Accuracy of the Lee–White Clotting Time Performed in the Hospital Routine to Detect Coagulopathy in *Bothrops atrox* Envenomation

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Abstract. Snake envenomation is a major public health problem in Brazil. Systemic complications that may arise from snakebites are mainly related to coagulopathy. The Lee–White clotting time (LWCT) is a simple and inexpensive test and available even in remote health facilities. However, the diagnostic value of such test needs to be evaluated to accurately diagnose coagulopathy in the clinical practice. This study aimed to assess the reliability of the LWCT performed in hospital routine to diagnose venom-induced coagulopathy. We studied 186 patients admitted at the Tropical Medicine Foundation Dr. Heitor Vieira Dourado in Manaus, Amazonas, Brazil, with *Bothrops* envenomation diagnosis. At admission, blood samples were collected for performing LWCT and the concentration of fibrinogen. Sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratios, diagnostic odds ratio, and accuracy were calculated with 95% confidence intervals. From the total, 85.5% had hypofibrinogenemia. The sensitivity of the LWCT to the diagnosis of hypofibrinogenemia was 78.0% and the specificity 40.7%. The accuracy of the test was 72.6%, and patients with a prolonged LWCT had 2.4 higher odds of developing hypofibrinogenemia. In addition, the LWCT was also compared with venom antigen levels and systemic hemorrhage. The LWCT showed moderate sensitivity to detect consumption coagulopathy and constitutes a valuable tool for the diagnosis of *Bothrops* snake envenomation and indication of antivenom therapy.

INTRODUCTION

Snakebite envenomation constitutes a major public health problem. In Brazil, more than 28,000 cases have been officially reported in the country yearly in the last decade, most of them in the Amazon region.^{1–3} *Bothrops* genus (common lance head), especially the species *Bothrops atrox*, is responsible for up to 90% of snakebites in the Amazon with the majority of the cases occurring in rural areas in an occupation-related manner.⁴

The coagulating activity of *Bothrops* venom results from components of the venom with thrombin-like activity, which directly hydrolyzes fibrinogen to fibrin, and procoagulant activity, which activates factors II and X of the coagulation, resulting in the formation of endogenous thrombin. Other clotting factors such as XIII and V are also activated by components of *B. atrox* venom.^{5,6} The Batroxase, a metalloproteinase, exerts fibrinolytic and thrombolytic activities and induces weak bleeding through breakdown of the extracellular matrix components such as laminin, type IV collagen, and fibronectin.⁷ The batroxrhagin also induces coagulation disorder, as well as atroxlysin-I, a PI class metalloproteinase.^{8–10} In addition, components that act on platelets function have been described.⁹ Consequently, the resulting incoagulable blood, combined with the damage in vascular endothelium caused by metalloproteinases, is responsible for the hemorrhagic episodes characterized by gingivorrhagia, hemoptysis, macrohematuria, and hematemesis observed in 16–18% of *Bothrops* snakebites.^{11–13} In some cases, bleeding in the central nervous system may also occur, with poor prognosis and death.¹⁴

Antivenom therapy is the only specific treatment available and its administration is based on the presence of clinical

manifestations and coagulation tests.^{3,15} Hemostasis parameters such as prothrombin time (PT) and activated partial thromboplastin time (APTT) are prolonged. Furthermore, total plasma fibrinogen concentration is decreased. Although these tests are used as diagnostic tools, they may not be readily available in resource-poor and isolated settings.¹⁶

Instead, bedside whole-blood clotting tests have been used as a cheaper alternative for standard laboratory tests, such as the 20-minute whole-blood clotting test (WBCT20).^{3,16,17} The Lee–White clotting time (LWCT), originally developed to monitor patients with hemophilia, has been used nationwide both as a diagnosis tool of snake venom–induced coagulopathy and to monitor antivenom therapy efficacy.^{15,18} The difference between the tests is basically the timing where the tube is checked for clots. In the WBCT20, the tube is left undisturbed and checked for clots only after 20 minutes. Differently, in the LWCT, it must be observed every following minute after 5 minutes undisturbed.

