EDORIUM Journals

Int J Blood Transfus Immunohematol 2024;14(2):1–6.
www.ijbti.com

RESEARCH ARTICLE PEER REVIEWED | OPEN ACCESS

Cytokines profile in occult hepatitis B virus infections in blood donors at the National Blood Transfusion Center of Lomé, Togo

Liza Koboyo Nadjir, Gnatoulma Katawa, Marthe Amessoudji, Maléwé Kolou, Lochina Feteke

ABSTRACT

Aims: Occult hepatitis B infection (OBI) was detected in blood donors at the National Blood Transfusion Center (CNTS) of Lomé. This constitutes a risk of transmission of the OBI to transfused patients since the detection of this infection is not systematic. The aim of this study is to describe the profile of cytokines in blood donors with OBI at CNTS of Lomé in Togo.

Methods: This is a prospective study during which the cytokines IL-6, TNFα, IL-5, IL-10, IL-17A, and IFNγ were measured by using a sandwich enzyme-linked immunosorbent assay (ELISA) at two time points: initially (D0) in August 2022 and again seven months later (+7 months) in April 2023, in blood donors with occult hepatitis B infection.

Results: At D0, the cytokine profile in sera of occult hepatitis B donors showed a significant reduced level of IL-6 (p=0.0009), TNFα (p=0.0025), IL-5 (p=0.0011), and IL-17A (p=0.0218) compared to non-occult hepatitis B donors. The inflammatory cytokine IFNγ and the regulatory cytokine IL-10 were also reduced but the differences were not significant. After seven months, the sera cytokine profile of occult hepatitis B donors had not changed, showing a significant reduced level of IL-6 (p=0.0005), TNFα (p<0.0001), IL-5 (p=0.0234), and IL-17A (p<0.0001). We noticed especially for IL-17A, undetectable levels.

Conclusion: Several authors have highlighted the presence of inflammatory cytokines in their studies; however, in our study, we noticed a downregulation or an absence of inflammatory cytokines in donors with occult hepatitis B.

Keywords: Blood donors, Lomé, OBI

Liza Koboyo Nadjir[1,2], Gnatoulma Katawa[3], Marthe Amessoudji[3], Maléwé Kolou[2], Lochina Feteke[1,2]

Affiliations: [1]National Blood Transfusion Center (CNTS), Lomé Togo; [2]Department of of Basic Sciences, Faculty of Health Sciences, Lomé, Togo; [3]Higher School of Biological and Food Techniques (ESTBA)-UL, Lomé, Togo.

Corresponding Author: Liza Koboyo Nadjir, BP: 4090, Lomé, Togo; Email: lizanadjir@yahoo.fr

Received: 26 July 2024
Accepted: 29 August 2024
Published: 12 October 2024

How to cite this article

Nadjir LK, Katawa G, Amessoudji M, Kolou M, Feteke L. Cytokines profile in occult hepatitis B virus infections in blood donors at the National Blood Transfusion Center of Lomé, Togo. Int J Blood Transfus Immunohematol 2024;14(2):1–6.

Article ID: 100084Z02LN2024

****

doi: 10.5348/100084Z02LN2024RA

INTRODUCTION

Occult hepatitis B virus infection (OBI) is defined by the presence of viral DNA in blood and tissues without detectable level of HBs Ag with or without anti-HBc Ab, or anti-HBs Ab, apart from the pre-seroconversion period [1], representing a serious public health threat [2]. From an immune response perspective, evidence suggests that both humoral and cellular responses are necessary for complete elimination of the virus. The cellular response and cytokines released by activated lymphocytes could play an important role in virus clearance without leading to death of the infected cell [3]. Cytokines are important chemical mediators that regulate the differentiation, proliferation, and function of immune cells, and the quality of immune responses are responsible for the elimination or persistence of hepatitis B virus (HBV) [2]. There are few studies on cytokine production profile in occult

hepatitis B patients and the mechanism of liver injury due to OBI is still unclear [4]. However, some studies describe that the persistence and transcription of HBV covalently closed circular DNA (cccDNA) in hepatocytes can lead to the production of cytokines, such as TNFα and INFγ, which can result in damage for hepatocytes [5, 6]. Occult hepatitis B virus infection was detected in blood donors at the National Blood Transfusion Center (CNTS) of Lomé [7]. The aim of this work is to describe the profile of cytokines in blood donors with OBI at CNTS of Lomé in Togo.

