

## Enigma of the high prevalence of anti-SARS-CoV-2 antibodies in HIV-positive people with no symptoms of COVID-19 in Burkina Faso

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### Abstract

The severe acute respiratory syndrome due to the new coronavirus (SARS-CoV-2), responsible for coronavirus disease (COVID-19), has severely tested the global health response capacity, with predictions of a fatality for developing countries. To evaluate the prevalence of anti-SARS-CoV-2 antibodies in People Living with HIV (PLHIV) with no COVID-19 symptoms in Burkina Faso. Seroprevalence was estimated by performing a qualitative screening test for SARS-CoV-2-specific immunoglobulins. The STANDARD™ Q COVID-19 IgM/IgG Combo Test kit from SD BIOSENSOR was used. Parameters like HIV plasma viral load, CD4 T cell count and C-Reactive Protein (CRP) expression were estimated. This study enrolled a total of 200 PLHIV aged 4-87 years who are asymptomatic for COVID-19. There were 36 (18%) positive for SARS-CoV-2 IgM and/or IgG of which three (1.50%) were

positive for SARS-CoV-2 IgM and 33 (16.50%) for IgG. Among participants diagnosed as IgM positive, 66.67% (2/3) had the highest HIV viral loads with the lowest CD4 T cell counts ( $p < 0.0001$ ). The expression of CRP was relatively higher in COVID-19 IgG positive individuals ( $7.95 \pm 12.5$  mg/L) than negative individuals ( $6.26 \pm 6.92$  mg/L;  $p = 0.37$ ). The rate of IgG and IgM SARS-CoV-2 immunoglobulin carriage (18%), accompanied by a relatively high CRP levels, was revealed in this study among PLHIV. This serologic evidence and mild inflammation suggest that Burkina Faso escaped the worst, not necessarily because there were not many SARS-CoV-2 infections in its population, but because factors including genetic and environmental, might have resulted in many asymptomatic carriers.

### Introduction

Severe acute respiratory syndrome due to the new coronavirus (SARS-CoV-2), responsible for coronavirus disease (COVID-19), is a global health threat. In December 2019, this new coronavirus was identified in the city of Wuhan<sup>1</sup> in patients with unexplained severe lung disease. In February 2020, the World Health Organization named COVID-19 the disease caused by this virus, originally called nCoV. After SARS-CoV in 2002 in China and MERS-CoV in 2012 in the Arabian Peninsula, which caused often fatal respiratory distress syndromes,<sup>2</sup> this is the third global health threat from a coronavirus in less than two decades.<sup>3</sup>

COVID-19 has induced panic, psychosis, skepticism and mistrust worldwide,<sup>4,5</sup> especially in Europe, Asia and the Americas, by resulting in incredible spectacles: empty streets and ghost towns. It thrived and spread in recreational places through such human contact as hugs and handshakes. The response to control it led to the confinement and closure of borders, markets and stores,<sup>6</sup> the bankruptcy of companies, factories, travel agencies, tourist offices, banks and even hospitals.<sup>7</sup> It has created large numbers of unemployed and has spread fear, anxiety and distress<sup>4</sup> around the world.<sup>5</sup> This climate of widespread crisis coupled with shortages of certain health products such as masks, hydro-alcoholic gel, hospital beds, respirators, and some essential drugs, testify that this SARS-CoV-2 pandemic has exposed and severely tested the response capacity of national, international and even global public health authorities.<sup>2</sup> With a limited supply of health products, countries with manufac-

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**Informed consent:** Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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turing capacity have put pressure on suppliers to serve domestic markets first. Some

governments have imposed export restrictions, forgetting the solidarity between nations and the duty to assist the most vulnerable.<sup>8</sup>

