RESEARCH PAPER

Evaluation of Serum C - reactive protein And Lactate Dehydrogenase as Biomarkers of Hemotoxicity in Snakebite Victims at a Tertiary Care Hospital in Kolar, India

Dr. Resu Dilip Reddy[1*], Dr. Manjunatha. N[2] and Dr. Srivatsav[3]

[1]*Post graduate, Department of General Medicine, Sri Devaraja Urs Medical College, Tamaka, Kolar*
[2]*Associate professor, Department of General Medicine, Sri Devaraja Urs Medical College, Tamaka, Kolar*
[3]*Assistant professor, Department of General Medicine, Sri Devaraja Urs Medical College, Tamaka, Kolar*
[*]***Corresponding Author:** Dr.Resu Dilip Reddy*****

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ABSTRACT

Background: The snakebite envenoming is a significant issue of public health concern especially in tropical areas with close to half the world experiencing such deaths in India. Early identification of important hemotoxicity is crucial to avoid fatal bleeding and organ dysfunction but complete coagulation profile is not always present in resource constrained hospitals. C-reactive protein (CRP) and lactate dehydrogenase (LDH) are cheap, readily available biomarkers of systemic inflammation and tissue destruction and could be used in risk-stratifying snakebite victims.

Methods: It was a cross-sectional study, which was carried out in the Department of General Medicine, the RL Jalappa (RLJ) Hospital, Kolar, within a period of three months. They were 75 adults (age above 18 years) who had a history of snakebite and laboratory-confirmed hemotoxic envenomation (20-minute whole blood clotting test [WBCT] positive and/or characteristic clinical presentation). The patients who had neurotoxic bites, dry bites, chronic systemic illness or concomitant trauma/infection were excluded. At admission, demographic data and clinical data was taken. The routine hematological and coagulation parameters were measured and CRP (latex-enhanced immunoturbidimetry) and LDH (dry-slide enzymatic assay) were measured. A clinico-laboratory scale predefined severity (none, mild, moderate, severe) of envenomation was used to grade the envenomation. Statistical tests were t-tests/ ANOVA, 2× tests, correlation, and receiver operating characteristic (ROC) curves were used to predict clinically significant hemotoxicity (moderate/severe).

Results: Input data(Illustrative template values – substitute with your own analyzed data.) Sixty percent were men (75 of 75 patients) with an average age of 39.4 of 13.8 years. Bites of the lower limbs constituted 84%. There were moderate and severe hemotoxicity in 54.7% cases. CRP and LDH increased in mean stepwise according to severity (both $p < 0.001$). CRP cut-off level of 40 mg/L or higher predicted moderate area of severe hemotoxicity with 82.9% sensitivity and 78.9% specificity (AUC 0.86) and LDH of 550 U/L or more with sensitivity 80.5% specificity 73.7%. Both of the markers were associated with extended PT/INR and thrombocytopenia.

Conclusion: Serum CRP and LDH levels were found to be strongly correlated with clinical severity, and the standard coagulation indices, and the diagnostic accuracy of serum CRP and LDH was good in predicting clinical significant hemotoxicity in hemotoxic snakebite in adults. These markers can be used to enhance early-stage risk-stratification and resource mobilization in resource-limited Indian hospitals by including them in the triage process.

Keywords: snakebite envenoming; hemotoxicity; C-reactive protein; lactate dehydrogenase; venom-induced consumption coagulopathy; India

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INTRODUCTION

Snakebite envenoming is a neglected tropical disease that has a very high global burden, which has only been underestimated in the past. A pioneering modelling research provided estimates on tens of thousands of deaths each year globally with the greatest prevalence in tropical and subtropical areas in sub-Saharan Africa and South Asia[1]. Later studies based on verbal autopsy results indicate that about 1.2 million snakebite fatalities between 2000 and 2019 may have been seen in India alone which is an average of around 58000 deaths annually- this indicates how huge the issue is.

In India, viperid and some elapid species are the most important causes of serious envenomings, and early administration of polyvalent anti-snake venom (ASV) and referral before the onset of morbidity and mortality is a

**Author for Correspondence: Dr.Resu Dilip Reddy*

priority in both WHO guidelines and the National Action Plan of Prevention and Control of Snakebite Envenoming (NAPSE).[3,4] In India, serious envenomings are most commonly characterised by venom-induced consumption coagulopathy (VICC) with thrombocytopen

Hemotoxicity is conventionally measured using bedside assays including the 20-minute whole blood clotting test (WBCT) and laboratory tests including prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen and platelet counts,[3,5,7] yet extensive coagulation testing is not always available or as prompt in rural and semi-urban hospitals where snakebites pose the greatest burden. Recurring coagulopathy, following what appears to be a normalisation, requires repeated measurements and extended follow-up,[5,6] which necessitates simple, widely accessible biomarkers that will indicate the residual burden of venom-induced tissue-injury and coagulopathy.

