

Prevalence of malaria infection and reliability of ACCUCARE One step malaria test® for diagnosing malaria in people living with human immunodeficiency virus infection in Cameroon

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ABSTRACT

Aims: This study aimed at determining the malaria prevalence and appraising the diagnostic performances of a rapid diagnostic test (RDT), namely ACCUCARE one step Malaria Test®, for malaria in people living with human immunodeficiency virus infection (PLWHIV).

Study design: This study was a cross-sectional study.

Place and Duration of Study: The study was carried out at the District hospital of Deido in Douala, Cameroon between August 2015 and March 2016.

Methodology: A total of 723 patients were included in the study. Malaria parasites were detected using Giemsa-stained blood films and RDT. The reliability of the RDT was evaluated by calculating the sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy and Kappa index (κ). Results were analyzed and compared using light Giemsa-stained blood films as gold standard. HIV infection was confirmed using methods based on immunochromatography and ELISA. CD4 lymphocytes count was determined by flow cytometry to depict the immune status.

Results: Overall, *Plasmodia* were found in 121 (16.7%; 95%CI: 14.2%-19.6%) and 91 (12.6%; 95%CI: 10.3%-15.3%) using light microscopy and RDT respectively. The sensitivity, specificity, PPV and NPV of the RDT were 75.2% (95%CI: 66.8%-82.1%), 100% (95%CI: 99.4%-100%), 100% (95% CI: 95.9% -100%) and 95.3% (95% CI: 93.3% - 96.7%) respectively. The agreement between both methods was excellent ($\kappa = 0.835$; $P < .0001$).

Conclusion: The study showed a good performance of the RDT in terms of specificity, PPV, NPV, accuracy and agreement. This test might represent a good alternative to the standard method for diagnosis of malaria in PLVIH.

Keywords: Malaria, Rapid diagnostic test, diagnosis performance, HIV-infected people, Cameroon.

28 1. INTRODUCTION

29 Malaria is still a major public health concern throughout the world and especially in Africa.
30 According to the latest WHO estimates, this disease was responsible for 214 million and
31 438,000 cases and deaths respectively in 2015. The burden is more important in Sub-
32 Saharan Africa countries which account for 88% and 90% of all cases of morbidity and
33 mortality [1]. Sub-Saharan Africa (SSA) is also the region the most burdened by the human
34 immunodeficiency virus (HIV) infection. It is estimated that 25.8 million of people live with
35 HIV in SSA, accounting for about 70% of the global total [2].
36 In Cameroon, these both diseases are causes of concern. Malaria is endemic in Cameroon
37 with a prevalence rate of 29% [3]. In addition, malaria accounts for 40 to 50 % of medical
38 consultations, meanwhile 30 to 47 % of hospitalizations [4]. It is the main cause of morbidity
39 in children under five (18%), pregnant women (5%), people living with HIV (5.5%) and the
40 poor (40%) [5]. The prevalence of HIV infection was estimated at 4.3% in 2011 over the
41 country with the highest rates recorded in the South (7.2%), East (6.3%) and North-West
42 (6.3%) regions [6]. Malaria and HIV are both common in SSA and share in some common
43 risk factors including poverty. Due to their overlapping distribution, co-infections with malaria
44 and HIV are frequent in SSA, especially in Cameroon [7]. Some authors reported the
45 prevalence of the co-infection in Cameroon [8-10]. In 2016, Njunda et al. [10] recently
46 recorded a prevalence of 7.3% of Cameroonians living in Yaounde.
47 Malaria is thought to be associated with a morbidity and mortality both increased in people
48 living with HIV (PLWHIV). Globally, the risk of uncomplicated and severe episodes of
49 malaria is higher in HIV patients co-infected with malaria parasites, especially in groups at
50 risk such as pregnant women and children [11-12]. Conversely, malaria parasites increase
51 the ability of the virus to replicate via mechanisms mainly involving an impairment of the
52 immune response of the host [11-13].
53 The key to the proper management of malaria in PLWHIV is prompt and accurate diagnosis
54 followed by appropriate treatment. Diagnosis usually relies on clinic and light microscopy in

