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PRO-INFLAMMATORY LOAD AND QUALITY ASSESSMENT OF RED BLOOD CELL CONCENTRATES PRODUCED AT THE YAOUNDE UNIVERSITY HOSPITAL BLOOD BANK FOR GOOD TRANSFUSION PRACTICES

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ABSTRACT

Blood transfusion is one of the most sensitive activities in a healthcare system. It is therefore vital to ensure the quality of the various labile blood products (LBP), which is why we were interested in the quality of the red blood cells (CGR) produced at the Yaoundé University Hospital Centre (CHUY). The study was prospective and descriptive, cross-sectional, and involved 150 RGCs. It took place from November 2023 to February 2024, with a collection period from March 2024 to June 2024. Our study focused on erythrocyte concentrates from labile blood products prepared and qualified by the CHUY blood bank. Erythrocyte concentrates judged valid after serological analysis and ready for distribution to the patient were included in this study. Expired RGCs, RGCs with cracked bags, RGCs containing clots and haemolysed RGCs were excluded from the study. We were able to obtain 150 samples, the majority from men (131, 87.33%) and only 19 from women (17.67%). The bags included were weighed to determine the volume of their contents. Haemoglobin and haematocrit were determined using the HumaCount 5D automated system. The accuracy and precision of the automated system were checked to ensure the reliability of the data analysed. Statistical analyses were performed using Excel graphpad 5.03 software. Of the RGCs obtained, 87.33% were from men and 17.67% from women. However, the majority were volunteers (92.66%). Nevertheless, 98.67%, 52.67% and 43.33% were compliant for volume, haemoglobin and haematocrit respectively. Considering the three parameters simultaneously, the compliance rate was 81.35% for male RGCs compared with 66.66% for female RGCs, and 86.66% for volunteer donor RGCs compared with 61.54% for family donor RGCs. In the course of this study, quality control enabled us to highlight non-compliances in the RGCs prepared and to make assumptions about the shortcomings in order to verify and remedy them. However, achieving a good percentage in terms of the quality of RGCs is an objective that can easily be achieved through vigilant and rigorous application of good transfusion practice. For this reason, it would be important for staff to be qualified and trained on an ongoing basis. It would therefore be enviable to broaden the scope of action by carrying out a similar study in all blood banks to assess all the problems linked to the quality of RGCs in order to find solutions. Ongoing staff training is recommended, as is their involvement in the establishment's quality policy. Continue to encourage the loyalty of blood donors, as blood from loyal donors offers greater transfusion safety, and extract all the plasma during the preparation of RGCs in order to correct the volume of the latter, and finally use anticoagulant solutions such as SAG-M.

KEYWORDS: Quality of red blood cells, Blood transfusion, Transfusion safety, Blood bank, Compliance rate.

