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Original Research Article

Contribution of anti-BHCG, CK18, hPL and Ki-67 antibodies in the diagnosis of choriocarcinoma in Senegal

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ABSTRACT

Background: Gestational choriocarcinoma (GC) is a rare malignant tumour derived from the trophoblast of women of childbearing age. The aim of this study was to determine the contribution of immunohistochemistry in the diagnosis of choriocarcinoma and to evaluate its ability to specify whether or not it is gestational in nature in order to establish a diagnostic algorithm for trophoblastic diseases in general.

Methods: This is a retrospective, descriptive, bi-centric study spanning eight (8) years from 1 January 2013 to 31 December 2020. All cases diagnosed on hysterectomy specimens and with a formal conclusion of gestational choriocarcinoma were included. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections using the manual method. We recorded the data collected in Excel 2007 software and the analysis was performed using Epi Info.

Results: We collected 25 cases of choriocarcinoma. The average annual frequency was 3.12. The average age of the patients was 38.1 ± 9.7 years (standard deviation). Multiparous women were the most common, accounting for 57.14% of cases. Mixed-site tumours (intracavitary and intramural) were the most common, accounting for 48% of cases. Patients in FIGO stage I accounted for 88% of cases. Immunohistochemistry was performed on 14 samples, revealing 100% positive staining for anti-hCG, CK18 and hPL.

Conclusions: Gestational choriocarcinoma (GC) is a proliferation of trophoblasts (cytotrophoblasts and syncytiotrophoblasts). This study demonstrated the indispensability of immunohistochemistry in confirming the diagnosis and in assessing both the progression and therapeutic prognosis of the disease.

Keywords: Choriocarcinoma, Hysterectomy, Immunohistochemistry, Trophoblast

INTRODUCTION

Gestational choriocarcinoma (GC) is a rare malignant tumour derived from trophoblasts. It is the most primitive

and least differentiated tumour that occurs after a molar pregnancy or, more rarely, a miscarriage, a normal pregnancy or even an ectopic pregnancy.¹

It belongs to the group of trophoblastic diseases that generally affect women of reproductive age, but are also described during perimenopause and menopause.² The trophoblast is a component of the placenta, which is a temporary and inaccessible organ, which probably explains why its pathology has never been researched as extensively as other organs that are studied by various medical specialities.³ The frequency of choriocarcinoma in Europe and North America is estimated at 0.2-0.7/1,000 pregnancies, and it accounts for 12.8% of gestational trophoblastic diseases.⁴⁻⁵

They are of particular interest because of their relatively high frequency in low socioeconomic populations and their potential to develop into malignant disease.

It is important to distinguish between gestational and non-gestational choriocarcinomas. The diagnosis is difficult to make when there is no recent history of pregnancy during the interview. This may go unnoticed and may have occurred several years earlier. The pregnancy preceding the choriocarcinoma may also not be the causal pregnancy.²

In Senegal, data on choriocarcinoma are not always available. The aggressiveness of choriocarcinomas, contrasting with the availability of all diagnostic and therapeutic means, has prompted us to pay particular attention to this pathology.

The aim of this study was to determine the contribution of immunohistochemistry in the diagnosis of choriocarcinoma and its ability to specify whether or not it is gestational in order to establish a standard algorithm for the diagnosis of trophoblastic diseases in general. Additionally, this study aimed to determine the frequency of choriocarcinomas and their distribution according to age, parity, pregnancy history, and characteristic clinical symptoms. It also sought to assess the distribution of gestational choriocarcinomas based on their location within the uterus. Furthermore, the study intended to classify the tumours according to the FIGO 2000 staging system. Another objective was to establish the immunohistochemical profile of choriocarcinomas using the following antibodies: anti-hCG, anti-hPL, anti-CK18, and Ki-67. Finally, the study compared the epidemiological, histological, and immunohistochemical profiles of choriocarcinomas observed in Dakar with those reported by other authors.

METHODS

Type of study and study site

It was a retrospective study covering an eight-year period, from January 1, 2013, to December 31, 2020.

This study was conducted in the histology, embryology, and cytogenetics laboratory at Cheikh Anta Diop University in Dakar, in collaboration with the anatomical

pathology laboratories at Idrissa Pouye General Hospital (HOGIP) and Aristide Le Dantec Hospital (HALD).