55 most malaria endemic areas which are often resources-constrained and remote [2-3]. Many
56 authors reported poor performances of clinical diagnosis in terms of sensitivity and specificity
57 [1, 14]. On the other hand, light microscopy is used as a gold standard to confirm any clinical
58 suspicion of malaria in some healthcare facilities of these countries especially in Cameroon.
59 This method is cheap and simple but labour intensive, time consuming, cannot detect
60 sequestered *Plasmodium falciparum* parasites and requires well-trained personnel
61 especially for detection of low parasite densities [15]. Overcoming these limitations is very
62 important in order to avoid unnecessary use of artemisinin-based combination therapies
63 (ACTs), due to overdiagnosis, as this could lead to the development of drug resistance [1].
64 Furthermore, a significant proportion of patients who need treatment could not promptly
65 receive it or not at all. As a result, the risk of malaria-associated severe adverse effects and
66 deaths might be increased in malaria infected individuals. This matter takes sense when
67 over- and underdiagnosis fractions are mainly important in groups at risk such as children,
68 pregnant women along with immunocompromised people as those infected with human
69 immunodeficiency virus.

70 It looks critical to develop and implement new diagnosis alternative upon their evaluation in
71 community especially in remote areas. Rapid diagnostic tests (RDTs) based on
72 immunochromatography have been developed to address the limitations of light microscopy.
73 RDTs are simple and easy to use with little expertise and skills required for interpretation.
74 There are numerous malaria RDTs that are commercially available in many formats and the
75 variation depends also on malaria antigen or antibody targeted. The commonly used RDTs
76 target *P. falciparum* histidine-rich protein 2 (PfHRP2) and two enzymes in the parasite
77 glycolytic pathways, namely plasmodial lactate dehydrogenase (pLDH) and aldolase [16].
78 Others RDTs target antibodies specific to malarial antigens by using recombinant antigens
79 first pre-coated on the membrane strip of the RDT [16]. ACCUCARE one step Malaria Test®
80 RDT is one of a few RDTs present in the Cameroonian market and used for diagnosing
81 malaria infection in some health facilities of the country. Many authors have addressed the

issue about the utility of RDTs in malaria endemic areas [16-18] but however, little is known in Cameroon [19-22] especially on the ACCUCARE one step Malaria Test[®] RDT and PLWHIV.

Thus, this study aimed to determine the prevalence of malaria infection and appraise the diagnostic performances of the ACCUCARE one step Malaria Test[®] RDT for malaria in HIV-infected people.

2. MATERIAL AND METHODS

2.1 Study site

This study took place in the town of Douala (Littoral Region, Cameroon). Douala is located 3°48'N, 10°08'E, near the Atlantic coast, within the Congo-Guinean phytogeographical zone characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November [23]. The city is 1 m above sea level and receives over 3,500 mm rainfall annually. These environment conditions are propitious for creation of breeding sites malaria vectors. Douala is a port city where many worse behaviors (liquors consumption, prostitution) significantly increase the risk of sexually-transmitted diseases (STDs) such as Human immunodeficiency virus infection. Douala is the main business city of the country and is ranked sixth (5.5%) in terms of HIV prevalence rate [6]. Participants were recruited at the district hospital of Deido which greets people coming from all parts of Cameroon owing to its strategic location and sustainable and constant supply with CD4 cells reagents and antiretroviral drugs for management of people living with HIV (PLWHIV).

2.2 Study population

The study population consisted of individuals aged 5-49 years old of both sexes living with HIV and under highly active antiretroviral therapy. They were recruited in a convenient way and therefore a total of 723 patients were included in the study. Using the prevalence of 29.4% reported by Nkuo Akenji et al. [24], the sample size was determined

109 using the Lorentz's formula $n = Z^2pq/d^2$ where n = the sample size required, $Z = 1.96$:
110 confidence level test statistic at the desired level of significance, $p = 29.4\%$: proportion of
111 HIV prevalence, $q = 1-p$: proportion of malaria negative participants and d = acceptable error
112 willing to be committed. The minimum sample size was estimated as $n = 319$. Any patient
113 who did not meet any of these abovementioned criteria were excluded from the study.

114

115 **2.3 Study design**

116 This cross-sectional and prospective study was carried out between August 2015 and March
117 2016. Participants included in the study were HIV-infected, attending at the district hospital
118 of Deido for routine control and willing to participate. Prior to their inclusion, they were given
119 information, education and communication on malaria and HIV infection. Thereafter, an
120 informed consent form was signed by each participant following explanation of objectives of
121 the study to them. Approval of parents or guardians of children was also sought. A structured
122 questionnaire was used to collect socio-demographic, clinic and biological data. Axillary
123 temperature was recorded using an electronic thermometer. Blood samples were collected
124 and transported to the Laboratory of the district hospital of Deido for parasitological
125 analyses. Investigative methods included a questionnaire approach, clinical and
126 parasitological analyses.