In general, WBCT20 had its accuracy estimated in experimental conditions rather than in clinical practice, which may lead to a mistakenly optimistic interpretation of the results in routine conditions.^{3,16,17} Besides, no studies so far had reported the accuracy of the LWCT on snake envenomation, and it is essential to compare this test with the standard laboratory assays. Thus, the present study aimed to analyze the accuracy of the LWCT performed in the clinical routine in diagnosing coagulopathy based on fibrinogen levels in *B. atrox* envenomation. This information is of paramount importance to use a sensitive diagnostic tool on venom-induced coagulopathy to avoid misuse of antivenom therapy.

MATERIALS AND METHODS

Study design. This is a prospective study to assess the diagnostic value of the LWCT performed in the clinical routine

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in *Bothrops* snakebite coagulopathy. This study was approved by the Ethics Review Board of the *Fundação de Medicina Dr. Tropical Heitor Vieira Dourado* (approval number 492.892/2013). All participants provided written and informed consent.

Participants and data collection. Samples were collected from 186 patients with clinically diagnosed snakebite envenomation, from 2014 to 2016, admitted at *Fundação de Medicina Tropical Dr. Heitor Vieira Dourado* in Manaus, Brazil, a reference center for snakebite envenoming treatment in the Amazon. For this study, inclusion criteria were the following: 1) LWCT performed at admission before antivenom therapy and 2) frozen (-80°C) plasma and serum aliquots, collected at the same time of LWCT on admission, available to perform further laboratory tests.

In addition to hemostasis evaluation, parameters such as age (years) and gender, time to medical assistance (hours), presence of local and systemic bleeding (conjunctival and gingival bleeding, hematuria, and hematemesis), and site of bite were recorded at admission. Antivenom therapy was initiated at the physician's discretion according to the severity of envenomation, classified according to the *Guidelines for Diagnosis and Treatment of Accidents by Venomous Animals* of the Brazilian Ministry of Health, which relies on the presence of local (pain, edema, and ecchymosis) and systemic (hemorrhage, shock, and anuria) signs.¹⁵

Hemostasis evaluation. All samples for LWCT, fibrinogen measurements, and venom antigen detection were obtained on admission (before antivenom administration), consecutively. The LWCT that has been recommended by the Brazilian Ministry of Health was modified from the test previously described by Lee and White.¹⁸ Briefly, 1 mL of venous blood was collected with a plastic unlubricated syringe and placed into a new glass tube (13×75 mm) without any anticoagulants or coating at room temperature (25°C). Using a stopwatch, the timing started as soon as the blood was drawn into the tube. The tube was left undisturbed for 5 minutes at room temperature (25°C) and then checked for clots every following minute by gently tilting the tube. The LWCT was considered normal when sample clotted in up to 9 minutes. The hospital's laboratory technicians performed the LWCT at admission as a routine test to detect coagulopathy.

For fibrinogen analysis, blood samples were obtained in 3.2% buffered sodium citrate tubes (Injex Surgical Industries, Sao Paulo, Brazil). They were centrifuged at 3,500 rpm for 15 minutes, and the resulting plasma was aliquoted and stored in a -80°C freezer until assayed. Fibrinogen concentrations were measured using an automated ACL TOP 300 Hemostasis Analyzer (Instrumentation Laboratory, Bedford, MA) according to the manufacturer's protocol. Samples were thawed in a 37°C water bath for 5 minutes and then immediately transferred into sampling cuvettes for further analysis. For this method, the reference range was 200–400 mg/dL as established by the manufacturer. The research personnel performed the fibrinogen concentration assays. The Lee–White clotting time and automated laboratory analysis were blinded.