Inclusion criteria

All cases diagnosed on hysterectomy specimens and with a formal conclusion of gestational choriocarcinoma were included.

Exclusion criteria

Cases in which choriocarcinoma was suspected but not formally diagnosed were not included.

Procedure

A standard histological technique was used to study the hysterectomy specimens, allowing for re-examination of the slides. The immunohistochemical study, which used a panel of antibodies consisting of anti-hCG, anti-CK18, anti-hPL, and Ki67, was performed on formalin-fixed, paraffin-embedded tissue sections using the manual method. This immunohistochemical study was performed at the pathology laboratory of Cheikh Anta Diop University in Dakar.

Ethical approval

The study was approved by the ethics committee of Cheikh Anta Diop University.

Statistical analysis

We recorded the collected data in Excel 2007, and the analysis was performed using Epi Info.

RESULTS

Epidemiological aspects

Frequency

During our study period, we collected 25 cases of choriocarcinoma, representing an average annual frequency of 3.12.

Age

The average age of patients was 38.1 years \pm 9.7 (standard deviation) with a median age of 39 years and extremes of 22 and 51 years. Patients under the age of 40 were in the majority, representing 52% (Figure 1).

Gestation and parity

Multigravida women were in the majority, accounting for 64.29% of choriocarcinomas, while nulligravida women were in last place, accounting for 7.14% of cases.

Multipara women were in first place, accounting for 57.14% of cases (Table 1).

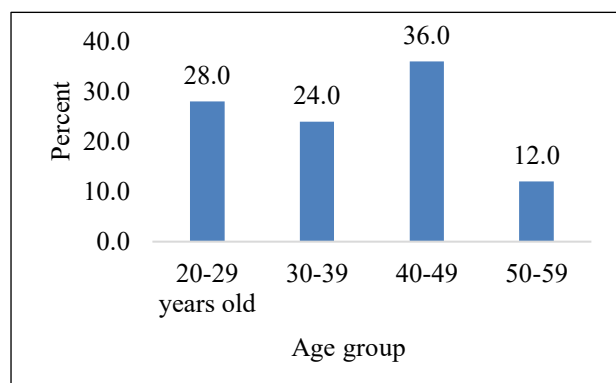


Figure 1: Distribution of patients by age group.

Table 1: Distribution of patients according to parity.

Gestation	Frequency (N)	Percentage (%)
Nulligravid	1	7.14
Paucigestes	4	28.57
Multigesture	9	64.29

History of molar pregnancy

A history of molar pregnancies was reported in 17 patients. Patients with a history of molar pregnancy accounted for 76.47%.

Clinical aspects

Metrorrhagia was the main symptom in 50% of cases, followed by pelvic pain in 25% (Figure 2).

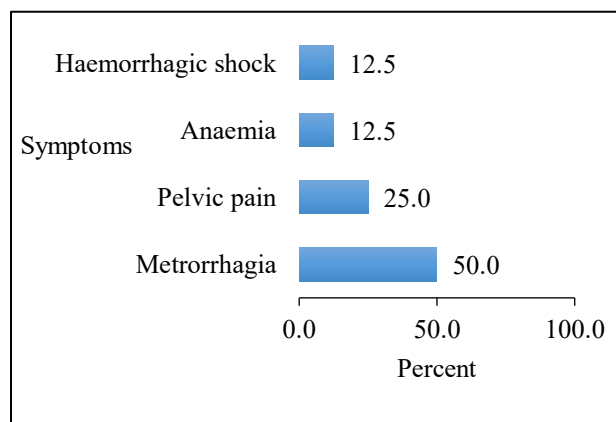


Figure 2: Distribution of patients according to symptoms (n=17).

Immunohistochemical characteristics

We performed immunolabelling on 14 samples.

Table 2: Immunomarking according to intensity.

Antibodies	Negative marking	Low marking	Moderate marking	Intense marking
Anti-beta HCG	0	1	0	13
Anti-CK 18	0	0	6	8
Anti-HPL	1	9	4	0
Ki 67	13	1	0	0
Total	14	10	10	21

Anti-hCG

Anti-hCG immunostaining was predominantly intense (Figure 3) in 92.86% of cases, with no negative results.