INTRODUCTION

Blood transfusion is a complex therapeutic procedure which consists of providing a patient, called the recipient, with the blood elements he or she temporarily lacks by intravenous infusion.[1] In transfusion therapy, RGCs are used primarily for symptomatic anaemia, with the aim of improving the patient's haemoglobin (Hb) level. To ensure an effective transfusion, the right product must be used in the right patient at the right time and in the right place.[2] Conformity assessment of the erythrocytes in blood bags produced in blood banks would guarantee maximum effectiveness and minimise any risk of contamination for the recipient. In view of the rules governing the effectiveness of these products in improving patient well-being. A great deal of progress has been made in recent years to improve the quality of erythrocyte concentrates. These include the popularisation of several international standards, including those of the African Society for Blood Transfusion (AFSBT) and WHO, whose aim is to provide a reference for the accreditation of establishments and to improve the quality and safety of blood transfusion in Africa.[3] The transfusion of RBCs is necessary in a variety of situations such as haemorrhage, erythrocyte disease, surgery or in the treatment of certain cancers.[4] Blood transfusion is the transfer of blood or blood components (donor) to another (recipient). It can be vital, which is why health services must ensure an adequate supply of safe, healthy blood, monitor the quality of erythrocyte concentrates produced in blood banks and ensure that they are used judiciously.[5,6,7] Blood safety and availability. Quality blood transfusion is ensured by blood safety and is part of modern healthcare worldwide. Blood banks collect almost 112.5 million units of blood every year. Almost 47% of these blood donations are collected in modern countries and represent less than 19% of the world's population (Baseline assessment findings of the Africa Society for Blood Transfusion Step-Wise Accreditation Programme in 10 sub-Saharan African countries 2016-2018 which is woefully inadequate for the world's population and causes blood shortages.[8] However, by fractionating blood into its various components, it will be possible to treat several patients with a single fraction of blood, transfusing the patient with the element he or she needs.[9] This will save the other fractions available. Erythrocyte concentrations are obtained by eliminating the plasma and white blood cells in the blood bag using a centrifugation technique in a bag containing an additive solution, or by apheresis. It is advisable to perform transfusions during surgery in the event of anaemia, depending on the individual.[10] In Cameroon, the process of producing, transporting and storing erythrocyte concentrates is compromised because demand outstrips supply. Around 400,000 bags of safe blood are needed each year to treat patients. It is important to note that the current supply is 90,000 bags.[11] The National Blood Transfusion Centre is therefore responsible for ensuring the blood supply for patients requiring transfusions, and is also responsible for guaranteeing the quality of blood and blood products for hospital use through the quality control programme. As a result of all these difficulties, the Cameroonian government is planning to build and equip a national blood transfusion centre. Until now, blood products have been produced in Cameroon by blood banks. In Cameroon, between 300,000 and 400,000 blood transfusions are not carried out each year due to a lack of blood.[11,12] In 2021, 140.207 blood bags were collected. This figure is not enough to meet national blood requirements, which are estimated at 400,000 blood bags a year.[11,13,14,15,16] In the light of these figures, the need for transfusion worldwide, and on the African continent in particular, is strongly felt in health facilities. Assessing the quality of each bag collected and its derivatives is becoming an essential step in the transfusion process. Red blood cell concentrate (RBC), also known as concentrated erythrocyte concentrate, is a labile blood product obtained from whole blood and mainly made up of erythrocytes. They are used for blood transfusions and can be phenotyped, counted, irradiated or deplasmatised, depending on the patient's needs.[13,14] It is used in several clinical situations such as acute anaemia, major bleeding or restoration of oxygen transport capacity. Given the importance and sensitivity of transfusion in general and of RBCs in particular, we proposed to carry out a study on the 'evaluation of the quality of red blood cell concentrates produced at the Yaoundé University Hospital blood bank', in which the main aim will be to determine the conformity of the red blood cell concentrates distributed at the Yaoundé University Hospital in relation to the references.

MATERIALS AND METHODS

Study setting

The study was conducted at the CHUY blood bank. The haematology/blood bank department is located on the first basement floor and has two large main rooms for processing the various samples and handling blood donors. This blood bank was chosen as the study site because of the quality criteria it meets.

2.2 Sampling and administrative procedures

We collected and analysed a total of 150 RGCs. We subsequently obtained collection authorisation from the HGOPY ethics committee, as well as ethical clearance N°4593CEI-UDo/08/2024/M from the ethics and institutional committee of the university of Douala.

Equipment and reagents

To determine the volume of RGCs, we used the 'Sartorius' balance, model 'TE6101', which is sensitive and well regulated. The various haematological parameters were determined using the Huma Count 5D haematology analyser. This instrument can operate either in CBC mode (blood cell count system), DIFF mode (CBC + leucocyte count) or RET mode (reticulocytes). The haemoglobin level and haematocrit of each RGC were determined with this machine using CBC mode. We also used a stripper to homogenise the blood in the bag

and tubing before taking the blood sample for analysis, and a sealer to close the tubing after taking the blood sample. During sampling, care gloves, cotton wool, adhesive tape, skin antiseptic, needles, tubes, blood bags and trays. A cooler was used to transport the blood bags. In terms of reagents, Huma Count 5D is used exclusively with the following reagents: HC5D-diluent for dilution, the sheathing and cleaning, HC5D-Clean for cleaning, HC5D CBC-Lyse and HC5D-Diff-Lyse for haemoglobin measurement and HC5D Control, HC-Calibrator for the concentrated cleaning procedure and a microscope for reading and smear slides to assess the shape of the red blood cells. The refrigerators, cold room and water bath must be well monitored. The centrifuge was well calibrated, and a micropipette was used for calibration. A sealing machine guaranteed protection against contamination of the RGCs. Reagents (check their effectiveness when each batch is opened).

Type of study

This was a prospective descriptive cross-sectional study. The study ran from November 2023 to February 2024, with a collection period of 8 months from March 2024 to June 2024. The objective was erythrocyte concentrates of labile blood products prepared and qualified by the CHUY blood bank.