MATERIALS AND METHODS

This is a prospective study that measured the titer of cytokines at an initial time (D0) in August 2022 and seven months later (+7 months) in April 2023, among 10 blood donors carrying OBI.

The identification of the 10 blood donors carrying OBI was made among 1015 donors consenting to participate in the study, on whom serological and virological tests were carried out.

Serological screening

We excluded donations that were found positive for at least one of the serological markers tested at the blood transfusion center: human immunodeficiency virus (HIV) screened by Genscreen™ ULTRA HIV Ag-Ab from BIORAD (REF 72561), hepatitis C virus (HCV) screened by Monolisa™ HCV Ag-Ab ULTRA V2 from BIORAD (REF 72346), HBV screened by Monolisa™ HBsAg ULTRA (REF 72348), and syphilis screened by RPR CARBON Slide agglutination of LABKIT (REF: 40130). The screening for total anti-HBc Abs (IgM and IgG) was carried out with Anti-HBc Total Elisa (KAPG4CBE3) from DIASOURCE.

After screening the sera of blood donors, 10 mL of serum from HBsAg-negative and anti-HBc Ab-positive donors were collected and stored at −35°C until further analysis, including viral DNA detection and cytokine titration.

Molecular diagnosis

The hepatitis B viral DNA detection was carried out by polymerase chain reaction (PCR) using the COBAS AmpliPrep/TaqMan 4800 amplification method (Roche diagnostics). Two viral loads were realized at initial time (D0) and seven months later (+7 months).

Cytokines measurement

The level of cytokines, including IL-6, TNFα, IL-5, IL-10, IL-17A, and IFNγ, was measured in the sera of blood donors using sandwich enzyme-linked immunosorbent assay (ELISA) with invitrogen cytokines ELISA Kits (Thermo Fisher Scientific, Bender MedSystems GmBH, Vienna, Austria), according to the manufacturer's instructions.

The validation of all the tests was carried out following the manufacturer's technical guidelines and protocols.

Statistical analysis

The statistical analysis was performed using GraphPad Prism version 5.02 (GraphPad Software Inc, La Jolla, USA). The Mann–Whitney *U* test was used to compare the different groups and p-value< 0.05 was considered as significant. SPSS software (IBM SPSS Statistics 21; Armonk, NY) was used for logistic regression analyses in order to investigate the cytokines profile associated with occult hepatitis B including the calculation of odds ratios (OR) and adjusted odds ratios (aOR). Thresholds of p-values < 0.2 and < 0.05 were considered for univariate and multivariate analyses respectively.

Ethics statement

The consent of blood donors and the agreement of the bioethics committee for health research (N° 052/2022/CBRS of December 16, 2022) have been obtained for this study.

RESULTS

Out of 1015 consenting donors who participated in blood donation during the study period, 10 were carriers of OBI.

Socio-demographic characteristics of occult hepatitis B donors

In this study, at least 90% of donors were male and they were not at their first blood donation (long-standing donors). Most of the blood donors carrying OBI were aged 29–39 years. Fifty percent of them work as office managers and 30% were students (Table 1).

Elevated inflammatory signals in HBc Ab positive donors

Figure 1 represents the plasmatic cytokines level in blood donors regarding their HBc Ab status. It was observed that HBc Ab positive donors had high levels of pro-inflammatory and inflammatory cytokines. Pro-inflammatory cytokines IL-6 ($p<0.0001$) and TNFα $p<0.0001$) were significantly higher in HBc Ab positive donors. Inflammatory cytokine IL-17A ($p<0.0001$) also was significantly higher in HBc Ab positive whereas it was undetectable in HBc Ab negative donors. No significant difference was observed for the non-inflammatory cytokine IL-5. Interestingly, the regulatory cytokine IL-10 was higher in HBc Ab negative donors, but the difference was not significant (Figure 1).