By September 29<sup>th</sup>, 2021, about 233,044,677 cases had been recorded worldwide including approximately 14,213 cases in Burkina Faso; with 4,770,268 deaths worldwide, with 183 in Burkina Faso.<sup>9</sup> With the highest African population growth rate in the world, problems to ensure adequate education,<sup>10</sup> problems of nutritional deficiency, and insufficient health care supply,<sup>11</sup> the continent's economy is such that it would not be able to cope with the pandemic situation of COVID-19. Western countries were already struggling to contain the pandemic because they needed more respirators, hospital equipment, protection strategies, better knowledge of the pathology. In regard to this, what could developing countries do? Thus, many researchers and politicians in Europe had predicted doom for Africa.<sup>12</sup> Since the worst of the predictions did not come true,<sup>11</sup> the question now was whether the majority of the population did not, acquire SARS-CoV-2 without manifesting the symptoms that could register them as suspicious cases leading to molecular testing for confirmation. Most people infected with SARS-CoV-2 produce antibodies (immunoglobulins or Ig) to a protein on the surface of the virus, the S (Spike) protein. The production of Ig type M (IgM) and type G (IgG) begins after the first week and peaks between the 2<sup>nd</sup> and 3<sup>rd</sup> week after infection. SARS-CoV-2 infection leads to an aggressive immune response<sup>13</sup> that weakens vital organs and leads to death.<sup>13</sup> Hypersensitivity is characterized, among other things, by a significant increase in C-Reactive Protein (CRP), associated with the deterioration of the health of patients with COVID-19 leading to progression to intensive care.<sup>14</sup> HIV-related immune depression is thought to result in high mortality from co-infection with COVID-19<sup>15,16</sup> because antiretroviral therapy does not appear to be protective against COVID-19 in some of cases.<sup>17</sup> In order to find out why Africa escaped the worst of the predictions, the objective of this study was to determine the prevalence of anti-SRAS-CoV-2 antibodies in vulnerable individuals who did not manifest the symptoms of COVID-19.

## Materials and Methods

### Study design and population

From November 2<sup>nd</sup> to November 30<sup>th</sup>, 2020, People Living with HIV (PLHIV) who receiving care at the Pietro Annigoni

Centre for Biomolecular Research (CERBA) and Saint Camille Hospital in Ouagadougou, Burkina Faso were included by the treating physician after obtaining informed consent.

Inclusion criteria: PLHIV regardless of age or sex and with no symptoms of COVID-19 according to national investigation tools (fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea). Considering that vulnerable individuals, including those infected with HIV, accounted for a large proportion of all admissions to COVID-19, the group of PLHIV was targeted for this study.

### Laboratory methods and tests

After administration of the questionnaire, venous blood (8mL) was collected and aliquoted in two EDTA tubes. The first tube was used for the CD4 T cell count. The second tube was centrifuged and the supernatant, plasma, was dispatched in cryotubes, two of which were reserved for viral load and COVID-19 serology. The COVID-19 diagnostic test, HIV viral load and CD4 T cell count were performed at CERBA.

For the HIV viral load assay, HIV-1 RNA was extracted using the Abbott kit and amplified by real-time PCR on the Abbott m2000rt thermal cycler following the manufacturer's protocol.

The CD4 T-cell count was performed using the BD FACS Count flow cytometer with the manufacturer's reagents and protocol.

The COVID-19 serological test was performed on STANDARD™ Q COVID-19 IgM/IgG Combo Test from SD BIOSENSOR. This test showed 81.8% of sensitivity and 96.7% of specificity. It is a rapid immuno-chromatographic test designed for the qualitative detection of specific antibodies (IgM and IgG) to SARS-CoV-2. The test consists of 3 pre-coated lines, control line "C", test lines "G" and "M" for the device on the surface of the nitrocellulose membrane. These two bands independently indicate the presence of IgG and/or IgM. The control line and the two test lines in the result window are not visible before applying any specimens. Plasma (10µL) was used in addition to three drops (90µL) of the buffer provided in the kit. The interpretation was done between 10 and 15 minutes after the diluent was deposited. The control line is used for procedural control, and the test was validated after the appearance of the violet control line. A violet test line would be visible in the result window if SARS-CoV-2 antibodies are present in the

specimen. If SARS-CoV-2 antibodies are not present in the specimen, then no color appears in the test line.