Selection criteria

This study included erythrocyte concentrates judged to be valid after serological analysis and ready for distribution to the desired patient. Expired RGCs, RGCs with cracked bags, RGCs containing clots and haemolysed RGCs were excluded from this study.

Sample size and sampling plan

The size of our sample is determined by the formula:

$n=z \times 2 \times p\ (1-p)/m \times 2$

n = sample size

z = confidence level according to the reduced central normal distribution (for the standard value of the 95% level will be 1.96 for =5%

p= estimated proportion of the population; m= tolerated margin of error = 5%

From the numerical application it emerges that:

$n= (1.96) \times 2 \times (0.5)\ (1-0.5)/(0.05) \times 2$

$n= 384.16$

This will be the number of RGC bags to be collected and processed for our sample to be representative of our population.

Methods

Variables studied

Volume of RGC contained in each bag: we weighed the RGC bags using the digital balance, which was calibrated. We also weighed the mass of an empty bag using the same balance. The values were recorded in the Excel file. The volume of RGC contained in each bag was obtained using the following formula: Volume of RGC= (Total mass of RGC-Mass of empty bag)/(Density of RGC). NB: RGC density = 1.06g/ml.

Quantity of haemoglobin and haematocrit in RGC bags

By stripping, the contents of the tubing and the contents of the RGC bag are homogenised. Using a sealing machine, the tubing is subdivided into small segments, each about 6 cm long, called 'boudi'. The last two 'boudi' were detached and their contents transferred into an EDTA tube, which was stored at between 2 and 6°C. The hemogram was then performed on the aliquot of the EDTA tube. The haemoglobin content of each bag of RGCs analysed was calculated using the following formula: Hb quantity (in g) = Hb level (in g/dL) x bag volume (in dL).

Sex and age of donors and previous history

During our study we recruited a total of 150 donors. Of these, 131 were male and 19 were female. During our study, among the 150 donors recruited, the average age of the sample was 31.78±8.81 years, with a minimum of 18 years and a maximum of 53 years.

Comparison of data with international standards

The data obtained were compared with the quality criteria for RGCs described in the European guide.[6] These include volume, haemoglobin and haematocrit. According to the European guide, the adult unit RGC must have a volume of 280±50ml, contain at least 45g of Hb and have a haematocrit of between 65 and 75%.

Expected results

The usual recommendations are as follows for standard red cell concentrates without additive solution (Table 1).

Table 1: Standard red cell concentrates without additive solution.

Parameter	Average standard	Test frequency
Volume	280± 50mL	1% of all units with at least 4 units per month
Hémoglobine Haemoglobin	Minimum 45g/unit	4 units per month
Haematocrit	0.65 to 0.75	4 units per month
Haemolysis at end of storage	< 0.8% of red blood cell mass	4 units per month

Note: If the EC contains an additive solution, its normal haematocrit will only be 0.50 to 0.70.

Table 2: Standard red cell concentrates with additive solution.

Parameter	Average standard	Test frequency
Volume	280±50mL	1% of all units with at least 4 units per month
Haemoglobin	Minimum 45g/unit	4 units per month
Haematocrit	0.50 to 0.70	4 units per month
Haemolysis at end of storage	< 0.8% of red blood cell mass	4 units per month

Methods

Provisional procedure for preparing blood components

As soon as the bag is received, variables are defined. The bag is then examined for anomalies such as clots, leaks, haemolysis, labelling, date and time of collection. A check is then made to ensure that the transport record for the batch of bags shows the same number of bags. At the same time, the transport temperature is recorded. At the same time, the label on each bag is checked for the time sampling began, the time sampling ended and the weight of the bag. At the end, the DIN for each bag was checked for leaks, clots and the colour of the blood. Shortly afterwards, we checked the DIN label on each tube. Finally, each tube was checked for leaks, clots and blood colour, the bag was weighed and its volume recorded in the register and on the bag.

Table 3: Variables.

