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Research Article

Joint effects of risk factors of adverse events associated with blood donations in western region of Cameroon

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Abstract:

Objective: To determine the risk factors of adverse events associated with blood donation in western region of Cameroon

Design: Cross sectional study

Setting: This study was carried out at the blood donors service, Regional Hospital West, West Region of Cameroon, from January 2020 to December 2021.

Participants: 252 cases of Donors were of body weight, ≧ 50kg and a hemoglobin (Hb) level of ≧13.0g/dL for males and a body weight of ≧45kg and an Hb level of ≧12.0g/dL for females. Hb screening was based on copper sulfate density. SBP was defined as 90 to160 mmHg and DBP as 50 to 95 mmHg. Pulse rate was between 60 and100 beats per minute.

Methods: The variables, age, BMI, pre-donation SBP, pre-donation DBP, and EBV were dichotomized by their median values. All statistical analyses in this study were performed using GraphPad Prism software version 8.0.2.

Interventions: No intervention was done during the period of study

Outcome measures: Donor records were obtained from participant questionnaire sheet. They included collection donation volume, and number of donations. BMI was calculated.

Results: A total of 252 whole blood donors accepted to participate in this study. The mean age (±SD) of the study population was 29.82 ± 8, 56 years, and the ages ranged from 18 to 61 years.

A significant difference with donation site [OR= 0.3796, 95% CI =0.1852 – 0.7994, p= 0.013] with patients donating at fixe site been more represented as presented. The distribution of characteristic according to donation site shows significant difference with pre-donation SBP [OR= 0.4373, 95% CI =0.2073 – 0.9419, p= 0.0411;], Pre-donation DBP [OR= 0.4094, 95% CI =0.1779 – 0.9337, p= 0.027;], as well as the site of collection [OR= Infinity, 95% CI= 1435 – Infinity, p<0.0001]. Multivariate analysis revealed pre-pulse groups<100 [(OR= 151.360, 95% CI= 1.550 – 14778.786, p= 0.032)] and mild adverse events [(OR= 3276.663, 95% CI= 193.264 – 55519.818, p= 0.0000)].

Conclusion: Although, no deaths occurred among donors with adverse events, adverse events affect the safety of blood donors and decrease donors' willingness to donate again. Thus, understanding risk factors of adverse events is important.

Key words: blood donation; adverse events; vasovagal reactions; site of donation

Introduction

Breast cancer is one of the most common malignant tumors in women, with the highest incidence (Sung et al., 2021). Breast cancer is the sixth cause of cancer-related death among women in China, with about 169,000 cases and 45,000 deaths due to breast cancer each year (He et al., 2019). The peak age of breast cancer patients in China is 45-55 years old, and the age of the incidence group is younger, accounting for 19.9% of all new cancer cases in Chinese women (Liu Z C et al., 2021). With the continuous progress of medical technology, there is a trend of diversification of breast cancer treatment, and the benefits and risks of different treatment methods are different, which seriously affects the quality of life of breast cancer patients (Association, 2021; Kang Y K & Yuan F, 2022). Patients face an increasingly complex clinical decision-making process, and they need to weigh the risks and benefits of different treatment options, quality of life and their own economic conditions, and it is not easy to find the right treatment plan for their own.

With the change of the medical model, the role of the patient in the treatment decision-making process has become increasingly important, and the degree of patient participation in the treatment decision-making has become an important indicator to judge the quality of medical care (Savelberg, Boersma, Smidt, & van der Weijden, 2021). Shared decision-making (SDM) involves the joint participation of patients and health care professionals in making health care decisions based on the best available evidence and patient preferences (Ter Stege et al., 2022). Patient decision aids (DA) are an effective knowledge translation tool in supporting SDM and achieving patient-centered care. Matsen conducted a study on attitudes toward SDM in young breast cancer patients, showed that most young women tend to take an active role in decision-making (Matsen, Lyons, Goodman, Biesecker, & Kaphingst, 2019). Fang conducted a questionnaire survey on 480 breast cancer patients with nursing decision participation, and the study showed that 57.3% of the patients believed that their nursing decision participation attitude was positive, 40.2% of the patients believed that their nursing decision participation was actually high (Fang et al., 2019). Yamauchi reported 87% of patients who were diagnosed with breast cancer preferred to play active or collaborative roles and 78% of the patients actually played such decisional roles (Yamauchi, Nakao, Nakashima, & Ishihara, 2017). Peng revealed 64.8% of breast cancer patients preferred to play collaborative decision-making role (Peng, 2016). These data suggest that patients with breast cancer are likely to perform affirmative involvement in the treatment decision-making process. While, China's medical staff have heavy workload and busy work, lack of time to communicate with patients, and difficulty to explain to patients the uncertain prognosis of disease treatment and other information, resulting in information asymmetry between doctors and patients, and patients are difficult to participate in treatment decision-making. Moreover, doctors do not recognize patients' preference to participate in treatment decision-making, and rarely encourage patients to participate in treatment decision-making and obtain patients' ideas (Sui, 2021), Thus affecting patients' participation in treatment decisions. Therefore, how to communicate effectively is a challenge for doctors, and how to make the most appropriate decision based on their preferences and values while fully understanding the pros and cons of various treatment options.