Venom antigen concentration. Venom antigen quantification and identification in patients' sera were assessed through enzyme-linked immunosorbent assay (Colombini, personal communication) at Instituto Butantan, a national reference research center for toxinology. Plates were sensitized with 25 $\mu\text{g/mL}$ of anti-*Bothrops/Laquesis* serum in

phosphate-buffered saline (PBS) overnight. Thereafter, the plates were washed and blocked with PBS containing 2% bovine serum albumin (BSA) for 2 hours at 37°C then incubated with homologous or heterologous venom diluted in PBS/BSA 1% or PBS/BSA 1% containing 1:5 diluted human normal serum (2.5 hours, 37°C).

After washing, the plates were incubated with anti-*B. atrox* biotin-conjugated mouse monoclonal IgG diluted 1:250 in PBS/BSA 1% containing 0.05% Tween-20 or a pool of anti-*Lachesis muta* biotin-conjugated mouse monoclonal IgG diluted 1:500 in the same buffer (2.5 hours, 37°C). The plates were washed and incubated with streptavidin–peroxidase diluted 1:250 for the anti-*B. atrox* mAb or 1:500 for the pool of anti-*L. muta* mAb, diluted in PBS/BSA 1% containing 0.05% Tween-20 (30 minutes, 37°C). The plates were revealed with an addition of the chromogenic substrate o-phenylenediamine dihydrochloride and hydrogen peroxide, and read at 490 nm. For this assay, the cutoff point for positivity was 1.19 ng/mL for *B. atrox* venom.

Statistical analysis. We assessed the accuracy of the LWCT compared with fibrinogen levels as the gold standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios, diagnostic odds ratio (DOR), and accuracy were calculated with 95% confidence intervals (CIs) using Stata v14 (StataCorp, College Station, TX).

RESULTS

Baseline demographic and clinical characterization. A total of 186 patients participated in the study. The majority of patients were male (82%) and the mean age was 35 years. Most patients (57.6%) took less than 3 hours to medical assistance. Regarding the severity of envenomation, 91 (48.9%) were moderate with the lower limbs being the local bitten in majority of the cases (84.4%). Gingival bleeding was the most present systemic sign (8.6%). From the total, only 55 patients presented detectable levels of venom antigens. All features based on the LWCT and fibrinogen results as well as the total number are shown on Table 1.

Test performance. At admission, the LWCT was prolonged in 140 patients and normal in 46. On the other hand, 159 patients had hypofibrinogenemia (< 200 mg/dL) and only 27 had normal levels of fibrinogen (> 200 mg/dL) (Figure 1).

Regarding coagulopathy, using fibrinogenemia as the gold standard, the test showed an overall sensitivity of 78% (95% CI: 70.7–84.2) and a specificity of 40.7% (95% CI: 22.4–61.2) with PPV of 88.6 (95% CI: 82.1–93.3) and NPV of 23.9 (95% CI: 12.6–38.8). Total accuracy of the test was 72.6% (95% CI: 65.7–78.5). Individuals with a prolonged LWCT had 2.4 times higher odds of presenting hypofibrinogenemia (DOR 2.4 [95% CI: 1.1–5.6]). Other variables such as likelihood ratios and prevalence are summarized in Table 2. The receiver operating characteristic (ROC) curve is shown in Figure 2, where fibrinogenemia had an area under the ROC curve of 0.7778.

DISCUSSION

The LWCT performed in routine clinical settings has a sensitivity of 78% to detect venom-induced consumption coagulopathy in patients with *B. atrox* envenomation. Despite its limitations, the LWCT is a rapid test to perform compared