Independent variables	Dependent variables	Population
Pouch integrity	Haemoglobin level	Pouch of red blood cell concentrate
Labelling	Haematocrit level	
Presence of clots	Leukocyte count	
Presence of red blood cell clots	Platelet count	
Volumes of concentrate bags		

During component preparation, refer to the component preparation and storage procedure: strip the tubing, seal the tubing every 10 cm, check for abnormalities (leaks, clots, haemolysis), label and store the bag in quarantine. In the preparation of standard erythrocyte concentrate, it was simply a matter of preparing this blood component, separating it from the whole blood unit after centrifugation and decanting it into a bag that could contain an additive solution. The sample here was whole blood collected in a double or triple bag, less than 18 hours old and stored between 18ºC and 20ºC. The procedure consisted of labelling all the satellite bags (donor identity, traceability number) and centrifuging the blood bag at 5.000 g for 15 minutes, then taking care to balance the centrifuge with a bag of approximately the same weight. After carefully removing the bag without shaking it, place the bag intended for the EC on the scales. Finally, decant the desired weight of plasma using the manual press. Leave the buffy coat in the EC bag. If bags containing this solution are available, strip and seal the tubing of the EC bag at several points, with segments separated by 10 to 15 cm, then release the bag. The sample used here was whole blood collected in a double or triple bag, less than 18 hours old and stored at between 18ºC and 20ºC. Finally, decant the desired weight of plasma using the manual press. Leave the buffy coat in the EC bag. If bags containing this solution are available, strip and seal the EC bag tubing in several places, with segments separated by 10 to 15 cm, then release the bag. Weigh the CGR bag to determine its volume and homogenise the blood in the bag and tubing before taking the blood sample for analysis.

Measure the various haematological parameters

Complete blood count and determination of haemoglobin and haematocrit levels for each CGR. The automated system we will use for our study is a HumaCount 5D, which uses Optimale Count technology to directly process capillary blood samples and provides excellent differentiation of neutrophils, basophils, eosinophils, monocytes, and lymphocytes using 3D differentiation technology. followed by accurate counting of immature cells and rapid switching from 5-population leukocyte formula mode to blood formula mode for each sample.

Statistical analysis

The collected data were entered into Word and Excel software. The collected data will be compared to the desired values of the HC5D-Control. A p-value of <0.05 was used as the statistically significant threshold.

RESULTS

During our analysis process, we performed blood counts and measured the volume of RBC concentrates, Hb levels and haematocrit after obtaining red blood cell concentrates. The results obtained are shown below:

Characteristics of donors whose RGCs were analysed

The RGCs in our study came from 150 donors. Of these, 131 were men and 19 were women. The figure shows that of the 150 people recruited in our study, 19 were women, i.e. 12.67% (n=19) % compared with 87.33% (n=131) obtained respectively from blood collected from women. The average age of the donors was 31.78±8.81 years, with a minimum of 18 and a maximum of 53 years. The majority of donors, 87.33% (n=131), were men aged between 18 and 39. Women accounted for 12.67% (n=19). Overall, 50.66% (n=76) of our donors

drank alcohol, compared with 49.34% (n=74) who did not. The results show that 25.34% (n=38) of our donors practised regular physical activity, compared with 74.66% (n=62) who did not. The majority of our participants had a university education (77.34%, n=116) compared with 17.33% (n=26) who had a secondary education and only 5.33% (n=8) who had a primary education. The results showed that the most represented group were students 48.03% (n=72) who donated more blood. This was followed by other sectors of activity such as bank employees, welders, firemen, teachers, mechanics, housewives, pupils, shopkeepers, drivers and others 51.97% (n=78). Finally, during our study, the majority of donations were voluntary, with 139 voluntary donors (92.66%) and 11 family donations (7.34%).

Blood count

The mean red blood cell count in our series was 3.75. $10^3/mm^3$ with extremes ranging from $2.10^3/mm^3$ to $5.10^3/mm^3$. The mean white blood cell count was $4.74.10^3/mm^3$ with extremes ranging from $3.73.10^3/mm^3$ to $5.76.10^3/mm^3$. Containing on average 52.08; 31.66; 5.02; 2.90; 1.18 multiplier per $10^3/mm^3$ for neutrophils, lymphocytes, monocytes, eosinophils and basophils respectively.

Volume of red blood cells (RGCs)

The average volume of red blood cells was 343.47±34.80 mL, with extremes ranging from 270 to 456 mL Only 2 (1.33%) did not comply in terms of volume, compared with 148 (98.67%) that complied with international standards.