There is evidence to support the use of das by breast cancer patients. we conducted a systematic review of DA for patients making a decision with respect to multiple treatment modalities. which included 22 studies revealed that Ads are helpful to breast cancer patients by decreasing decisional conflict (Gao, Jin, Yu, Wu, & Han, 2021). Given the advantage, we decided to develop breast cancer DA and conduct pilot study, we therefore conducted a pilot testing to develop and evaluate a WeChat mini program-based breast cancer decision aid.

Methods

Study Design and Population

This cross- sectional study was conducted from January 2020 to December 2021 targeting blood donors at the blood bank unit of the Bafoussam Regional Hospital known to be the referral hospital of the Mifi Division, West region of Cameroon. The Bafoussam Blood Bank Unit area is approximately 1.146000, with 350 blood donations attendance per month and serves more than 27 medical institutions. All blood donors that accepted to participate in this study voluntarily signed an informed consent. There was no change in the standard procedure for blood donations during this study.

The criteria for eligibility of blood donation are in accordance with Cameroon Ministry of Health and Welfare guidelines.

Donor's selection was based on anthropometric information such as age, height, weight, gender, BMI. Interview regarding past medical history, lifestyle and a limited clinical examination such as BP, pulse, hemoglobin level was conducted. Donors were of body weight, ≧ 50kg and a hemoglobin (Hb) level of ≧13.0g/dL for males and a body weight of ≧45kg and an Hb level of ≧12.0g/dL for females. Hb screening was based on copper sulfate density. Systolic blood pressure (SBP) was defined as 90 to160 mmHg and diastolic blood pressure (DBP) as50 to 95 mmHg.

Pulse rate was between 60 and100 beats per minute. There were 2 volumes of whole blood (WB) donations, 250mL and 450mL. Donation intervals were 3 months. All the participants in this study were eligible to donate blood. Half a liter of mineral water was offered to each participant prior to donation and snacks, coffee, juice or milk were issued at post donation during their resting for 10 to 15 minutes.

Data and Specimen Collection

Donor records were obtained from participant questionnaire sheet. They included collection (date, status and site), donation volume, and number of donations. BMI was defined as body mass divided by the square of body height and estimated blood volume (EBV) was calculate using the following equations (height, in meters; weight, in kg). Female donors: blood volume (L)=0.3561(Height)3 + 0.03308(Weight)+0.1833).

Male donors: Blood volume (L)= 0.3669 (Height)3 +0.03219 (Weight)+0.6041.

Ascertainment of Adverse events

Adverse donor reaction:

A list of observed and/or donor reported signs/symptoms that occurred during or up to a week after donation were recorded. Signs/symptoms were categorized as mild adverse events (chills, nausea, pallor, dizziness, vomiting, nervousness, headache) and severe adverse events

(hypotension, muscle contractions, convulsions, fainting, syncope, respiratory problems or emesis) [1, 10-13]. In case the donor manifested both mild and severe signs/symptoms simultaneously, they were accounted as severe adverse events.

Statistical analysis: The variables, age, BMI, pre-donation SBP, pre-donation DBP, and EBV were dichotomized by their median values. All statistical analyses in this study were performed using GraphPad Prism software version 8.0.2. Chi-squared test and t test were respectively used for binary variables and continuous variables to compare the baseline demographic characteristics of the case and control groups statistical software. A univariate logistic regression model was then used to analyze the strength of association between potential factors and adverse events. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Multiple logistic regression analysis was performed to identify the independent risk factors for adverse reactions to adjust for potential important confounders. The P-value of the test was 2-tailed with a level of significance (a)=0.05. A P-value of less than 0.05 indicated statistical significance.

