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Point-of-care derived INR does not reliably detect significant coagulopathy following Australian snakebite

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Running head: Point of care testing for coagulopathy in snakebite

Abstract

Introduction: Point-of-care international normalised ratio (INR) has been suggested as a way to screen for venom-induced consumption coagulopathy following snakebite, but has not been validated for this. This study aimed to assess the diagnostic reliability of point-of-care INR for venom-induced consumption coagulopathy.

Methods: This was a prospective study of snakebite patients recruited between January 2011 and May 2012 where a point-of-care INR was done and compared to an INR done on a laboratory coagulation analyser, as part of a quality assurance exercise. Data was obtained for each patient, including demographics, information on the snake bite, the point-of-care INR results and any laboratory derived coagulation studies. Snake identification was confirmed by expert identification or venom specific enzyme immunoassay.

Results: There were 15 patients with a median age of 29 years (2 to 68y) and 13 were male. Four of the 7 patients with venom-induced consumption coagulopathy had an abnormal point-of-care INR (3 false negatives) and 1 of the 7 non-envenomed patients had an abnormal point-of-care INR (1 false positive). The patient with a falsely elevated point-of-care INR was given antivenom prior to formal coagulation studies. The point-of-care INR was also negative in the patients with an anticoagulant coagulopathy.

Conclusions: The study shows that point-of-care INR testing devices should not be used in suspected snakebite cases in Australia to diagnose venom-induced consumption coagulopathy.

Keywords: Snakebite, coagulopathy, INR, point-of-care testing, envenomation, diagnostic tests

Abbreviations: INR – international normalised ratio; PT – prothrombin time; POC - point-of-care; aPTT – activated partial thromboplastin time; VICC – venom induced consumption coagulopathy; IQR – interquartile range;

Introduction

Snake envenomation is a major health problem globally and is now recognised as a neglected tropical disease.[1] Major envenomation syndromes following snake bite include neurotoxicity, coagulopathy, myotoxicity, cytotoxicity and acute kidney injury. Venom induced consumption coagulopathy (VICC) is common worldwide[2] and is the most important clinical envenomation syndrome in Australia.[3] VICC is an acquired clotting factor deficiency. The specific factors that are consumed depends on the procoagulant toxin in the snake venom, which may be a thrombin-like enzyme, prothrombin activator or factor X activator, to name the most common.[2] Brown snakes (*Pseudechis* spp.), tiger snakes (*Notechis* spp.) and taipans (*Oxyuranus* spp.) are the major Australian snakes causing VICC and their venoms contain prothrombin activators. These cause deficiencies in factor V, factor VIII and fibrinogen.[4] Treatment is principally with antivenom, with the aim to allow the coagulopathy to recover and prevent major bleeding complications.[2] Factor replacement has been shown to speed the recovery of clotting function but not to improve clinical outcomes.[5]

In Australia there are thousands of suspected snakebites every year but only a small number result in systemic envenomation, over three-quarters of these causing VICC and requiring antivenom.[3] Detection of coagulopathy in patients presenting with a suspected snakebite is therefore an integral component of both initial assessment and ongoing management.[6] The presence or absence of VICC is used to guide subsequent medical care in the majority of cases, including retrieval of patients from rural or remote locations, administration of antivenom[7] and possible provision of clotting factor replacement.[5] Antivenom does not come without the risk of systemic

hypersensitivity reactions and anaphylaxis, so it is important that patients without VICC are not given antivenom unnecessarily. Coagulation testing in snake envenomation is therefore a key investigation in the assessment and treatment snake bite, to ensure only patients with proven VICC receive antivenom.

The majority of snakebites occur in rural and regional centres and health care facilities in these areas do not always have access to on-site laboratory coagulation testing. In many of these hospitals point-of-care (POC) devices are being used in place of a standard laboratory coagulation profile to detect VICC. The use of such POC devices in haemostasis is well established for monitoring the international normalised ratio (INR) in patients taking oral vitamin K antagonists,[8] such as warfarin. However, the use of POC-derived INR to screen for VICC in patients presenting following snakebite has not been formally studied, and its validity has not been established.

Following a case in which a POC-derived INR (iSTAT[®]) failed to detect clinically overt VICC in a patient presenting with bleeding following snakebite, a quality assurance activity was initiated to investigate if a POC-derived INR is able to detect VICC following snakebite.

Materials and Methods

This was a prospective study of patients with snakebite where a POC INR was done and compared to an INR done on a laboratory coagulation analyser as part of a quality assurance exercise. The study was conducted in accordance with the regulatory requirements of the Royal Brisbane and Women's Hospital Human Research Ethics Committee. Additional clinical information was obtained as part of the Australian snakebite project (ASP) which has approval through multiple Human Research and Ethics Committees to cover all involved institutions.[7]

The study was undertaken in Queensland where pathology is provided to all public hospitals and health services throughout the state by a governmental pathology service, incorporating a networked system of 33 laboratories. In public health facilities without a 24 hour on-site laboratory the iSTAT[®] (Abbott Group) testing device is used to provide INR testing. Cases presenting to Queensland Health facilities were recruited between January 2011 and May 2012 where both a POC INR result was available and a laboratory derived INR (either at the time or on frozen citrate plasma) was done.