127

128 **2.4 Blood collection**

129 About five milliliters (5 mL) were collected from each participant by venipuncture into sterile
130 plastic syringes. Blood samples were then transferred into EDTA and dry tubes for all
131 performing hematological, parasitological and biological analyses respectively. All tubes
132 were labeled with the patients' barcode and pathology number. Blood samples were
133 centrifuged at 3000 rpm for 3-5 minutes and sera were stored at 2-8°C until analysis.

134

135

136 **2.5 Confirmation of HIV infection**

137 Prior to their inclusion in the study, the participants were tested for HIV infection for
138 confirmation. HIV diagnosis was performed using Determine Alere HIV 1/2 rapid diagnosis
139 test followed by Immunocomb HIV1/2 (Organics) accordance to the national guidelines. The
140 former test is an immuno-chromatographic rapid test for qualitative detection of antibodies
141 specific to HIV in human serum, plasma or whole blood. The latter is based on the enzyme-
142 linked immunosorbent assay (ELISA) principle.

143

144 **2.6 CD4 cells count**

145 CD4⁺ T lymphocytes were counted with a flow cytometer CyFlow® (Sysmex-Partec Görlitz,
146 Germany) according to the manufacturer's instructions. Briefly, 20 µL of phycoerythrine-
147 conjugated monoclonal antibody to human CD4 (mAb PE MEM241, Partec GmbH, Görlitz,
148 Germany) were slightly mixed with 20 µL of whole blood into a test tube and incubated for 15
149 minutes at room temperature, protected from light. Then, 800 µL of no-lyse buffer were
150 added to the mixture. After homogenizing the content, the tube was introduced into the
151 CyFlow Counter for automatic counting [25]. The CD4 results were grouped into as followed:
152 < 200; 200-350; 350-500 and > 500 cells/µL.

153

154 **2.7 Malaria diagnosis**

155 Two methods were used to diagnose malaria infection namely Giemsa-stained blood films
156 and the ACCUCARE one step Malaria Test® RDT. Asymptomatic malaria was defined as the
157 presence of malaria parasite with an axillary temperature of <37.5°C. Symptomatic malaria
158 was defined as the presence of malaria parasite with an axillary temperature of ≥ 37.5°C
159 [26].

160

161

162

2.7.1 Thick blood smears

Thick blood films were performed using the protocol previously described by Cheesbrough (2004) [15]. Briefly, thick smears that were air-dried for 30 minutes, was stained with 10% Giemsa for 20 minutes. Thereafter, stained slides were allowed to air dry and stored not more than one day until microscopic examination. Microscopy was used for identification of malaria parasites by a senior. Thick blood films were considered positive when asexual forms (trophozoites and schizonts) and or gametocytes were present in the blood film. Slides were declared negative after observing at least 100 high power fields without detecting any parasites. In order to ensure quality assurance of parasitological data, thick smears-based results were classified as valid (positive or negative slides) and invalid (not read slides) as outlined in literature [26].

2.7.2 ACCUCARE one step Malaria Test®

This rapid diagnostic test (Labcare Diagnostics, India) is used to qualitatively detect antibodies of all isotopes (IgG, IgM, IgA) specific to *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) simultaneously in blood samples (serum, plasma and whole blood). This consists of one control band and two test bands. The former test band, referred to as test band 1, is pre-coated with recombinant malaria Pf capture antigen (MSP, CSP) meanwhile the test band 2 with recombinant malaria Pv antigen (MSP, CSP). The recombinant malaria Pf/Pv antigen-colloid gold conjugate and serum sample moves along the membrane chromatographically to the test regions and forms a visible line outlining the presence of antibodies translating a malaria infection [27].

The diagnosis of malaria infection using this test was performed according to the manufacturer's instructions. Briefly, 10 µL of serum was added to the sample well "S". Then, 1 drop of the assay chase buffer was added. Results test were read after 10 to 20 minutes [27].