All demographic characteristics converted to dichotomous variables were coded as 0 or 1 by the medium values.

Results

Demographic Characteristics of Study Participants

A total of 252 whole blood donors accepted to participate in this study. The mean age (±SD) of the study population was 29.82 ± 8, 56 years, and the ages ranged from 18 to 61 years.

Of the study population, donors age <35 were more represented with 77% compare to ≧35 (23%). Male represented more with 80% compare to female (20%). Blood donors having secondary level of education were highly represented with 48.8% follow by those with tertiary level of education with 40.1% whereas those with primary level of education represented least with 11.1%. The distribution of the donors according to profession revealed that skilled workers where more represented with 46% follow by students 26.6% whereas unskilled were less represented with 6%. The unmarried were more represented with 60.3% unlike 39.7% for the married. Majority of the donors were for replacement 53.2% whereas 46, 8% were voluntary as represented in table 1

0	Frequency	Percentage
Age		
<35	194	77.0
≧35	58	23.0
Gender		
Female	52	20.6
Male	200	79.4
Educ level		
Primary	28	11.1
Secondary	123	48.8
Tertiary	101	40.1
Profession		
Skilled Workers	116	46.0
Unskilled workers	54	21.4
Students	67	26.6
Unemployed	15	6.0
Marital status		
Married	100	39.7
Unmarried	152	60.3
Don type		
Voluntary	118	46.8
Replacement	134	53.2
Total	252	100.0

Table 1: Sociodemographic characteristics of blood donors

Characteristics of adverse events of study subjects.

The characteristics of adverse events of study subject show a significant difference with donation site [OR= 0.3796, 95% CI =0.1852 – 0.7994, p= 0.013] with patients donating at fixe site been more represented as presented as shown in table 2.

	Positive		Negative				
	Effective	%	Effective	%	p-value	OR	95% CI
Gender							
Female	18	7.14	34	13.49	0.299	1.468	0.7614 - 2.777
Male	53	21.03	147	58.33			
Age							
<35	60	23.81	134	53.17	0.0958	1.913	0.9338 - 4.009
≧35	11	4.37	47	18.65			

Predonation SBP (mmHg)							
<124	38	15.08	81	32.14	0.0958	1.913	0.9338 - 4.009
≧124	33	13.10	100	39.68			
Predonation DBP (mmHg)							
<75	42	16.67	99	39.29	0.5738	1.2	0.6888 - 2.060
≧75	29	11.51	82	32.54			
Prepulse							
<100	69	27.38	173	68.65	0.7298	1.595	0.3767 - 7.612
≧100	2	0.79	8	3.17			
BMI groups							
<24	19	7.54	56	22.22	0.5441	0.8156	0.4498 - 1.485
≧24	52	20.63	125	49.60			
Number of donations							
First-time	42	16.67	84	33.33	0.0925	1.672	0.9616 - 2.872
Repeated	29	11.51	97	38.49			
donation site							
Fixed	55	21.83	163	64.68	0.0131	0.3796	0.1852 - 0.7994
Mobile	16	6.35	18	7.14			
Collection volume							
450	34	13.49	91	36.11	0.7802	0.9088	0.5201 - 1.577
250	37	14.68	90	35.71			

Table 2: Characteristics of study subjects

Adverse events occurred in donors who were not significantly associated in Mean biomarkers for donors with and without adverse events as presented in table 3.

Variables overall	Adverse events n=71 (mean ± SD)	No adverse events n=181 (mean ±SD)	P-value
Age, years	28.51 ± 8.64	30.33 ± 8.64	0.1282
BMI, kg/m^2	26.59 ± 3.92	27.19 ± 5.05	0.3857
Predonation SBP (mmHg)	124.1 ± 14.97	126.9 ± 17.42	0.232
Predonation DBP (mmHg)	72.54 ± 12.08	73.78 ± 16.13	0.557
Pulse	75.45 ± 11.67	77.13 ± 12.83	0.3382

Table 3: Mean biomarkers for donors with and without adverse events.