Amount of haemoglobin in RGCs

The average amount of haemoglobin (Hb) was 48.50±9.30g with respective extreme values of 29.7g and 81g. Of these red blood cell concentrates, 47.33% (n=71) had a haemoglobin level of less than 45g, compared with 79 (52.67%) who had the correct level of haemoglobin.

Haematocrit of RGCs

The mean haematocrit of the RGCs was 65.41±8.16% with extreme values of 52% and 74.72%. Of these, 65 (43.33%) had a haematocrit in compliance, i.e. between 65% and 75%; 85 (56.67%) were not in compliance. Compliance rates for the various parameters are shown in Table 1.

This table 4 shows that the majority of our bags were compliant in terms of volume (148 bags, i.e. 98.67%), haemoglobin content (115 bags, i.e. 76.66%) and haematocrit (65 bags, i.e. 43.33%). In total, of the 150 RGCs analysed, 62 (41.33%) were compliant for all three parameters analysed, i.e. volume, haemoglobin content and haematocrit, compared with 88 (58.67%) that were not compliant for all three parameters.

Table 4: Conformity of RGC rates according to the different parameters tested.

	Mean ± S	European standards	Percentage of compliant units (%)
CGR volume (mL)	343.47 ± 34.80	280 ± 50280 ± 50	98.67
Hb quantity of RBCs (g)	48.50 ± 9.30	≥ 45	76.66
Haematocrit of RBCs (%)	65.41 ± 8.16	65 to 75	43.33

Compliance of bags according to parameters

Compliance of RGCs by gender according to compliance

The table 5 shows that the majority of compliant bags came from men (81.35%), compared with 66.66% from women. Only 12 did not comply.

Table 5: Compliance rate of RGCs with compliance according to gender.

Compliance	Men		Women	
	Number	Percentage	Number	Percentage
Compliant	48	81.35%	2	66.66%
Pas conforme	11	18.65%	1	33.34%
Total	59	100%	3	100%

Compliance of RGCs according to type of donation

The table 6 shows that the majority of compliant bags came from volunteer donors (86.66%), compared with 61.54% from family donors. Moreover, 38.46% of non-compliant bags also came from family donors.

Table 6: Compliance rate of RGCs according to type of donation.

Compliance	Family		Volunteers	
	Number	Percentage	Number	Percentage
Compliant	9	61.54%	39	86.66%
Non-compliant	5	38.46%	10	13.34%
Total	13	100%	45	100%

DISCUSSION

Our study was conducted with the aim of assessing the quality of red blood cell concentrates (RBCCs) produced at the Yaoundé University Hospital Centre. The bags included were weighed to determine their volume. Haemoglobin and haematocrit levels were obtained using an automated method (using the Pentra XLR automated analyser from Horiba Medical). Using this methodology, we obtained 150 samples, the majority of which came from men (131, or 87.33%) compared to only 19 women (17.67%). This result is similar to that of Minkoro et al.[17] who obtained 91.34% male donors in Bamako in 2020. This can be explained by the fact that men can donate at any time, unlike women, who are sometimes prevented from doing so by physiological constraints. The average age of our donors was 31.78±8.81. The majority of our participants had a higher level of education (77.34%), and the majority of donations were voluntary, with 139 voluntary donors, or 92.66%. These two results are also consistent with those reported by Elionora et al.[19] in Tanzania and Minkoro et al.[18] in Bamako. However, our results differ in terms of the type of donation; we found that the majority of donations were voluntary (92.66%). This can be explained by the fact that the CHUY blood bank implements numerous strategies to encourage volunteers to donate. This can be explained by the fact that the CHUY blood bank implements numerous strategies to encourage volunteers to donate blood. Of the 150 bags studied, the average volume of red blood cell concentrates was 343.47±34.80 ml, which is slightly higher than that reported by Hèzouwè et al.[20] in Lomé; as well as Eiman et al. in 2014[21], which were 280.33±32.1 ml and 275±35.5 ml, respectively. Of the 150 bags studied, 98.67% had a volume that complied with standards. This rate is slightly higher than that reported by Hèzouwè et al.[20] in 2019 which was 79.90%. However, Mbanya et al.[14] in Cameroon in 2007 found a compliance rate of 57% with regard to the volume of RBCs. This rate was lower than our result and could be explained by the time between their study and ours; in addition, the total volume of blood collected from the donor had been adjusted. Also, most of our RBC concentrates were prepared by centrifugation. Regarding the haemoglobin content of RBC concentrate bags, the average was 48.50±9.30g with a compliance rate with international standards of 76.66%. This rate is very close to those reported by Fétéké et al.[22] in 2006 Hèzouwè et al.[20] in 2019, who found compliance rates of 81.86% and 80.74%, respectively. However, Mbanya et al.[13] in Cameroon in 2007 found that 66% of RBC bags were compliant in terms of their haemoglobin content. This difference can be explained by the inclusion of pre-donation Hb level testing during the medical selection of blood donors. Despite this progress, the compliance rate of at least 90% recommended by the European guidelines[37] has not yet been achieved. The average haematocrit level was 65.41±8.16%, similar to the result reported in Pakistan in 2018 by Sultan et al., which was 65.41±8.16%. The average haematocrit rate was 65.41±8.16%, similar to the result reported in Pakistan in 2018 by Sultan et al.[23], which was an average of 69.5±7.24%.