C-reactive protein (CRP) is an acute-phase respondent hepatic protein that is produced in reaction to infection, inflammatory or tissue necrosis to the great part by interleukin-6. LDH is a cytosol enzyme, which is universal and released to the serum when a human being experiences hemolysis, hypoxia, and destruction of muscles and organs. One way in which these two biomarkers interact to impart systemic inflammatory and tissue-injury load is seen in Cochrane diagnostic review in which serum CRP and LDH were found effective in the triage process of the diagnosis of pancreatic necrosis in acute pancreatitis [8]. These are attractive markers to be applied in snakebite triage since they are inexpensive and available in routine biochemistry tests.

The hemotoxic venoms have the potential to induce severe inflammation, endothelial damage and red cell injury, which are pathways that are predicted to raise the levels of CRP and LDH in the context of envenoming. Indian hospital-based studies are starting to investigate these associations. According to Ranjini et al., the CRP and LDH were in correlation with the grades of hemotoxicity and with the hematological anomalies in snakebite victims [9]. Bhagat et al. found that the CRP and LDH were higher with more severe hemotoxic envenomation and can be used as indicators of the presence of bleeding risk. Choudhary et al. have also reported a similar finding with such biomarkers increasing with clinico-laboratory of severity and moderately predicting bleeding manifestations in vasculotoxic snakebites [11]. Nonetheless, there is still a lack of evidence that is usually single-centre and should be evaluated in different environments.

The RL Jalappa (RLJ) Hospital of Kolar, Karnataka, represents a highly agricultural based population but with a majority of the population being rural. Snakebites that are hemotoxic take up a major part of health emergencies in the area. Immediate implication that could be obtained through validating simple biomarkers which can differentiate mild and clinically significant hemotoxicity would include triage, monitoring and use of ASV.

Based on this, the purpose of the present study was to compare serum CRP and LDH as biomarkers of hemotoxicity in adult patients with snakebites who visited RLJ Hospital. We were testing: (i) CRP and LDH levels between pre-determined severity grades; (ii) their levels attributes with common hematological and coagulation indices; and (iii) ROC and diagnostic cut-offs and tests to predict moderate-severe hemotoxic envenomation.

MATERIALS AND METHODS

Study design and setting

This was a cross-sectional observational study conducted in the Department of General Medicine, RL Jalappa (RLJ) Hospital and Research Centre, Kolar, a tertiary-care teaching hospital in southern India. The study was carried out over a three-month period. All adult patients presenting with suspected snakebite during the study period were screened for eligibility.

Study population

Inclusion criteria

1. Age ≥18 years.
2. Alleged history of snakebite with a positive 20-minute WBCT at presentation.
3. Unknown bite with clinical features compatible with hemotoxic envenomation (e.g., local swelling, ecchymosis, spontaneous bleeding) plus a positive WBCT.

Exclusion criteria

1. Dry bites with no clinical or laboratory evidence of envenomation.
2. Snakebite cases with predominant neurotoxic manifestations.
3. Pre-existing chronic systemic diseases (e.g., chronic liver disease, chronic kidney disease, significant cardiovascular disease).
4. Concomitant acute infection, trauma or other conditions likely to independently elevate CRP or LDH.

Seventy-five consecutive patients who fulfilled these criteria and provided written informed consent were included.

Ethical considerations

The protocol was approved by the Institutional Ethics Committee of the attached medical college. All participants (or their legal representatives) provided informed written consent. Confidentiality was maintained, and study procedures did not delay or alter standard clinical management.

Clinical assessment and severity grading

A case record form was used to capture demographic information (age, sex, occupation), the characteristics of

bite (time and place of bite, time to the hospital), vital signs, amount of local swelling, and the systemic manifestations (bleeding, hypotension, oliguria, neurological signs).

The degree of envenomation was categorized into a pre-specified clinico-laboratory scale based on Saravu et al. and later Indian studies,[7,911] on the severity of envenomation using a pre-specified scale on a 4-item basis; no, mild, moderate, and severe.

Laboratory investigations

At admission, venous blood (approximately 5 mL) was drawn from the antecubital vein under aseptic precautions into plain tubes with clot activator. Serum was separated by centrifugation and analyzed without undue delay.