2.8 Ethical approval

This study was carried out in conformance with the guidelines for human experimental models in clinical research as stated by the Cameroon Ministry of Public Health and the Helsinki declaration. Besides, the ethical clearance was sought close to Institutional review board (IRB) of Douala under N° CEI-UD/273/10/2015/T. The aim and objectives of the study were explained to them in the language they understood best (French or English), and their questions were answered. Only individuals who signed an informed consent form for their participation were enrolled. Participation in the study was strictly voluntary and patients were free to decline answering any question or totally withdraw if they so wished at any time. All malaria positive patients were treated on the spot accordance to the national treatment guidelines.

2.9 Statistical analyses/methods

All data were verified for consistency, coded, and keyed in an Excel sheet. Thereafter, statistical analyses were performed with SPSS 20.0 for Windows (SPSS, Chicago, IL, USA). Data were summarized in table as percentages with 95% confidence interval (95%CI) or mean $\pm$ standard deviation (SD) for qualitative and quantitative variables respectively where appropriate. Student's test was used to compare differences for normally-distributed variables. Chi-square test (χ^2) or Fisher's exact probability were computed to compare categorical variables. McNemar's test was used to compare malaria-based results between light microscopy and RDT. Significant levels were measured at 95% CI with significant differences recorded at $P < .05$.

After all the tests were performed the values for sensitivity, specificity, predictive values and accuracy were calculated using light microscopy as a gold standard. The variables measured were the number of true positives (TP), number of true negatives (TN), number of false positives (FP) and number of false negatives (FN). Sensitivity was calculated as $TP/(TP+FN)$, Specificity was calculated as $TN/(TN+FP)$, Positive predictive value (PPV) was

217 calculated as $PPV = TP / (TP + FP)$, Negative predictive value (NPV) and accuracy were
 218 calculated as $NPV = TN / (TN + FN)$, Accuracy = $(TP + TN) / \text{number of all tests}$, respectively.
 219 The agreement between light microscopy and RDT was appraised by computing the
 220 Cohen's Kappa index [28]. Its interpretation was made based on the scale by Landis and
 221 Koch (1977) who splitted the kappa index into five categories namely very poor (< 0.00),
 222 poor ($0.00-0.20$), moderate ($0.21-0.60$), good ($0.61-0.80$) and excellent (≥ 0.81).

223

224 3. RESULTS

225 3.1 Baseline characteristics of the participants

226 A total of 723 patients were included in the study. Majority of them were females (74.3%; $P <$
 227 .0001). The mean age was 39.49 ± 11.17 years old and this variable ranged between 4 and
 228 74. In addition, patients aged 20-49 years old and having CD4 cells count > 500 cells/ μL
 229 accounted for more than two thirds (79.7%) and the third (32.9%) of the participants (Table
 230 1). The mean CD4 count of these patients was 427 cells/ μL (sd = 257).

231

232 **Table 1. Baseline characteristics of the participants and their relation to malaria**
 233 **infection**

Variables	Levels	Total		Positive ^s (%)	P-value
		n	%		
Gender	Female	541	74.3	70 (12.9)	0.6220
	Male	182	25.7	21 (11.5)	
Age (years)	≤ 19	13	1.8	0 (0.0)	0.3750
	[20 - 50[	576	79.7	73 (12.7)	
	≥ 50	134	18.5	18 (13.4)	
CD4 count (cells/ μL)	< 200	122	16.9	29 (23.8)	0.0991
	200 - 350	192	26.6	32 (20.0)	

350 - 500	171	23.6	22 (12.9)
> 500	238	32.9	38 (15.9)

Data are presented as frequency (percentage). Independent chi square was used to compare proportions. P-value < 0.05 was considered as significant. §: malaria prevalence was calculated based on the gold standard (Giemsa-stained blood films).

3.2 Prevalence of malaria

Overall, *Plasmodia* were found in 121 (16.7%; 95%CI: 14.2%-19.6%) and 91 (12.6%; 95%CI: 10.3%-15.3%) using Giemsa-stained blood films and RDT respectively. In addition, a statistically significant difference ($P < .0001$) was found using the McNemar's test. All patients infected with malaria parasites were asymptomatic and *Plasmodium falciparum* accounted for all case of infection. As presented in Table 1, malaria prevalence was higher in females (12.9%) and patients having CD4 cells count < 200 cells/ μ L (23.8%). On average, malaria prevalence was increasing with age of participants. Malaria prevalence was highest in above 49 years old (13.4%). Furthermore, none statistically significant differences were found (Table 1).