The distribution of characteristic according to donation site shows significant difference with pre-donation SBP [OR= 0.4373, 95% CI =0.2073 – 0.9419, p= 0.0411;], Pre-donation DBP [OR= 0.4094, 95% CI =0.1779 – 0.9337, p= 0.027;], as well as the site of collection [OR= Infinity, 95% CI= 1435 – Infinity, p<0.0001].

characteristics	Fixed		Mobile				
	Effectif	%	Effectif	%	p-value	OR	95%IC%
Gender							
Female	44	17.46	8	3.17	0.6516	0.8218	0.3547 - 1.846
Male	174	69.05	26	10.32			
Age							
<35	168	66.67	26	10.32	>0.9999	1.034	0.4647 - 2.364
≧35	50	19.84	8	3.17			
Pre SBP							
<124	97	38.49	22	8.73	0.0411	0.4373	0.2073 - 0.9419
≧124	121	48.02	12	4.76			
Pre DBP							

<75	116	46.03	25	9.92	0.0273	0.4094	0.1779 - 0.9337
≧75	102	40.48	9	3.57			
Prepulse groups							
<100	208	82.54	34	13.49	0.3665	0	0.000 - 2.184
≧100	10	3.97	0	0.00			
BMI groups							
<24	65	25.79	10	3.97	>0.9999	0.9957	0.4570 - 2.169
≧24	153	60.71	24	9.52			
Number of donations							
First-time	114	45.24	12	4.76	0.0959	2.01	0.9340 - 4.236
Repeated	104	41.27	22	8.73			
donation site							
Fixed	218	86.51	0	0.00	<0.0001	Infinity	1435 - Infinity
Mobile	0	0.00	34	13,49			
Collection volume							
450	111	44.05	14	5,56	0.3573	1.482	0.6962 – 3.187
250	107	42.46	20	7,94			

Table 4: Mean biomarkers for donors with and without adverse events

	Estimation	**Standard Error**	**Wald**	**p-value**	**OR**	**95% CI**
Gender(Female)	1.818	1.306	1.938	0.164	6.161	0.48 - 79.71
Age groups(<35)	0.477	1.097	0.189	0.664	1.611	0.19 - 13.83
Number of donations(First time)	-2.174	1.079	4.059	0.044	0.114	0.01 - 0.94
volume collected (450 ml)	-0.602	0.807	0.558	0.455	0.547	0.11 - 2.66
donation site (Fixed)	0.180	1.074	0.028	0.867	1.197	0.15 - 9.83
PRE-SBP	0.020	0.029	0.485	0.486	1.020	0.96 - 1.08
PRE-DBP	0.041	0.031	1.688	0.194	1.042	0.96 - 1.11
BMI	0.005	0.095	0.002	0.962	1.005	0.83 - 1.21
Level of ADR			35.839	0.000		
Mild	-7.480	1.249	35.839	0.000	0.001	0.00 - 0.007
Moderate	-26.981	10692.870	0.000	0.998	0.000	NA
Severe	-27.327	10441.869	0.000	0.998	0.000	NA
Pre-pulse groups (<100)	-4.135	1.889	4.793	0.029	0.016	0.00 - 0.65
Constance	3.299	5.054	0.426	0.514	27.081	

Table 5: Odds ratios and 95% confidence intervals from univariate logistic

Donors With Adverse Events

Risk factors identified as predicting a responsive outcome from the donors with adverse events were the first-time donation [(OR= 0.0114, 95% CI= 0.01 – 0.94, p= 0.044)], the mild adverse event [(OR= 0.001, 95% CI= 0.00 – 0.007, p= 0.000)], the pre-pulse groups (<100) [(OR= 0.016, 95% CI= 0.00 – 0.065, p= 0.029)] (table 6).