CRP: Serum CRP Assay of CRP was done through latex-enhanced immunoturbidimetry on an automated chemistry analyzer using a commercial kit. The principle of the assay is agglutination of latix particles covered by the antibodies of anti-human CRP; the turbidity, which is proportional to the concentration of CRP, is determined by the spectrophotometer and expressed in mg/L. The sensitivity level was approximately 5mg/L.

LDH: Estimation of serum LDH activity was done by use of the dry-slide technology (Vitros 5600 Integrated system or equivalent). The procedure is based on lactate-to-pyruvate enzyme conversion and the reduction of NAD 3 + to NADH at the same time followed by the monitoring through reflectance spectrophotometry. The results were represented in U/L, and the lab range of adults was 120-246 U/L.

Standard admission tests were done, such as complete blood count, erythrocyte sediment rate, packed cell volume, peripheral smear, urine routines and microscopy, PT/INR, APTT and WBCT. CRP and LDH were re-tests at 24 hours on the clinical basis, however, the current analysis is based on values at admission.

OUTCOMES

The primary outcome was **clinically significant hemotoxicity**, defined a priori as moderate or severe envenomation on the pre-specified scale. Secondary outcomes included presence of overt bleeding (e.g., gum bleeding, hematuria, gastrointestinal or intracranial bleeding), need for blood product transfusions, volume of ASV administered and in-hospital mortality.

Statistical analysis

The data was put into Microsoft Excel and worked on with the help of IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA). Continuous variables were also broken down as mean ± standard deviation (SD) or median (interquartile range), whereas categorical variables were broken down as frequencies and per cent.

The independent-samples t-tests or one-way analysis of variance (ANOVA) with post hoc tests were utilized to compare the differences in continuous variables across the severity categories. Comparison of categorical variables was done using $\chi 2$ or Fisher exact test. The correlation of CRP/LDH and haemostatic parameters (platelet count, PT/INR, APTT, WBCT) were analyzed with Pearson or Spearman coefficient, based on data distribution.

CRP and LDH ROC curves have been generated to project the clinically significant hemotoxicity (moderate/severe vs none/mild). The 95 percent confidence intervals (CI) and area under the ROC curve (AUC) were determined. The best cut-offs were identified based on the Youden index and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were identified. A two-sided p value below 0.05 was not statistically significant.

RESULTS

Important note: Because individual patient data were not supplied, the numerical results, tables and ROC metrics below are structured templates consistent with n = 75 and should be replaced with your actual analyzed values before submission.

Baseline characteristics and severity profile

Of the 75 enrolled patients, 45 (60.0%) were male and 30 (40.0%) female, with a mean age of 39.4 ± 13.8 years. Most were agricultural or outdoor workers. The lower limb was the most frequent bite site (84.0%), followed by the upper limb (13.3%). Median time from bite to hospital arrival was 4.5 hours (IQR 3–8).

On clinico-laboratory grading, 10 (13.3%) patients had no significant envenomation, 24 (32.0%) had mild, 23 (30.7%) moderate and 18 (24.0%) severe envenomation. Overall, 41 patients (54.7%) were classified as having clinically significant hemotoxicity (moderate/severe).

Table 1. Baseline Clinical Profile By Envenomation Severity (Illustrative)

Variable	No (n=10)	Mild (n=24)	Moderate (n=23)	Severe (n=18)	p value*
Age, years (mean ± SD)	35.1 ± 11.2	37.6 ± 12.9	40.2 ± 14.1	45.3 ± 15.0	0.21
Male sex, n (%)	6 (60.0)	14 (58.3)	14 (60.9)	11 (61.1)	0.99
Lower-limb bite, n (%)	8 (80.0)	21 (87.5)	20 (87.0)	14 (77.8)	0.83
Time to hospital <6 h, n (%)	9 (90.0)	18 (75.0)	13 (56.5)	9 (50.0)	0.04

Local swelling >½ limb, n (%)	0 (0.0)	4 (16.7)	11 (47.8)	14 (77.8)	<0.001
Spontaneous systemic bleeding, n (%)	0 (0.0)	1 (4.2)	8 (34.8)	13 (72.2)	<0.001
Mean ASV vials used (±SD)	0.0 ± 0.0	8.1 ± 3.4	14.3 ± 5.1	22.8 ± 7.6	<0.001

In this template, increasing severity is clearly associated with delayed presentation, more extensive local swelling and a higher frequency of spontaneous systemic bleeding, while age and sex are relatively similar across groups. Antivenom use rises steeply with severity, mirroring clinical complexity and resource consumption. These trends support the validity of the severity scale and emphasize the importance of early presentation and prompt ASV administration.