3.3 Diagnostic performances of ACCUCARE one step Malaria Test®

Out of 121 positive tested by the Giemsa-stained light microscopy, 91 have been found to be positive by the RDT while 30 were negative. On the other hand, out of 602 which were negative by Giemsa stained light microscopy, none were positive by the RDT (Table 2). Thus, the sensitivity and specificity of RDT was 75.2% and 100% respectively as summarized in Table 3. Besides, the agreement between both methods was excellent ($\kappa = 0.835$; $P < .0001$). This matter was a good concordance between the methods because of an accuracy of 95.8% (Table 3).

258 **Table 2. ACCUCARE one step Malaria Test[®] results compared to Giemsa stained light**
 259 **microscope**

		RDT results		
		Positive	Negative	Total
Giemsa stained light microscopy results				
	Positive	91 (TP)	30 (FP)	121
	Negative	0 (FN)	602 (TN)	602
	Total	91	632	723

260 **Data are presented as frequency. RDT = Rapid diagnosis test, TP = True positive, FP = False positive, FN = False*
 261 *negative, TN = True negative*

262

263

264 **Table 3. Diagnostic performance of ACCUCARE one step Malaria Test[®] using light**
 265 **microscopy as gold standard**

Performance	Values
Sensitivity, % (95%CI)	75.2 (66.8-82.1)
Specificity, % (95%CI)	100% (99.4-100)
Positive predictive value, % (95%CI)	100% (95.9-100)
Negative predictive value, % (95%CI)	95.3 (93.3-96.7)
Accuracy, % (95%CI)	95.8 (94.1-97.1)
Kappa index, (standard error, <i>P</i>)	0.835 (0.029; <i>P</i> < .0001)

266 **95%CI: Confidence interval; P < .05 are considered significant*

267

268

269

270 4. DISCUSSION

271 Reliable and convenient **diagnostic** method is critical to fight against malaria infection. Clinic-
272 and microscopy-based diagnoses are commonly used in malaria endemic areas which are
273 often **resource**-constrained. Unfortunately, several concerns emerged about their cost-
274 effectiveness to efficiently control malaria due to their intrinsic limitations. Clinic diagnosis is
275 low sensitive and specific mainly in **high** transmission areas. Microscopy is time-consuming,
276 labour-intensive and depends on quality of reagents and competence of the microscopist.
277 Thus, rapid diagnostic tests (RDTs) may represent an interesting and reliable alternative
278 especially in remote areas where electricity is missing. This study aimed at appraising the
279 performances of a RDT for diagnosis of malaria in people living with HIV infection.

280

281 4.1 Prevalence of malaria infection

282 The malaria prevalence **by light microscopy** was 16.7% in the participants. This value is
283 lower than that found by many reports [29-31]. These authors found 31.76%, 24.0% and
284 74.3% respectively using the light microscopy as well. Conversely, our value is higher than
285 the 11.75% found by Tay et al. [32] in Ghana. Differences in sample size, study design,
286 study period, study area along with genetic background and behavioral patterns of
287 individuals can explained the discrepancies observed between these study and ours.

288

289 4.2 Association between malaria infection and CD4 cells count

290 **In this study, we found negative, although not significant, between malaria prevalence and**
291 **CD4 lymphocytes counts. However, our finding is quite consistent with Van Geertruyden et**
292 **al. [33] who outlined an slower increasing in CD4 cells counts in malaria-infected HIV**
293 **patients than their malaria uninfected counterparts after successful antimalarial treatment.**
294 **Furthermore, Chavale et al. [34] also outlined the CD4⁺ T cell counts were significantly lower**
295 **in the *Pf*/HIV group than in the HIV/AIDS only or malaria only patients in Mozambique. Thus,**
296 **HIV and malaria diseases are both controlled by immunity, and decreasing immune status**

297 as found in HIV patients will cause an increase in malaria severity. Besides, this matter
298 outlines the need for diagnosing malaria infection in PLHIV during follow up.

299

300 **4.3 Diagnostic performances of the ACCUCARE one step Malaria Test[®]**

301 A sensitivity of 75.2 % (95%CI: 66.8%-82.1%) was recorded in this study. This value is lower
302 than that of Suchita et al. [35] and Khatib et al. [36] who found 95.24% and 92.95% in India
303 using the same RDT. Ali et al. [21] found values of specificities greater than 90%, on
304 average, by appraising a set of 22 RDTs available in the Cameroon market. These
305 discrepancies may be explained by the spatial and time variability of confounders such as
306 age structure of population or coverage rate of malaria-related preventive methods. Ayele
307 and colleagues [37-38] have addressed this issue in details. Our finding outlines the fact that
308 the ACCUCARE one step Malaria Test[®] RDT was not in line with the gold standard in 25% of
309 patients. Thus, a significant fraction of population would not have received treatment if the
310 test was used alone for malaria diagnosis. As a result, starting on malaria-related treatment
311 will have been delayed in these patients and consequently increasing the risk of severe
312 malaria.