TABLE 1
Demographic data and clinical features

Variable	LWCT n (%)		Fibrinogen n (%)		Total (186) n (%)
	Normal	Prolonged	Normal	Low	
Number of patients					
Male	36 (23.5)	117 (76.5)	19 (12.5)	134 (87.5)	153 (82.0)
Female	10 (30.3)	23 (23.0)	8 (24.2)	25 (75.8)	33 (18.0)
Age range (Mean $\pm$ SD)	—	—	—	2–75 (35 $\pm$ 18.3)	
Time to medical assistance (hour)					
Up to 3	24 (22.4)	83 (77.6)	17 (15.8)	90 (84.2)	107 (57.6)
4–6	13 (26.5)	36 (73.5)	7 (14.3)	42 (85.7)	49 (26.4)
7–12	6 (50.0)	6 (50.0)	3 (25.0)	9 (75.0)	12 (6.5)
13–24	3 (16.6)	15 (83.4)	—	18 (100.0)	18 (9.7)
Severity on admission					
Mild	17 (21.3)	63 (78.7)	13 (16.3)	67 (83.7)	80 (43.0)
Moderate	26 (28.6)	65 (71.4)	13 (14.3)	78 (85.7)	91 (48.9)
Severe	3 (20.0)	12 (80.0)	1 (6.7)	14 (93.3)	15 (8.1)
Systemic signs on admission					
Hematemesis	—	3 (100.0)	—	3 (100.0)	3 (1.6)
Conjunctival bleeding	—	1 (100.0)	—	1 (100.0)	1 (0.5)
Gingival bleeding	1 (6.2)	15 (93.8)	1 (6.2)	15 (93.8)	16 (8.6)
Hematuria	—	5 (100.0)	—	5 (100.0)	5 (2.7)
Venom antigenemia (ng/mL)					
Up to 50	7 (20.0)	28 (80.0)	6 (17.1)	29 (82.9)	35 (63.6)
51–100	2 (16.7)	10 (83.3)	2 (16.7)	10 (83.3)	12 (21.8)
101–150	2 (50.0)	2 (50.0)	—	4 (100.0)	4 (7.3)
More than 151	—	4 (100.0)	—	4 (100.0)	4 (7.3)
Fibrinogenemia (mg/dL)					
Up to 50	21 (15.8)	112 (84.2)	—	133 (100.0)	133 (71.5)
51–100	3 (37.5)	5 (62.5)	—	8 (100.0)	8 (4.3)
101–150	4 (57.1)	3 (42.9)	—	7 (100.0)	7 (3.7)
151–199	7 (63.6)	4 (36.4)	—	11 (100.0)	11 (5.9)
More than 200	11 (40.7)	16 (59.3)	27 (100.0)	—	27 (14.5)

with laboratory-based coagulation tests and the accuracy of 72.6% may not be disregarded when assessing venom-induced coagulopathy in snakebite envenomation. In tropical countries, snakebites usually occur in rural areas and patients may be treated in remote health centers without laboratory

facilities.¹⁹ Thus, bedside clotting tests may be the only auxiliary tool to confirm snakebite envenomation. This may allow prompt clinical decision-making, prevent any delay in antivenom therapy, when it is available, and costs to transport the patient or blood samples to secondary hospital units.

Several investigators have found that the WBCT20 is a sensitive indicator of severe coagulopathy with greater accuracy after test standardization.^{16,17,20,21} Conversely, for Russell's viper venom-induced consumption coagulopathy, WBCT20 showed a lower sensitivity and the negative results led to delayed antivenom therapy.²² These findings highlight the importance of the standardization of the procedures and materials used among clinical institutions, including the use of standard tube size, the same volume of blood collection, and trained operators undertaking the test.

TABLE 2
Reliability of the Lee–White clotting time

Variable	Fibrinogen levels	
	% (95% CI)	
Sensitivity	78.0 (70.7–84.2)	
Specificity	40.7 (22.4–61.2)	
Positive predictive value	88.6 (82.1–93.3)	
Negative predictive value	23.9 (12.6–38.8)	
Prevalence	85.5 (79.6–90.2)	
Accuracy	72.6 (65.7–78.5)	
Diagnostic odds ratio	2.4 (1.1–5.6)	
Positive likelihood ratio	1.3 (0.9–1.8)	
Negative likelihood ratio	0.5 (0.3–0.9)	

CI = confidence interval.

FIGURE 1. A flowchart of reference and standard testing. A total of 186 patients were analyzed, where 159 had coagulopathy and 27 did not have coagulopathy as characterized by fibrinogen levels.