For each case recruited to the study basic demographic information was obtained as part of the quality assurance exercise, including information on the snake bite, the POC INR results and any laboratory derived coagulation studies. Patients recruited at centres without on-site automated coagulation analysers **had citrate samples double spun and frozen** and transported **with the next available courier to the central laboratory** for later INR determination. Additional clinical and treatment information was extracted from the ASP database for each case. Snake identification was confirmed by either expert identification of the snake or venom specific enzyme

immunoassay if pre-antivenom blood was available. All patients recruited to ASP have serum collection, frozen and transported to the research laboratory for venom immunoassays.[7, 9]

All POC INR were done on an iSTAT[®] (Abbott Group) POC testing devices. Laboratory derived INR (prothrombin time [PT]), activated partial thromboplastin time (aPTT) and fibrinogen testing were done on the coagulation analyser available on-site at the hospital. When an on-site coagulation analyser was not available the INR, aPTT and fibrinogen were done at the closest hospital laboratory or on frozen citrate samples at a central laboratory in Brisbane (Sysmex CA560, Sysmex CA1500 and Stago Star). INR was also confirmed by a manual clotting time.

A sample from a patient with congenital afibrinogenaemia was used as a positive (abnormal) control. **The reason for this was to confirm that the lowered INR seen with the i-STAT was not simply due to an inability of a clot based endpoint laboratory analyser to reliably detect an imperfect clot in a patient with a consumptive coagulopathy and to test the i-STAT analysis system, which relies on an electrochemical substrate which is split by thrombin in place of fibrinogen, and should therefore theoretically produce a “normal” INR even in the complete absence of fibrinogen, as long as other factors are present in normal amounts.**

Descriptive data is presented as medians with interquartile ranges (IQR) and ranges.

Results

During the study period there were 15 patients who were recruited to the study. The median age was 29 years (2 to 68y) and 13 of the 15 patients were male. The demographic details, snake identification and clinical features are provided in table 1. There were seven patients with venom induced consumption coagulopathy, seven patients with no evidence of coagulopathy and formal coagulation studies and one patient with a black snake envenomation with an anticoagulant coagulopathy (**abnormal aPTT**). One of the seven patients with no evidence of coagulopathy was a suspected death adder envenomation which is an envenomation syndrome not associated with coagulopathy.

Only 4 of the 7 patients with VICC had an abnormal POC INR (3 false negatives) and 1 of the 7 non-envenomed patients had an abnormal POC INR (1 false positive). The patient with a falsely elevated POC INR **of 2.8** was given antivenom **based on the POC INR at a rural hospital** prior to **transfer to a larger hospital for** formal coagulation studies. The POC INR was also negative in the patient with an anticoagulant coagulopathy, **but this was consistent with the laboratory INR also being normal**. The POC INR is compared to the laboratory INR in table 2. The POC INR was normal in the positive control sample from a patient with congenital afibrinogenaemia.

Discussion

The study found that in a small series of cases the POC INR result was normal in almost half of snake envenomed patients with VICC, with an unrecordable INR done on a laboratory coagulation analyser. This confirms previous reports of cases of false negative POC INR results and current concern that these devices cannot be used in suspected snakebite to test for VICC. In addition, there was one patient who received unnecessary antivenom based on a false positive POC INR result done in a small hospital **before formal coagulation studies could be done.**

VICC is the most important effect of Australian elapid envenomation, and three of the five major snake groups are able to induce this syndrome.[2, 3] Laboratory tests in VICC demonstrate prolongation of INR/PT and aPTT to beyond the limit of quantification, markedly reduced or undetectable fibrinogen and elevated fibrinogen degradation products.[4] Detection of envenomation relies on clinical history and examination, and results of directed laboratory testing.[6] Coagulation tests are the most important laboratory tests in snake envenomation because of VICC. The availability of POC coagulation testing would improve the treatment of suspected snakebite allowing the early treatment and disposition of patients in the rural and regional setting without the need for retrieval.

There are at least two previously reported cases of POC INR testing being unable to detect VICC.[10, 11] In the more recent case a POC D-Dimer result was also inaccurate with a series of normal results compared to a 100-fold rise in the laboratory derived D-Dimer result.[11] This series adds weight to the evidence suggesting that these devices should not be used for detecting VICC. Unfortunately there is currently no alternative bedside test for snakebite coagulopathy. The 20 minute whole blood

clotting test has also been shown to be unreliable in one recent study[12] and glass test tubes are rarely available in Australia.