For the determination of C-Reactive Protein (CRP) in vitro, a Roche Hitachi Cobas C system 6000 series automaton was used. For each sample 6µL of plasma was required for the determination of human CRP which agglutinates on latex particles coated with anti-CRP monoclonal Ac, the precipitate is then measured by immunoturbidimetry, with a functional sensitivity of 0.3mg/L and a so-called normal value between 0 and 6mg/L.

### Statistical analysis

Survey data and test results were entered into Excel 2016 and then statistical analysis were done using Standard Statistical Package for Social Sciences (SPSS) version 21 and EPI Info version 6.0. The statistical significance level was set at  $p < 0.05$ .

### Ethical considerations

The research protocol was approved by the national ethics committee with the ethical clearance authorization document n° 2020-10-240. All study participants gave free and informed consent. Anonymity and confidentiality were respected throughout the study. The respondents' information, collected by the research team in the questionnaire, included socio-demographic characteristics, COVID-19 symptoms and medical history. Patients who tested positive for IgM entered the national COVID-19 case management system after confirmation by PCR.

## Results

The questionnaire was completed by 200 PLHIV and on antiretroviral treatment. At the time of inclusion, participants were not vaccinated against COVID-19. Participants were between 4 years and 87 years of age with 65% female and 35% male. Those between 31 and 45 years of age were in the majority (48.50%) compared to those under 30 years of age (22.50%;  $p < 0.05$ ) and those over 45 years of age (29.00%;  $p < 0.05$ ). Within the most represented age group (31-45 years), the majority (49.30%) had an HIV viral load of less than 20 copies per mL ( $p < 0.05$ ) and a CD4 T cell count of less than 500 cells per µL ( $p < 0.05$ ). Among the participants, there were 36 (18%) positive for SARS-CoV-2 IgM and/or IgG including three (3) positive for SARS-CoV-2 IgM and 33 for IgG. One of the participants under 30 years of age was IgM and IgG positive, with an HIV viral

load of approximately 2,000 copies per mL. Among the patients, those who were anti-SARS-CoV-2 antibody-positive, were under 45 years of age. There was no statistically significant difference in IgG positive results across age groups ( $p>0.05$ ). These data are summarized in Table 1.

There was a statistically significant difference in seroprevalence observed between participants with a HIV viral load less than or greater than 20 copies/mL ( $p<0.05$ ). Among the IgM-positive participants, 66.67% (2/3) had the highest HIV viral loads with the lowest CD4 T cell counts ( $p<0.0001$ ); which was not the case for participants testing positive for IgG ( $p<0.05$ ) of whom 75.76% had a low ( $<20$  copies/mL) or even undetectable viral load and 90% had CD4 T-lymphocyte levels of more than 500 cells/ $\mu$ L (Table 2).

Among participants, there was no statistically significant difference in mean CRP between those who had COVID-19 and those who did not ( $p>0.05$ ). However, there was a statistically significant difference when ranked by normal value: the difference was statistically significant for the mean values between those with a normal CRP and those with a CRP greater than 6mg/L ( $p<0.001$ ). The mean CRP values are shown in Table 3.

## Discussion

Among the participants, the majority (65%) were women. The most common age group was 31-45 years of age, a group in which HIV plasma viral load was high, TCD4 lymphocyte count was low, and IgG

positive was significantly higher. Among other things, authors have noted that older people living with HIV with low CD4 T-cell counts are most likely to develop COVID-19.<sup>18</sup> Studies have shown that HIV was associated with mortality by COVID-19,<sup>19</sup> with similar risks in all HIV viral load and immunosuppression ranges.<sup>20</sup> This shows that more attention needs to be paid to strategies to prevent COVID-19 in people with weaker immune systems. The seroprevalence of IgM and/or IgG antibodies to SARS-CoV-2 in people living with HIV (PLHIV) was 18% (36/200). Considering the adjustment proposed by Sempos and Tian on the basis of the kit specificity and sensitivity,<sup>21</sup> this prevalence could become 18.72% instead of 18%. None of the patients in our study population exhibited symptoms suggesting active COVID-19