FIGURE 2. Receiver operating characteristic (ROC) curves for different cutoff points of reference test. The Lee-White clotting time was compared with fibrinogen levels (AUC = 0.7778) as the gold standard. AUC = area under ROC curve. This figure appears in color at www.ajtmh.org.

In our study, there were 35 false negatives. In fact, mild venom-induced consumption coagulopathy may be present even with a normal LWCT measure, as observed in 63% of patients with a normal LWCT and fibrinogenemia between 151 and 199 mg/dL (Table 1). Such an event may also occur if the sample is left for prolonged periods and not seldom seen in some types of venom and sites of activity on the coagulation cascade.^{23,24} The consequence could be a delay in the antivenom administration or even no antivenom therapy. In such a case, the patient should be maintained under observation at the health service and the bedside clotting test should be repeated after at least 6 hours to confirm the coagulation status.

The presence of false positives may lead to over administration of antivenom in normal patients and exposure to animal-derived immunoglobulins that might induce early or late reactions.^{16,25} Morais et al.²⁶ showed up to 100-fold increase in IgM and IgG levels against antivenom components in a dose-dependent manner. In Brazil, 18–25% of patients develop early adverse reactions to antivenom.^{11,27,28} Thus, it reinforces the need of a sensible diagnostic tool on snake envenomation. Increased sensitivity may be achieved once standardization is achieved or with the establishment of parallel or serial test combinations to increase sensitivity and specificity.²⁹

The LWCT performed in clinical settings should be linked to clinical signs. Local signs such as local bleeding and edema and systemic signs that include hematemesis, hematuria, and gingival and conjunctival bleeding are present depending on the severity of the accident and may aid to confirm envenomation.^{11,12,30} Although it is true that increased reliability may be achieved in the presence of clinical signs, the administration of antivenom solely based on LWCT result should not be disregarded, especially in cases where the onset of disease is not yet established. In the face of the first evidence of envenomation, whether clinical or laboratorial, the antivenom would minimize possible complications of circulating venom. However, the patient should be kept under close medical supervision for the following hours to avoid early adverse reactions to antivenom.

Reasons for criticisms of the bedside clotting tests, including the LWCT and WBCT20, are related to the lack of knowledge of the correct performance of the test in cases of snakebite envenomation. The Lee-White clotting time may be unreliable and insensitive as a result of several deviation variables from the original description that include the number of syringes used, whether one discards some of the blood, the degree and frequency of tilting the tubes, the type and diameter of the tubes, the volume of the blood, prerinsing the tubes with saline, and temperature. The test may also be invalidated by a traumatic venipuncture, delayed flow or bubbling of the blood, failure to remove the needle before delivery of the blood, and by squirting the blood too rapidly into the tube.³¹ Therefore, because the LWCT was performed by the hospital laboratory technicians at admission with no supervision to access its reliability under real circumstances, it might have affected the real sensitivity.

A point-of-care test that detects especially *Bothrops* venom might be a future option to detect circulating venom components, to guide antivenom choice, and to refrain from using antivenom in bites without envenomation (dry bite). The *Bothrops* genus is responsible for most cases of envenomation in the Amazon, so it would be useful as a rapid diagnostic tool, especially in isolated areas.^{1,4} Whenever possible, determination of PT and APTT should be included, but its absence should not prevent clinician's judgment based on a thorough clinical diagnosis in addition to a simple bedside clotting test.

CONCLUSION

The LWCT showed moderate sensitivity to detect consumption coagulopathy and constitutes a valuable tool for the diagnosis of *Bothrops* snake envenomation and indication of antivenom therapy. Care should be taken to prevent delay in antivenom administration in case of false negative results or to promote its unnecessary use in false positive patients. Future studies using a standardized method should be performed to assess the true sensitivity of the test and also to compare with the alternative WBCT20, as to monitor the restoration of blood coagulability, with tests performed before and after antivenom therapy.

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