Table 1: Socio-demographic characteristics of occult hepatitis B carrying donors

Characteristics		Number (%)	p-value
Gender			1
	Male	**9 (90.0)**	
	Female	1 (10.0)	
Type of donor			0.293
	Long-standing	**9 (90.0)**	
	New	1 (10.0)	
Age			0.868
	[18–28]	2 (20.0)	
	[29–32]	**3 (30.0)**	
	[33–39]	**3 (30.0)**	
	[40–59]	2 (20.0)	
Profession			0.672
	Student	3 (30.0)	
	Manager	**5 (50.0)**	
	Informal	2 (20.0)	

For our analysis, the HBc Ab positive donors were used to differentiate occult hepatitis B donors (HBc Ab positive – DNA positive) from non-occult hepatitis B donors (HBc Ab positive – DNA negative).

Bold values signify socio-demographic characteristics of occult hepatitis B carrying donors.

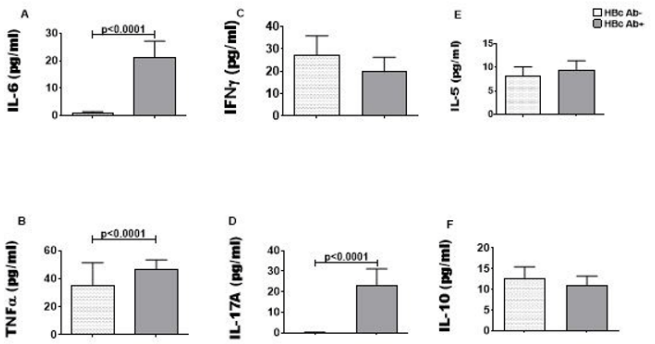

Figure 1: HBc Ab-based cytokine profile. Cytokines were measured in the sera of HBc Ab negative (n=640) and positive (n=327) donors. Graphs show sera levels of (A) IL-6; (B) TNFα; (C) IFNγ; (D) IL-17A; (E) IL-5; (F) IL-10. Box whiskers (Tukey) with outliers indicate the concentration of cytokines in each group. The Mann–Whitney *U* test was performed to compare groups and the difference was significant for p-value < 0.05.

Downregulation of inflammatory cytokines in occult hepatitis B donors

In this section, we tried to elucidate the cytokine profile at the initial time point (D0) and seven months later (+7 months) during the presence of the viral DNA.

At D0, the cytokine profile in sera of occult hepatitis B donors showed a significant reduced level of IL-6 (p=0.0009), TNFα (p=0.0025), IL-5 (p=0.0011), and IL-17A (p=0.0218) compared to non-occult hepatitis B donors. The inflammatory cytokine IFNγ and the regulatory cytokine IL-10 were also reduced but the differences were not significant (Figure 2).

After seven months, the sera cytokine profile of occult hepatitis B donors had not changed, showing a significant reduced level of IL-6 (p=0.0005), TNFα (p<0.0001), IL-5 (p=0.0234), and IL-17A (p<0.0001). We noticed especially for IL-17A, undetectable levels. No significant difference was observed in the cytokine profile comparing the production at D0 and seven months later (Figure 3).

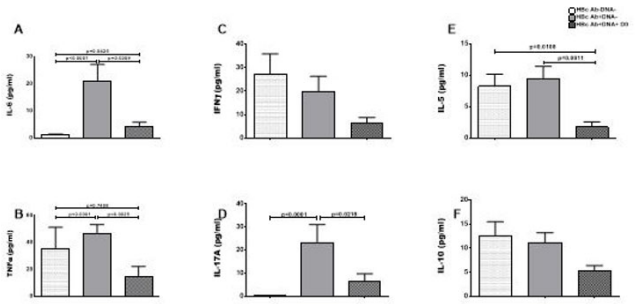

Figure 2: Cytokine profile in occult hepatitis B donors at D0. Cytokines were measured in the sera of HBc Ab-DNA– (n=33), HBc Ab+DNA– (n=31) and HBc Ab +DNA+ D0 (n=10). Graphs show sera levels of (A) IL-6; (B) TNFα; (C) IFNγ; (D) IL-17A; (E) IL-5; (F) IL-10. Box whiskers (Tukey) with outliers indicate the concentration of cytokines in each group. The Mann–Whitney *U* test was performed to compare groups and the difference was significant for p-value < 0.05.