**Table 1. Results of the parameters studied by age group.**

		$\leq 30$ (%)	31-45 (%)	$> 45$ (%)
Sex	Female (n=130)	21 (16.15)	72 (55.39)	37 (28.46)
	Male (n=70)	24 (34.29)	25 (35.71)	21 (30.00)
HIV plasma viral load (copies/mL)	$\leq 20$ (n=144)	31 (21.53) <sup>a</sup>	71 (49.30) <sup>b</sup>	42 (29.17) <sup>c</sup>
	$> 20$ (n=56)	14 (25.00) <sup>d</sup>	26 (46.43) <sup>e</sup>	16 (28.57) <sup>f</sup>
CD4 T cell counts (cells/ $\mu$ L)	$\leq 500$ (n=173)	39 (22.54) <sup>a</sup>	83 (47.98) <sup>b</sup>	51 (29.48) <sup>c</sup>
	$> 500$ (n=27)	6 (22.22) <sup>d</sup>	14 (51.85) <sup>e</sup>	7 (25.93) <sup>f</sup>
IgM	Positive (n=3)	2 (66.67)	1 (33.33)	0 (0)
	Negative (n=197)	43 (21.83) <sup>a</sup>	96 (48.73) <sup>b</sup>	58 (29.44) <sup>c</sup>
IgG	Positive (n=33)	7 (21.21) <sup>a</sup>	15 (45.45) <sup>b</sup>	11 (33.34) <sup>c</sup>
	Negative (n=167)	38 (22.75) <sup>d</sup>	82 (49.10) <sup>e</sup>	47 (28.15) <sup>f</sup>
Total	n=200	45 (22.50) <sup>a</sup>	97 (48.50) <sup>b</sup>	58 (29) <sup>c</sup>

p-value: HIV plasma viral load: p(a  $\rightarrow$  b)= 0.012; p(b  $\rightarrow$  c)= 0.031; CD4 T cell counts: p(a  $\rightarrow$  b)= 0.008; p(b  $\rightarrow$  c)= 0.032; Serology IgM: p(a  $\rightarrow$  b)= 0.002; p(b  $\rightarrow$  c)= 0.017; Serology IgG: p(d  $\rightarrow$  e)=0.009; p(e  $\rightarrow$  f)=0.019; Age: p(a  $\rightarrow$  b)= 0.003; p(b  $\rightarrow$  c)=0.019.

**Table 2. SARS-CoV-2 serology results according to HIV viral load and CD4 T-cell count.**

Biological parameters	HIV viral load (copies/mL)			CD4 T cell counts (cells/ $\mu$ L)		
	$\leq 20$ n=144 (%)	$> 20$ n=56 (%)	p value	$\leq 500$ n=173 (%)	$> 500$ n=27 (%)	p value
IgM	Positive (n=3)	2 (66.67)	-	2 (66.67)	1 (33.33)	-
	Negative (n=197)	143 (72.59)	$<0.0001$	171 (86.80)	26 (13.20)	$<0.0001$
IgG	Positive (n=33)	8 (24.24)	0.029	30 (90.91)	3 (9.09)	-
	Negative (n=167)	119 (71.26)	$<0.0001$	143 (85.63)	24 (14.37)	$<0.0001$

**Table 3. Plasma CRP concentration and serological status of COVID-19.**

COVID-19	CRP mg/L	p value
Results		
Negative (n=25)	6.26 $\pm$ 6.92	0.37
Positive (n=36)	7.95 $\pm$ 12.5	
Total (n=61)	7.25 $\pm$ 10.54	
CRP value		
0 to 6mg/L (n=43)	2.18 $\pm$ 1.70	$<0.001$
$>6$ mg/L (n=18)	19.38 $\pm$ 12.81	
Total (n=61)	7.25 $\pm$ 10.54	

CRP: C-reactive protein.