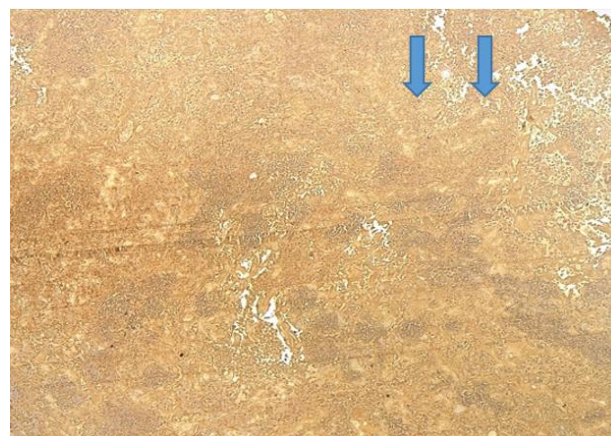


Figure 3: Anti-hCG immunostaining (X10, blue arrows indicating intense membrane staining greater than 50%).

Anti-CK18

Anti-CK18 immunostaining was predominantly intense (Figure 4) in 57.14% of cases, with none being negative.

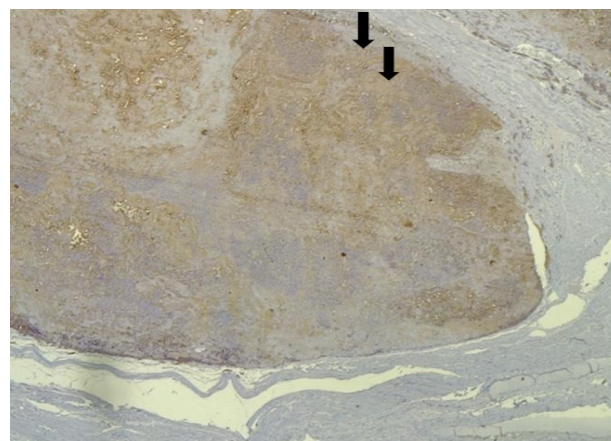


Figure 4: CK18 immunostaining, (X10, black arrows indicating intense membrane staining of the syncytiotrophoblast greater than 50%).

Anti-hPL

Anti-hPL immunostaining was predominantly weak in 64.29% of cases and moderate in 28.57% (Figure 5).

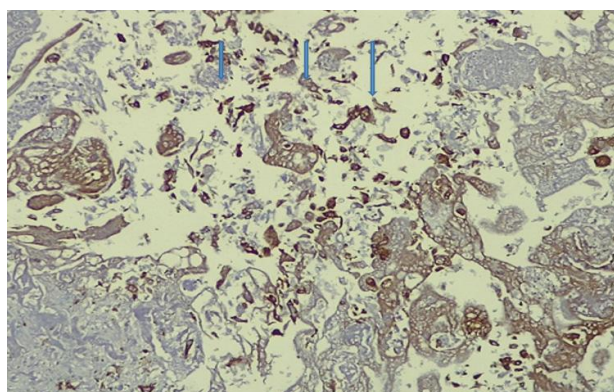


Figure 5: hPL immunostaining (X10, blue arrows indicating moderate membrane staining of 30% of the intermediate trophoblast).

Anti-Ki67

Ki67 immunolabelling was predominantly negative at 92.86%.

DISCUSSION

Epidemiological aspects

Gestational choriocarcinoma is a malignant tumour of the villous trophoblast, lacking placental villi.^{6,7}

Studies have shown a high incidence of gestational choriocarcinoma ranging from 23 to 335 per 100,000 pregnancies.^{8,9,14} Teoh, in Singapore, also reported a rate of 1 per 4,298 deliveries.¹⁵ Gueye et al in Senegal recorded 878 cases of patients with choriocarcinoma between 2011 and 2017.¹⁶ In the United States, 203 cases of choriocarcinoma were collected by Louise between 1973 and 1982.¹⁷

However, other authors observed a low frequency of gestational choriocarcinomas, with rates varying between 2 and 7 per 100,000 pregnancies.^{13,18} In Pital's 2002 study (England), only one case of choriocarcinoma was noted out of 5,976 cases of trophoblastic disease.¹⁹

We collected 25 cases of gestational choriocarcinoma, representing an annual frequency of 3.12, which can be considered low.