	Estimate	Standard Error	Wald	p-value	OR	95% CI
Constante						
PRE-SBP	-0,012	0,045	0,074	0,785	0,988	0,904 - 1,080
PRE-DBP	0,000	0,062	0,000	0,995	1,000	0,886 - 1,129
Pre Pulse	0,022	0,036	0,361	0,548	1,022	0,952 - 1,097
BMI	-0,109	0,147	0,545	0,460	0,897	0,672 - 1,197
Gender						
Female	-1,687	1,385	1,483	0,223	0,185	0,012 - 2,795
Male	0[b]					-
Age						
<35	-0,915	1,153	0,630	0,427	0,400	0,042 - 3,835
≧35	0[b]					-
Type of donation						
Voluntary	-0,957	1,052	0,828	0,363	0,384	0,049 - 3,019
Replacement	0[b]					-
Pre SBP						
<124	0,366	1,329	0,076	0,783	1,442	0,107 - 19,503
≧124	0[b]					-
Pre DBP						
<75	1,631	1,651	0,976	0,323	5,112	0,201 - 130,082
≧75	0[b]					-
Pre-pulse						
<100]	5,020	2,337	4,612	0,032	151,360	1,550 - 14778,786
≧100	0[b]					-
BMI						
<2]	-0,956	1,219	0,615	0,433	0,384	0,035 - 4,191
≧24	0[b]					-
Number of donation						
First time	2,154	1,151	3,504	0,041	8,619	0,904 - 82,212
Repeated	0[b]					-
Volume of Blood						
450 ml	-0,119	0,927	0,017	0,898	0,887	0,144 - 5,460
250m]	0[b]					-
ADR						
Mild	8,094	1,444	31,420	0,000	3275,663	193,264 - 55519,818
Moderate	28,129	0,000			1645509709456,500	1645509709456,500 - 1645509709456,500
Severe	27,525	0,000			899473078512,125	899473078512,125 - 899473078512,125
No	0[b]					-

Table 6: Odds ratios and 95% confidence intervals from multiple logistic

Demographic characteristics	Adverse events n=181	No adverse events n=71	OR	OR 95%CI	p-value
Female, <35, First-time	11	12	0,3181	0,133 - 0,759	0,0131
Female, <35, Repeated	12	4	1,189	0,370 - 3,818	>0,9999
Female, ≧35, First-time	3	0	1,189	0,370 - 3,818	>0,9999
Female, ≧35, Repeated	8	2	1,595	0,330 - 7,702	0,7298

Male, <35, First-time	61	28	0,7807	0,442 - 1,376	0,464
Male, <35, Repeated	50	16	1,312	0,688 - 2,501	0,4314
Male, ≧35, First-time	9	2	1,312	0,688 - 2,501	0,4314
Male, ≧35, Repeated	27	7	1,714	0,709 - 4,141	0,3097

Table 7: Joint effect of gender, age and number of donations on adverse events

Results of multiple logistic regression analysis are shown in Table7. Multivariate analysis revealed pre-pulse groups<100 [(OR= 151.360, 95% CI= 1.550 – 14778.786, p= 0.032)] and mild adverse events [(OR= 3276.663, 95% CI= 193.264 – 55519.818, p= 0.0000)].

At mobile donation sites, the independent risk factors of pre-pulse (<100) and mild adverse event contribute more to adverse events.

The ORs of 3 major risk factors (age, gender, and donation status) were analyzed simultaneously as demonstrated in Table 8. Male repeated donors aged ≧35 years were treated as the reference group.

Male repeated donors aged <35years (age effect) were associated with increased odds of adverse events (OR, 1,312, 95% CI, 0,688 - 2,501), as were male first-time donors aged ≧35 years (OR, 1,312, 95%CI, 0,688 - 2,501) and female repeat donors aged ≧35years (gender effect) (OR, 1,189, 95% CI, 0,370 - 3,818). Female first-time donors aged <35years (joint effects of age, gender, and donation status) were associated with increased odds of adverse events compared with male repeated donors aged ≧35years (OR, 0,3181, 95% CI, 0,133 - 0,759, p=0,0131).

Discussion

Our findings showed that the most significant risk factor for adverse events is first-time blood donor with pre-donation DBP and level of adverse reaction were two significant factors with strong association. In a research conducted in Taiwan, first-time donors are not recommended to donate 500 mL of WB or platelets apheresis to prevent adverse reaction. From 2010 to 2014, 1.86% of males and 0.013% of females aged 20 to 65 years donated 500 mL as first-time donors at the Taichung Blood Center but also in the study conducted by Wand et al. 2019 [7]. From the findings of this study, factors associated with adverse events related to blood donation include Pre-Diastolic Blood Pressure and level of adverse reaction blood donation.