disease. As with many other viral infections, asymptomatic disease is present in a significant fraction of people who are unaware of it.<sup>22,23</sup> This serological proportion found in asymptomatic PLHIV confirms the scale of the spread of the SARS-CoV-2. Oliveira *et al.* found that 13.9% (61/439) of outpatients were IgG positive even though 32.8% of patients testing positive for anti-SARS-CoV-2 IgG antibodies were asymptomatic<sup>24</sup> while among health-care professionals, the seroprevalence of IgM/IgA and IgG anti-SARS-CoV-2 antibodies was 24.24%.<sup>25</sup> Also, the expression of CRP, an indicator of the degree of inflammation in an organism, was relatively higher in COVID-19 IgG positive individuals (7.95±12.5mg/L) than negative individuals (6.26±6.92mg/L; p=0.37).

Therefore, more attention should be given to the general population regardless of symptoms in epidemiological studies. The participant who screened positive for anti-SARS-CoV-2 IgM and IgG antibodies can be considered as positive case for COVID-19 in the absence of PCR as suggested by some studies.<sup>26</sup> The presence of IgM indicates recent or intermediate infection with COVID-19, which can be considered as a positive case. As for IgG, this would be a possibility of past contact with the virus and may be detectable up to 3 weeks later.<sup>27</sup> Of the participants who tested positive for IgM, 66.67% (2/3) had the highest HIV viral loads with the lowest CD4 T cell counts (p<0.0001); in contrast, for participants who tested positive for IgG (p<0.05), 75.76% had a low (<20 copies/mL) or undetectable viral load and 90% had high CD4 T cell counts (>500 cells/μL).

There are two patterns that would require further study: could the SARS-CoV-2 virus have induced additional immunosuppression in HIV-infected individuals resulting in increased HIV viral load? Authors had found that HIV viral load increased significantly after recovery from COVID-19.<sup>28</sup> It is also possible that the high viral load may have facilitated co-infection with SARS-CoV-2 in HIV-infected individuals. This second scenario hypothesizes that co-infection with SARS-CoV-2 is more common among PLHIV as suggested by Byrd *et al.*<sup>29</sup> The possibility of virus infection was due to the frequent contact of these participants with hospital settings, which increased the potential risk of exposure to the virus.<sup>30</sup> Indeed, Alharbi *et al.* found a seroprevalence of SARS-CoV-2 of 24.24% among healthcare workers.<sup>25</sup> Antiretroviral treatment of PLHIV may also have had an effect in the asymptomatic presentation of COVID-19 in these infected

subjects. But more data are needed to answer these questions.

A number of possibilities have been proposed to explain the general mild cases as seen in Africa during this pandemic. These have included heat or the warm weather which has been thought to have a protective effect,<sup>31</sup> as the disease has grown exponentially in the West during this period and less so tropical countries.<sup>32</sup> It is also possible that genetic regulation or cross-reaction may have occurred that made CoV-2-SARS infection non-acute.<sup>33</sup> But in any case, the problem in Africa remains to be elucidated. These results show that in Burkina Faso there was a large number of infections, but due to multiple factors, there was not a wide spread of the disease.

## Conclusions

Among people living with HIV, not showing symptoms of COVID-19, 18% were positive for SARS-CoV-2 IgM and/or IgG serology. An increase in CRP and variations in viral load and TCD4 lymphocyte levels were also observed. Probably genetic, epigenetic and environmental factors would have contributed to protect Africans including Burkinabe from the fearsome pandemic that is COVID-19. The significant prevalence of IgM/IgG (18.0%) in this study would indicate that the importance of surveillance for CoV-2-SARS should not only be based on symptoms or syndrome, but also on serology in order to better assess the progression and spread of the disease.

## Study limitation

At the beginning of the spread of COVID-19 in Burkina Faso, with the increase in the number of cases and the ever-increasing demand for testing, many measures to control the pandemic were taken but some were abandoned due to certain constraints, including financial ones.<sup>34</sup> In addition, there was no rush for voluntary testing because of the psychosis due to the mandatory containment of confirmed cases in hospitals that was instituted at those times. This could explain why we did not have more volunteers for the screening during this period.

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