The high incidence of gestational choriocarcinoma observed in some studies may be due, on the one hand, to diagnosis by imaging (ultrasound and MRI) on ovular debris and hysterectomy specimens and, on the other hand, to the probable inclusion of other trophoblastic diseases, particularly invasive mole.

Low socioeconomic status may be a factor contributing to the occurrence of gestational choriocarcinoma due to the lack of follow-up of high-risk patients.

The low incidence of gestational choriocarcinoma in our study, as in other series, could be explained by the rigorous recruitment of choriocarcinoma cases, as the diagnosis was based solely on hysterectomy specimens.

Improvements in diagnostic and therapeutic resources in many countries may play a decisive role in the decline in the incidence of gestational choriocarcinoma.

In general, gestational trophoblastic disease rarely occurs in patients who are past reproductive age.²⁰ African studies have reported a mean age of patients with gestational choriocarcinoma of 36 years or less and found that the majority of cases occurred in women under 40 years of age.^{8,10}

In our cohort, 52% of patients were under 40 years of age, with a mean age of 38.5 years, consistent with other African studies.

In some European countries and in the United States, cases of gestational choriocarcinoma in patients over 40 years of age have been observed.^{5,21,22} In England, Pital found 13 cases of choriocarcinoma in patients aged 45 and over. Jane (USA) reported a very high rate among mothers aged 40 or over, where the risk (171.7 cases per 100,000 women) was approximately 13 times higher than the overall risk (12.4 per 100,000) among women under 40.²³ This occurrence of choriocarcinoma in people over the age of 40 is thought to be linked to the higher risk of molar pregnancy in this age group. There is an increased risk of choriocarcinoma associated with pregnancies in older women.²²

Multiparous women ranked first, accounting for 57% of cases in our series. There is a very strong correlation between multiparity and the occurrence of choriocarcinoma. Boufettal, in Morocco, reported 62.5% of gestational choriocarcinomas in multiparous patients, 12.5% in nulliparous patients and 25% in pauciparous patients.⁹ This high frequency of gestational choriocarcinomas in multiparous women was also noted in a Senegalese study, which found 78.7% of women to be multiparous.⁸

Choriocarcinoma most often follows a molar pregnancy or persistent gestational trophoblastic disease (50% of cases) but can occur after a spontaneous miscarriage (25% of cases) or after a normal full-term pregnancy (22.5% of cases). Rare cases (2.5%) have been described in ectopic pregnancies, particularly tubal pregnancies. Exceptional intraplacental choriocarcinomas have been diagnosed incidentally after histopathological analysis of mature or immature placentas, sometimes associated with foeto-maternal transfusion or foetal metastases.⁵

Hydatidiform moles (HM) represent a real public health problem due to their incidence and their risk of developing into gestational choriocarcinoma.²⁴

Hydatidiform mole is the main risk factor for choriocarcinoma (relative risk of 2,000 to 4,000, making it probably one of the greatest known risk factors for any disease).¹¹

In countries with poor healthcare systems, hydatidiform moles can become invasive or, in rare cases, lead to gestational choriocarcinomas.^{25,26}

Choriocarcinomas following molar pregnancies accounted for 76.47% of cases in our study.

This preponderance of choriocarcinomas in molar pregnancies has been reported by several other authors (Table 3).

Table 3: Distribution of history of hydatidiform mole in patients with gestational choriocarcinoma according to the authors.

	This series	Boufettal et al ⁹	Cisse et al ⁸	Khabouze et al ³⁰	Dreyfus et al ³¹
History of mole (%)	76.4	5	10	100	50
Number of cases (N)	25	43	61	24	124

Choriocarcinoma develops after 2-3% of hydatidiform moles, more often after a complete mole than after a partial mole.¹³ After a complete hydatidiform mole, approximately 15 to 20% of patients are treated for gestational trophoblastic neoplasia.²⁷ An invasive mole is observed in 15% of cases and metastatic disease in 5% of cases.²⁷ After a partial mole, local invasion occurs in up to 3 to 5% of cases and metastatic disease is rare.²⁸ The overall incidence of gestational trophoblastic neoplasia is approximately 1/40,000 pregnancies.²⁹