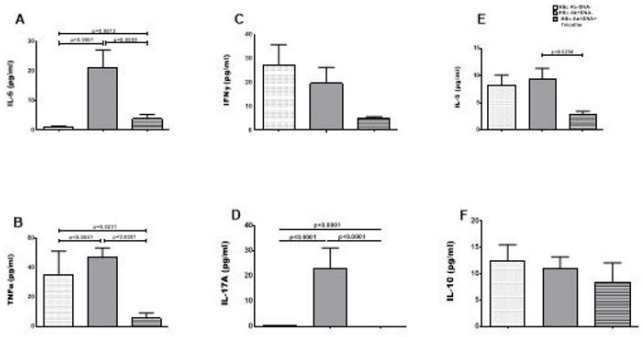

Figure 3: Cytokine profile in occult hepatitis B donors 7 months later. Cytokines were measured in the sera of HBc Ab-DNA– (n=33), HBc Ab+DNA– (n=31) and HBc Ab+DNA+ 7months (n=9). Graphs show sera levels of (A) IL-6; (B) TNFα; (C) IFNγ; (D) IL-17A; (E) IL-5; (F) IL-10. Box whiskers (tukey) with outliers indicate the concentration of cytokines in each group. The Mann–Whitney *U* test was performed to compare groups and the difference was significant for p-value < 0.05.

IFNγ low level associated with viral DNA persistence in occult hepatitis B infection

The associations showed by the logistic binary regression are summarized in Table 2. At D0, the low level of TNFα (aOR=0.946, CI 95% [0.901–0.993] p=0.026) was associated to the presence of the viral DNA in occult hepatitis B donors.

Seven months later, the low level of TNFα still associated to the presence of the viral DNA (aOR=0.891, CI 95% [0.807–0.984] p=0.022). Interestingly, not only the TNFα but the low level of IFNγ was also associated to the persistence of the viral DNA (aOR=1.437, CI 95% [1.003–2.058] p=0.048) in occult hepatitis B donors (Table 2).

DISCUSSION

This study aimed to determine the cytokines profile of 10 blood donors with OBI at the National Blood Transfusion Center (CNTS) of Lomé.

The socio-demographic characteristics of our study population revealed that, among the 10 blood donors who were positive for OBI, 9 were men and they are long-standing donors. It is known that in Togolese context, men are more likely to be blood donors than women. Moreover, it has been shown that during HBV infection, male subjects are more likely to be healthy carriers, whereas female subjects produce HB antibodies which contribute to the viral elimination. Therefore, males are more likely to develop OBI than females, justifying this male predominance in our population [7].

Antiviral immune responses in OBI are continuously stimulated by persistent/intermittent low concentrations of HBV antigens and cytokines can play an important role in controlling HBV replication [8, 9]. We measured several cytokine levels including IL-6, TNFα, IL-5, IL-10, IL-17A, and IFNγ in the sera of blood donors to better understand the immune system of OBI infected donors.

In donors who were positive for HBc Ab (HBc Ab+), the levels of IL-6, TNFα, and IL-17A were higher compared to donors who were negative for HBc Ab (HBc Ab–). The differences in IFNγ, IL-5, and IL-10 levels were not significant. The HBc Ab+ group was separated into two groups: healthy blood donors with an undetectable viral load (HBc Ab+ viral DNA–) and blood donors with detectable viral load (HBc Ab+ viral DNA+) that means donors with OBI. At D0, compared to healthy donors, donors with OBI showed a very low and significant production of pro-inflammatory cytokines IL-6, TNFα, and IL-17A and anti-inflammatory cytokine IL-5. There was no significant difference for the production of IFNγ

Table 2: Logistic regression associating cytokines profile with the viral DNA in HBc Ab+ donors

Cytokines		Univariate		p-value	Multivariate		p-value
		OR	CI 80%		aOR	CI 95%	
D0							
	IL-5	0.686	(0.501–0.938)	**0.018**	0.532	(0.247–1.150)	0.109
	IL-10	0.839	(0.677–1.041)	**0.111**	0.929	(0.662–1.303)	0.668
	TNF-α	0.947	(0.902–0.994)	**0.027**	0.946	(0.901–0.993)	**0.026**
	IL-17A	0.963	(0.902–1.028)	0.256			
	IL-6	0.79	(0.608–1.026)	**0.077**	1.072	(0.726–1.585)	0.725
	IFN-γ	0.91	(0.796–1.041)	**0.169**	1.025	(0.788–1.303)	0.854
7 months							
	IL-5	0.755	(0.573–0.995)	**0.046**	0.963	(0.475–1.953)	0.917
	IL-10	0.974	(0.895–1.060)	0.545			
	TNF-α	0.86	(0.772–0.957)	**0.006**	0.891	(0.807–0.984)	**0.022**
	IL-17A	0	NA	0.408			
	IL-6	0.714	(0.492–1.036)	**0.076**	0.712	(0.435–1.168)	0.179
	IFN-γ	0.787	(0.607–1.02)	**0.071**	1.437	(1.003–2.058)	**0.048**