In our series, we found a compliance rate for haematocrit of 43.33%. This rate is consistent with that found by Minkoro et al.[17] who found a compliance rate of 47.62%, while Mbanya et al.[14] found a rate of 95% of units compliant with haematocrit. This difference can be explained by the fact that the technique used to separate blood products from whole blood was centrifugation, which resulted in an insufficient amount of plasma. The percentage of bags compliant with the three parameters (volume, haemoglobin quantity and haematocrit) was 41.33%, which is similar to the result reported by Hèzouwè et al.[20] in 2019 in Lomé, i.e. 42.16%. However, it is low compared to that found in Côte d'Ivoire in 2014 by Yao et al.[16] which was 93.85% of CGR bags compliant with the three parameters.[20] This difference can be explained by the fact that their study was the result of a three-year experiment involving the establishment of a quality control laboratory for labile blood products. Saloni et al. also argued that periodic quality control of blood products was essential to verify the adequacy and safety of these products and was part of good transfusion practices. With regard to the compliance rate by gender, we noted that the majority of these bags came from men (81.35%). This difference can be explained by the fact that their study was the result of a three-year experiment involving the establishment of a quality control laboratory for labile blood products. Saloni et al.[17] also argued that periodic quality control of blood products was essential to verify the adequacy and safety of these products and was part of good transfusion practice. With regard to the compliance rate by gender, we found that the majority of these bags came from men (81.35%). However, we noted a compliance rate of 66.66% among women. These results are consistent with those of Minkoro et al.[19,24] This difference could be explained, on the one hand, by the physiological state of women (menstruation, childbirth) and, on the other hand, by the fact that men's haemoglobin levels are slightly higher than those of women. Our results concerning compliance according to the type of donation contradicted those of the previous author, as our results showed that compliance was high among voluntary donors, at 86.66%. This difference can be explained by the fact that the CHUY has implemented a system to retain its voluntary donors.

CONCLUSION

Blood transfusion is one of the most sensitive activities in a healthcare system. It is therefore essential to always ensure the quality of the various blood products, which is why we focused on the quality of red blood cell concentrates. During this study, quality control enabled us to highlight non-conformities in the prepared RBC concentrates and to make assumptions about the shortcomings in order to verify and remedy them. However, achieving a good percentage in terms of RBC concentrate quality is an objective that can be easily

achieved by vigilantly and rigorously applying good transfusion practices. For this reason, it is important that staff are well qualified and receive ongoing training. It would therefore be desirable to broaden the scope of action by conducting a similar study in all blood banks to assess all issues related to the quality of GCRs in order to find solutions.

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AUTHOR CONTRIBUTIONS

Authors Minoue Kuum Marc Germain, Messa Tchudjo Stéphanie Manuella and Biwole Sida Magloire participated in designing the study, validating the study protocol, and progressively revising the article. Authors Messa Tchudjo Stéphanie Manuella and Minoue Kuum Marc Germain carried out the preparation, collection and analysis in the laboratory, then participated in the writing of the article. Authors Minoue Kuum Marc Germain, Messa Tchudjo Stéphanie Manuella and Biwole Sida Magloire performed the statistical analysis. The authors Temdie Guemmogne Joel Romeo, Gisèle Atsang à kiki, Kada Sanda Antoine and Biwole Sida Magloire, the team's sponsor, contributed to the writing and various corrections of the article, the English translation of the abstract, and the formatting of the final document. All authors have read and approved the final version of the manuscript.

COMPETING INTERESTS

No conflict of interest declared.

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