Patients with recurrent molar pregnancies should undergo germline genetic testing for mutations in NLRP7 and KHDC3L.²¹

The time between the last pregnancy and gestational choriocarcinoma is an important parameter. This time interval was not studied in our series. However, according to Cissé, the median time between molar abortion and diagnosis of choriocarcinoma was three months.⁸

Other risk factors, such as ABO blood groups, have been reported in an American study showing a predominance of group A in women with gestational choriocarcinomas and in a Moroccan study.^{21,10}

The majority of samples received by pathological anatomy laboratories came from hospitals, which constitute the top of the healthcare pyramid, accounting for 53% of cases, followed by health centres, which accounted for 35.4% of samples received. This distribution of sample origins can be explained by the fact that in Senegal, the organisation of the healthcare pyramid prohibits facilities at the base from having operating theatres.³² As a result, no cases came from health posts or health huts. However, hospitals, as referral centres, are responsible for performing hysterectomies and then sending the samples to PCA laboratories.

Blood group O was the most common, accounting for 54.1% of cases.¹⁰

This blood group factor was not taken into account in our study.

Clinical aspects

Metrorrhagia is the main warning sign of choriocarcinoma and manifests as unexplained and persistent bleeding for 6 weeks.³³

In our series, half (50%) of the patients consulted for metrorrhagia. According to Régis (France), metrorrhagia was the most common warning sign of choriocarcinoma despite its polymorphic symptomatology.³⁴ In a study conducted at the Ibn Rochd University Hospital Centre in Casablanca, Morocco, metrorrhagia accounted for 79.2% of symptoms.¹⁰

Other signs may be indicative of the disease, such as pelvic pain, complications (anaemia, haemorrhagic shock) and abnormally high beta hCG levels in the follow-up of patients who have had a molar pregnancy.⁸ Beta hCG levels were not studied in our cohort. It is important to note that clinical and imaging findings alone are not sufficient to diagnose any trophoblastic disease, as shown by O. Faye in Senegal in his study, where out of 43 suspected molar pregnancies at the clinic, only 37 were confirmed by histology.³⁵ In another study by the same author, out of 17 cases suspected of being moles at the clinic, 12 cases were confirmed by histology.³⁶

Immunohistochemical aspects

Anti-hCG antibody

Our study revealed that the anti-hCG antibody was positive in all cases (100%), which corroborates the findings of numerous authors. Notably, an Iranian study

revealed intense syncytiotrophoblast staining in a patient who presented with cervical choriocarcinoma. Indeed, beta hCG staining indicates the presence of trophoblast-derived structures constituting tumour proliferation.³⁷ In other words, this staining confirms the diagnosis of choriocarcinoma without specifying whether it is gestational or non-gestational. This is why non-gestational choriocarcinomas have been confirmed thanks to this staining. This is the case in studies conducted by Moran on eight cases of choriocarcinoma in male patients, all of whom had positive HCG immunomarking.³⁸ This was also the case in a Japanese study which confirmed the diagnosis of choriocarcinoma in a male subject through intense HCG staining on syncytiotrophoblasts.³⁹ The importance of this immunostaining in diagnosis is even more evident in cases of tumour combinations, i.e. cases where choriocarcinoma coexists with another type of malignant proliferation. In a study conducted by the Japanese researcher Sohei, we note a patient who presented with malignant tumour proliferation in the lungs, with intense positive expression of beta hCG indicating cytotrophoblast and syncytiotrophoblast proliferation.³⁹ Two diagnoses were therefore made: pulmonary adenocarcinoma and non-gestational choriocarcinoma. The same type of combination was found in the study by Goussot, which involved a combination of gastric adenocarcinoma and choriocarcinoma, as evidenced by intense beta hCG staining of syncytiotrophoblasts.⁴⁰

Unusual locations of choriocarcinomas are most often diagnosed by this immunostaining, as in Satinder's study, where, in the case of a mediastinal tumour, strong staining within the syncytiotrophoblasts led to the diagnosis of choriocarcinoma in this individual.⁴¹ In addition, intense beta hCG staining was observed in a vaginal choriocarcinoma.⁴²