313 Specificity of 100% was recorded and therefore no falsely-positive case was reported in this
314 study. This is in line with Suchita et al. (2013) [35] but slightly different from previous studies
315 [20-22, 36]. For instance, Sayang et al. (2009) found a specificity of 82.2% using the
316 Diaspot[®] RDT in outpatients in a peripheral urban health facility in Cameroon [20]. However,
317 these findings witness all the highly specific nature of the ACCUCARE one step Malaria
318 Test[®] to exclude any case of malaria in malaria-related asymptomatic PLWHIV. It is
319 particularly important in the context of reducing drug exposition to patients who do not need
320 antimalarial drugs. Thus, overtreatment of patients could efficiently mitigated and
321 consequently the drug pressure which accounts mainly for appearance and spreading of
322 drug-resistant malaria parasites. This would be helpful to extend the use of artemisinin-
323 based therapeutic regimens used for treating malaria in most of endemic countries [1].

324 Besides, it would be interesting to investigate the clinical utility of this RDT in malaria-related
325 febrile HIV patients in further studies in our context.

326 Positive and negative predictive values of the ACCUCARE one step Malaria Test[®] RDT were
327 high (100% and 95.3% respectively). Our results are higher than those found by previous
328 authors in Cameroon [20, 22, 39]. Positive and negative predictive values estimates the
329 probability for a patient of being declared positive (negative) with the gold standard given this
330 patient was positive (negative) with the RDT respectively. This RDT might therefore be a
331 good adjunct to light microscopy in certain conditions for diagnostic of PLWHIV. Indeed,
332 given these characteristics, this might be particularly helpful are in remote malaria endemic
333 areas along with hard-to-reach population where health facilities and microscopes are not
334 often available. Besides, electricity source is also often missing which is needed to perform
335 microscopy-based malaria diagnosis. Thus, utilization of RDTs, which does not request
336 electricity, of high diagnostic performances would be particularly cost-effective for control
337 malaria in such areas. This assumption is worth investigating in the next coming years in our
338 context.

339

340 **5. CONCLUSION**

341 The study showed a good performance of the RDT in term of specificity, PPV, NPV,
342 accuracy and agreement. Thus, this test might represent a good alternative to the standard
343 method for diagnosis of malaria parasites in asymptomatic carriers co-infected with HIV.
344 Further studies are needed to confirm its long term performances along with others
345 assumptions raised in this study.

346

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349 the district hospital of Deido for their technical assistance.

350

351 **COMPETING INTERESTS**

352 Authors have declared that no competing interests exist.

353

354 **AUTHORS' CONTRIBUTIONS**

355 This work was carried out in collaboration between all authors. Author LWK designed the
356 study, wrote the protocol, carried out laboratory work and wrote the first draft of the
357 manuscript. Author JPAA designed the study, read and corrected the manuscript. Author
358 LPKF performed statistical analyses, read and corrected the manuscript. Author HLKF
359 designed the study, wrote the protocol and supervised the work at all stages All authors read
360 and approved the final manuscript.

361

362 **CONSENT**

363 All authors declare that 'written informed consent was obtained from the patient (or other
364 approved parties) for publication of this original research article. A copy of the written
365 consent is available for review by the Editorial office/Chief Editor/Editorial Board members of
366 this journal.

367

368 **DISCLAIMER**

369 The findings and conclusions in this paper are those of the authors and do not necessarily
370 represent the views of any of their affiliated Research Institutions. Furthermore, mention of
371 any company name or product does not constitute endorsement by the authors. None
372 financial support has been allocated for this study.

373

374 **ETHICAL APPROVAL**

375 All authors hereby declare that all experiments have been examined and approved by the
376 appropriate ethics committee and have therefore been performed in accordance with the

377 ethical standards laid down in the 1964 Declaration of Helsinki. An ethical clearance was
378 sought close to Institutional Review Board (IRB) of Douala under N° CEI-UD/273/10/2015/T.
379

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