Bold values are significant p-values.

and IL-10 in the study groups. The results of studies carried out in Mexico (where the prevalence of HBV genotype H is high) and China are similar to ours, with low levels of pro-inflammatory cytokines, notably IL-6, IL-17A, and TNFα [10, 11]. Unlike us, after stimulation of periferics blood mononuclear cells (PBMCs) with HBV core peptides, Zhang et al. also observed a low level of extracellular TNFα production but rather a high level of IL-17A production in subjects with OBI compared to cured or uninfected hepatitis B subjects [12]. Martin et al. observed a reduction in pro-inflammatory cytokines, such as TNFα, in patients who resolved HBV infection when compared to healthy individuals [5], and IFNγ was noticeably decreased, especially in monoinfected patients with hepatitis C virus (HCV) genotype 1b according to Han et al. [13]. In our study, the low level of IFNγ was associated to the persistence of the viral DNA (aOR=1.437, CI 95% [1.003–2.058] p=0.048) in blood donors with OBI.

Furthermore, high levels of TNFα, IL-10, and IL-6 were found in patients co-infected by chronic hepatitis C [2].

The roles of IL-17A in inducing appropriate immune responses against viral infections are controversial, IL-17A may play a positive role in antiviral immune responses in several diseases [14]. However, for chronic hepatitis B, it is well known that IL-17A is associated in HBV-mediated inflammation and the development of liver cirrhosis and hepatocellular carcinoma (HCC) [15, 16].

Other cytokines possibly involved in OBI, like increased interleukin 10 (IL-10), can lead to reduced expression of IL-12, stromal cell-derived factor (SDF)-1α, and C–C chemokine receptor (CCR), which lead to the interruption of T and natural killer cells (NK cell) activation and the recruitment of immune cells to the infected liver [17].

In our study population, after seven months follow-up, we noticed an indetectable level of IL-17A, a reduction in IL-6 production, and an increased level of IL-5 and IL-10. A study about the impact of the OBI on HIV patients revealed a decreased numbers of $CD4^+$ T cells in co-infected patients compare to HIV monoinfected ones [18]. Limited data are available on the immune profile of long-term OBI but knowing that IL-6 is an important cytokine involved in the regulation of the balance between IL-17A-producing Th17 cells and regulatory T cells (Treg). This result is awoken us on the role of TH17 cells in the OBI [19].

The correlation between the serum cytokines levels and the presence of the HBV DNA revealed that the low levels of IFNγ and TNFα are strongly associated with the persistence of the viral genome in OBI donors. Xia et al. demonstrated that stimulating HBV-infected cells with IFNγ and TNFα led to a reduction and a characteristic modification in the DNA sequences [20].

CONCLUSION

Several authors have highlighted the presence of inflammatory cytokines in their studies; however, in our study, we noticed a downregulation or an absence of inflammatory cytokines in occult hepatitis B donors. The mechanisms involved need further investigation in future studies.