The anti-CK18 antibody

Immunohistochemical staining with CK 18 showed 100% positivity in our study, with mostly very strong intensity. It is a very important marker in the diagnosis of choriocarcinoma, although it is not specific. Several studies have shown that this staining was intensely positive. This was the case in the Chinese study, which found that CK 18 was strongly positive in 100% of 15 cases of gestational choriocarcinoma.⁴³ The Iranian study on a case of cervical choriocarcinoma also revealed strong staining on the syncytiotrophoblast.³⁷ In all these cases of gestational choriocarcinoma, the staining is positive, although the locations differ. In addition, cases of non-gestational choriocarcinoma showed intense positivity for this staining. This was the case in a Chinese study which diagnosed non-gestational ovarian choriocarcinoma in two women, with CK18 staining being intense at 100%.⁴⁴ However, this immunomarking does not only diagnose choriocarcinoma, as in another Chinese study where the diagnosis was an epithelioid trophoblastic tumour, the marking was strong. CK 18 staining therefore does not rule out other trophoblastic diseases.⁴⁵ Rather, this staining

reflects the degree of differentiation of the epithelial cells. Indeed, numerous studies have revealed that CK 18 staining was more intense when the trophoblast cells were well differentiated.^{37,36,43} This is why the staining was intense at the syncytiotrophoblast level and very weak or negative at the intermediate trophoblast level.

Anti-hPL antibody

Our study revealed that immunolabelling was positive in 92.86% of cases. This labelling confirms that the choriocarcinoma is gestational. This is why, in all cases of gestational choriocarcinoma, the labelling is strongly or moderately positive depending on the cell group labelled. The Chinese study, which looked at 15 cases, revealed a 100% positivity rate, leading to a diagnosis of gestational choriocarcinoma.⁴³ This is all the more important when the site is atypical, as in another Chinese study where the site was the cervix and hPL immunostaining was moderately positive, confirming the gestational nature of the choriocarcinoma.⁴⁵

In cases where the gestational nature of choriocarcinoma has been established on the basis of previous pregnancies, it can only be formally confirmed if the immunostaining is positive; otherwise, the choriocarcinoma would be non-related. This was the case in a study of two cases of ovarian choriocarcinoma in two patients who had previously given birth.⁴⁴ The hPL immunostaining was negative, which allowed the diagnosis of non-gestational choriocarcinoma to be formally confirmed. This is of paramount importance in confirming whether or not the choriocarcinoma is gestational, regardless of the patient's obstetric history. It should be noted that some patients may have early miscarriages that go unnoticed, meaning that these patients are considered nulligravid and, as a result, a diagnosis of non-gestational choriocarcinoma is often made as a first-line diagnosis. In these cases, hPL staining is very important because, if positive, it confirms a history of pregnancy. Our study is a perfect illustration of this, as in one of our patients considered nulligravida with doubts about the likelihood of a history of miscarriages, hPL marking was positive, which confirmed with certainty the gestational nature of the choriocarcinoma.

Ki67

Mitotic index staining is very important in the complementary diagnosis of choriocarcinoma. It neither confirms nor refutes the diagnosis, but shows the extent of mitotic activity in the cells. In our series, Ki67 was predominantly negative, which does not correlate with several studies. Indeed, Ki67 was strongly positive in a study of 15 hysterectomies with intense staining of both the cytotrophoblast and the intermediate trophoblast, but staining was weak and sometimes absent in the syncytiotrophoblast.⁴³ These differences with our results could be explained by the fact that our samples were taken as part of a preventive measure, so the pathology was not very advanced, which could be consistent with a low or

absent mitotic index. On the other hand, it could be explained by the fact that in our samples, the intermediate trophoblast was underrepresented, even though it is the cell contingent that responds best to Ki 67.

This study has few limitations. Missing data in the patient information sheet were not studied. These included blood group (ABO system), serum beta hCG level, and time between the last pregnancy and the onset of gestational choriocarcinoma. Not all patients were able to benefit from immunohistochemistry because 11 paraffin blocks containing patient samples could not be found. The immunohistochemical study was only conducted on 14 samples.

CONCLUSION

Gestational choriocarcinoma (GC) is a rare malignant tumour derived from the trophoblast. It is the most primitive and least differentiated tumour that occurs after a molar pregnancy or, more rarely, after a miscarriage, a normal pregnancy or even an ectopic pregnancy.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee of Cheikh Anta Diop University, Dakar, Senegal

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