzo Puro, Vincenzo Schininà, Giuseppe Ippolito, and Francesco Vaia revised the manuscript. All authors give the final approval of the version to be submitted.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author.

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