First-time donors might be more anxious and fearful than repeated donors as they have had no experience donating blood. Anxiety has direct emotional consequences that can lead to VVR [14]. More experienced blood donors have less fear [15]. Fear may be a predictor of adverse events [16, 17]. Almutairi H et al also reported that first-time donors have a 2.2-fold increased risk of adverse events [18]. Moreover, first-time donors with adverse reaction experience maybe less likely to donate again [19]. Many studies have shown that female gender is associated with VVRs, highlighting the gender differences in incidences of adverse reactions [20]. Gender differences in autonomic functions are associated with differences in BP. There is also gender differences in the renin angiotensin system and the effects of bound angiotensin II type2 receptor on renal vascular resistance. Renal sympathetic nervous activity is the main cause of vascular resistance in the evaluation of BP in female subjects. [20]. In this study, we also found a higher risk of VVR among female donors than among male donors.

In addition, blood donors who donated at mobile site had higher risk of VVR than those who donated at fixed site. The reasons maybe less space and less relaxed environment at mobile sites. It is important to ensure that mobile site shave adequate ventilation and space for blood donors to rest for at least 15 minutes after donation.

To illustrate the joint effect of the 3 most significant factors, a multiple logistic regression model was used to assess the combinations of age, gender, and donation status. We found that the combined effects of any 2 or 3 factors resulted to a stronger association than any 1 factor alone.

Adverse events are thought to be caused by various physical (e.g., standing up after donating blood) and psychological reasons (e.g., pain, fear). [22]

Moreover, neural mediated reflex, relatively mediated reflex, relative hypovolemia, posture change, [23] and evaluated serum protein and Hemoglobin [24] can lead to adverse events.

Aging of the population is a global challenge for blood services [25,26]. Effective strategies for recruitment and retention of young, first-time blood donors are very important. Prevention of adverse events is also of importance to blood centers as blood donors who experience adversary are less likely to give blood again. Reducing adverse events improves donor retention [27]. VVR is the most common adverse event among WB donors. [28]. Therefore, it is important to understand and prevent adverse events related to blood donation and to improve blood donation safety.

This study has several limitations. Teenage blood donors have significant risk of adverse reactions and injuries after blood donation when compared with adults. Secondly, one of the criteria for blood donors was pulse rate of 60 to 100 beats per minute. Pulse Rate is measured at blood centers, but this data is not recorded. Third, Hb screening for blood donors was based only on copper sulfate, meaning no quantitative Hb data. A previous study showed that higher Hb level is associated with adverse events for WB donations. Blood donors are healthy with normal cardio-vascular and renal functions. They can manage as lightly negative balance in water normally, but not when it is due to blood loss. If there is a negative balance of water during blood donation, loss of intravascular volume may not be supported [18]

Conclusion and Recommendation

Although, no deaths occurred among donors with adverse events, adverse events affect the safety of blood donors and decrease donors' willingness to donate again. Thus, understanding risk f actors of adverse events is important.

Pre-pulse <100, level of adverse effects, first time donors, donation at fixe site of blood donation area risk of adverse event. In addition, a novel finding of this study is that first-timed donors. Moreover, drinking 500 mL of water or isotonic drink before donation is useful for preventing adverse reactions in blood donors. At blood donation sites of the Bafoussam Blood Center, blood donors are suggested to drink water before phlebotomy to promote better intra vascular volume.

After controlling for other important demographic and health factors, VVRs are more likely to occur among fearful blood donors. At Bafoussam Blood Center donation sites, first-time donors are given a silicone bracelet to wear before phlebotomy. This bracelet Tre minds staff members to pay more attention to them. They explain the process and chat with donors to divert their attention and reduce psychological stress. Providing a comfortable and friendly environment for donors is important. Based on the results of this study, we can educate staff at donation sites regarding risk factors and identification of those at risk to prevent adverse events. The collection staff should be well trained in collecting techniques to minimize adverse reactions such as nerve injury or pain. Further, if appropriate interventions such as practicing applied muscle tension for increasing BP are carried out, we speculate that incidences of adverse reactions can be reduced.

Ethical Considerations

Ethical clearance was obtained from the National Ethical Committee for Research in charge of Human Health from the University number under the application number1244-04 from the18th May 2020. Research authorizations were also obtained from the Director of regional Hospital Bafoussam and consents were obtained from each participant before inclusion in the study.

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