A prothrombin time measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. POC machines calculate INR/PT using a recombinant human thromboplastin with electrochemical detection of the clotting end point. This is achieved through thrombin cleaving an electrochemical substrate rather than fibrinogen as is the case in traditional laboratory analysers that have clot formation as the end point. The INR is calibrated using the same international sensitivity index (ISI) as is used for laboratory thromboplastin reagents. One possibility is that the presence of procoagulant toxins, such as prothrombin activators, in the snake venom and therefore the patient's sample interferes with the electrochemical substrate.

It is less clear why there was a false positive POC INR result in one patient but this may have been due to errors in sample collection such as the sample being collected in a heparized blood gas collection tube. The negative POC INR result in the patient with an anticoagulant coagulopathy due to black snake envenomation is not unexpected. Black snake anticoagulant coagulopathy is characterised by an isolated elevation of the aPTT and the INR is rarely abnormal except in the most severe cases of black snake envenomation.[13, 14]

There were a number of limitations of the study. The study had a small sample of cases despite a potentially much larger number of suspected snakebite cases in the State during the same period. This is most likely a result of a significant proportion of cases presenting directly to large regional hospitals which have on-site laboratories.

However, the study identified false negative POC INR results in almost half of the cases of VICC. A larger study is unlikely to change the conclusion that POC INR devices are insensitive to VICC. The results of the study cannot be immediately applied to snakes outside of Australia and other POC INR testing devices. However, any POC testing device that uses a similar assay method should be considered unreliable for the detection of VICC without appropriate studies. **In some of the cases the laboratory INR was analysed on citrate samples that had been frozen which may have affected the results. Previous studies of snake bite patients with VICC have shown that measurement of clotting studies in frozen samples is reliable,[4] so this is unlikely to have influenced the results of this study.**

The study shows that POC INR testing devices should not be used in suspected snakebite cases in Australia to diagnosis VICC. Further study is required to determine if this holds for all POC devices and snakes in other parts of the world.

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Table 1: Demographic details, clinical effects and antivenom treatment for the 15 cases in the study.

Case	Sex/Age	Snake ID	Clinical features	Antivenom
1	M37	Brown	Hypotension, collapse, VICC, systemic symptoms [†]	BSAV
2	M30	Brown	Asymptomatic, VICC	BSAV
3	M2	Taipan	Neurotoxicity, myotoxicity, VICC, acute renal failure and thrombotic microangiopathy	PVAV/TAV
4	M68	Brown	Partial VICC, systemic symptoms [†]	BSAV
5	M27	Tiger	VICC	TSAV
6	M17	Black	Nil	Nil
7	M55	Death adder	Systemic symptoms [†]	DAAV
8	M20	Unknown	Nil	Nil
9	M51	Brown	Hypotension, collapse, VICC, systemic symptoms [†]	BSAV
10	F42	Unknown	Nil	Nil
11	M24	Unknown	Nil	Nil
12	M54	Black	Anticoagulant coagulopathy, myotoxicity	BISAV
13	F29	Brown	Partial VICC, abnormal creatinine, systemic symptoms [†]	BSAV
14	M3	Unknown	Nil	BSAV/TSAV

15	M18	Unknown	Nil	Nil
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[†] Systemic symptoms include nausea, vomiting, headache and diaphoresis;

◆ POCT INR test repeated by POCT testing facility; BSAV – brown snake antivenom; TSAV – tiger snake antivenom; TAV – taipan antivenom; DAAV – death adder antivenom; BISAV – black snake antivenom; PVAV – polyvalent antivenom; VICC – venom induced consumption coagulopathy;

Table 2: Comparison of the POC INR results to an INR, aPTT and fibrinogen done on a laboratory coagulation analyser.

Case	Diagnosis	APTT (s)	PT (s)	Fib (g/L)	Lab INR	POC INR
1	VICC	>200	>100	<0.4	>10.0	3.2
2	VICC	>200	>100	<0.4	>10.0	1.2 (1.2) ¹
3	VICC	>200	>100	<0.4	>10.0	1.5 (1.4) ¹
4	VICC	117	19	2	1.8	0.9
5	VICC	>200	>100	<0.4	>10.0	1.2
9	VICC	>200	>100	<0.5	>10	2.6 (2.0) ¹
13	VICC	133	34	<0.5	3.4	1.8
6	Nil	36	17	1.7	1.2	1
7	Nil	32	13	2.3	1.3	1.1 (1.2) ¹
8	Nil	28	12	3.0	1.0	1.0
10	Nil	31	12	3.5	1.1	0.9
11	Nil	28	11	2.7	0.9	1.0
14	Nil	37	14	2.9	1.1	2.8 ²
15	Nil	37	16	2.4	1.4	1.1
12	AC	56	12	4.9	1.1	1.3
Control ³	-	>200	>100	<0.4	>10.0	1

¹ Result in brackets is a replicate test done between 10 and 90 minutes after the first POC INR; ² The false positive result may be related to pre-analytical errors such as contamination with heparin; ³ Plasma sample from a patient with congenital afibrinogenemia; VICC – venom induced consumption coagulopathy; AC – anticoagulant coagulopathy **which is characterised by an elevated APTT, with a normal or mildly elevated INR and normal fibrinogen.**