REFERENCES

1. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis 2002;2(8):479–86.
2. Ribeiro CRA, Beghini DG, Lemos AS, et al. Cytokines profile in patients with acute and chronic hepatitis B infection. Microbiol Immunol 2022;66(1):31–9.
3. Hoffnagle JH, Seeff LB, Buskell-Bales Z, et al. Serologic responses in HB. In: Vyas GN, Cohen SN, Schmid R, editors. Viral Hepatitis: A Contemporary Assessment of Etiology, Epidemiology, Pathogenesis and Prevention. Philadelphia: Franklin Institute Press; 1978. p. 219–42.
4. Ribeiro CRA, de Almeida NAA, Martinelli KG, et al. Cytokine profile during occult hepatitis B virus infection in chronic hepatitis C patients. Virol J 2021;18(1):15.
5. Martin CM, Welge JA, Shire NJ, Shata MT, Sherman KE, Blackard JT. Cytokine expression during chronic versus occult hepatitis B virus infection in HIV co-infected individuals. Cytokine 2009;47(3):194–8.
6. Germanidis G, Hytiroglou P, Zakalka M, Settas L. Reactivation of occult hepatitis B virus infection, following treatment of refractory rheumatoid arthritis with abatacept. J Hepatol 2012;56(6):1420–1.
7. Nadjir LK, Katawa G, Kolou M, et al. Anti-HBc antibodies and occult hepatitis B infection (OBI) among blood donors in Lomé, Togo. Int J Blood Transfus Immunohematol 2024;14(1):11–6.
8. Blumberg BS. The curiosities of hepatitis B virus: Prevention, sex ratio, and demography. Proc Am Thorac Soc 2006;3(1):14–20.
9. Raimondo G, Locarnini S, Pollicino T, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol 2019;71(2):397–408.
10. Fierro NA, Roman S, Realpe M, Hernandez-Nazara Z, Zepeda-Carrillo EA, Panduro A. Multiple cytokine expression profiles reveal immune-based differences in occult hepatitis B genotype H-infected Mexican Nahua patients. Mem Inst Oswaldo Cruz 2011;106(8):1007–13.
11. Chen Sx, Liu X, Liu H, Lou Jl. The incidence rate, serological characteristics and cytokines profile of occult HBV infection in clinic. Research Square Platform LLC. 2022.
12. Zhang W, Luo S, Li T, et al. Hepatitis B virus-specific cellular immunity contributes to the outcome of occult hepatitis B virus infection. Front Microbiol 2022;13:850665.
13. Han ZQ, Huang T, Deng YZ, Zhu GZ. Expression profile and kinetics of cytokines and chemokines in patients with chronic hepatitis C. Int J Clin Exp Med 2015;8(10):17995–8003.
14. Ma WT, Yao XT, Peng Q, Chen DK. The protective and pathogenic roles of IL-17 in viral infections: Friend or foe? Open Biol 2019;9(7):190109.

15. Du WJ, Zhen JH, Zeng ZQ, et al. Expression of interleukin-17 associated with disease progression and liver fibrosis with hepatitis B virus infection: IL-17 in HBV infection. Diagn Pathol 2013;8:40.
16. Wu JF, Wu TC, Chen CH, et al. Serum levels of interleukin-10 and interleukin-12 predict early, spontaneous hepatitis B virus e antigen seroconversion. Gastroenterology 2010;138(1):165–72.e1–3.
17. Arababadi MK, Nasiri Ahmadabadi B, Kennedy D. Current information on the immunologic status of occult hepatitis B infection. Transfusion 2012;52(8):1819–26.
18. Coffin CS, Mulrooney-Cousins PM, Osiowy C, et al. Virological characteristics of occult hepatitis B virus in a North American cohort of human immunodeficiency virus type 1-positive patients on dual active anti-HBV/HIV therapy. J Clin Virol 2014;60(4):347–53.
19. Kimura A, Kishimoto T. IL-6: Regulator of Treg/Th17 balance. Eur J Immunol 2010;40(7):1830–5.
20. Xia Y, Stadler D, Lucifora J, et al. Interferon-γ and Tumor Necrosis Factor-α Produced by T Cells Reduce the HBV Persistence Form, cccDNA, Without Cytolysis. Gastroenterology 2016;150(1):194–205.

****

Acknowledgments
Immunology and immunomodulation research unit (UR2IM), Higher School of Biological and Food Techniques (ESTBA)-UL, Lome, Togo

Author Contributions

Liza Koboyo Nadjir – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Gnatoulma Katawa – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Marthe Amessoudji – Acquisition of data, Analysis of data, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Maléwé Kolou – Design of the work, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Lochina Feteke – Conception of the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Guarantor of Submission
The corresponding author is the guarantor of submission.

Source of Support
None.

Consent Statement
Written informed consent was obtained from the patient for publication of this article.

Conflict of Interest
Authors declare no conflict of interest.

Data Availability
All relevant data are within the paper and its Supporting Information files.