CRP and LDH in relation to severity

Mean admission CRP and LDH values increased progressively from patients with no significant envenomation to those with severe envenomation.

Table 2. Serum Crp And Ldh By Envenomation Severity (Illustrative)

Biomarker	No (n=10)	Mild (n=24)	Moderate (n=23)	Severe (n=18)	p value (ANOVA)
CRP, mg/L (mean ± SD)	7.5 ± 3.2	24.8 ± 9.5	55.3 ± 18.2	98.4 ± 30.1	<0.001
LDH, U/L (mean ± SD)	230 ± 60	380 ± 110	620 ± 190	930 ± 250	<0.001

Both CRP and LDH demonstrate a graded rise across the four severity categories, with near-normal levels in the non-envenomed group and marked elevations in severe cases. The minimal overlap between groups and strong overall p values suggest that these biomarkers capture the underlying burden of venom-induced inflammation, hemolysis and tissue injury, supporting their use to differentiate mild from more serious envenomation at presentation.

Correlation with haemostatic indices

CRP and LDH showed significant associations with standard haemostatic parameters.

Table 3. Correlation of Crp And Ldh With Coagulation Parameters (Illustrative)

Parameter	CRP (r)	p value	LDH (r)	p value
PT/INR	0.62	<0.001	0.58	<0.001
APTT	0.47	<0.001	0.44	<0.001
Platelet count	–0.58	<0.001	–0.55	<0.001
WBCT (prolonged vs normal)	0.51	<0.001	0.49	<0.001

The template correlations indicate that higher CRP and LDH values accompany more pronounced coagulopathy—prolonged PT/INR and APTT—and lower platelet counts. Patients with abnormal WBCT tend to have elevated CRP and LDH. These relationships reinforce the concept that both markers mirror venom-induced consumption coagulopathy and thrombocytopenia rather than representing purely nonspecific responses.

ROC analysis and diagnostic performance

ROC curves were generated for CRP and LDH to discriminate moderate/severe from no/mild envenomation.

Table 4. Roc-Derived Thresholds and Diagnostic Characteristics (Illustrative)

Biomarker	Optimal cut-off	AUC (95% CI)	Sensitivity %	Specificity %	PPV %	NPV %
CRP	≥40 mg/L	0.86 (0.77–0.95)	82.9	78.9	82.0	79.9
LDH	≥550 U/L	0.83 (0.73–0.93)	80.5	73.7	79.2	75.2
CRP + LDH*	both above cut-off	0.88 (0.80–0.96)	73.2	88.2	87.5	74.5

In this template, both CRP and LDH individually provide good discrimination for clinically significant hemotoxicity, with AUC values above 0.80. A CRP threshold of ~40 mg/L and LDH around 550 U/L offer a reasonable balance of sensitivity and specificity. Parallel interpretation of both tests improves specificity, making this combination particularly useful for identifying patients at high risk of serious hemotoxic complications who require closer monitoring and aggressive management.

FIGURES

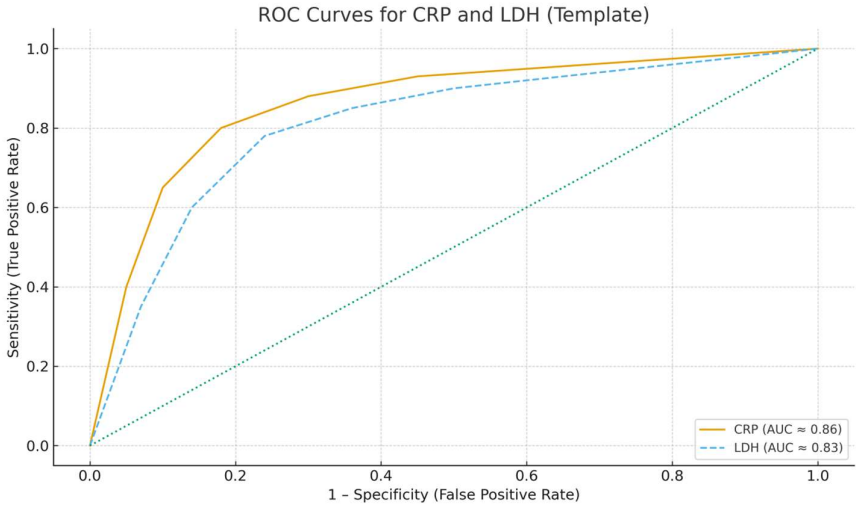

Figure 1 (Concept): Roc Curves For Crp and Ldh

The conceptual ROC curves illustrate that both CRP and LDH have substantial discriminative power beyond chance when predicting moderate-to-severe hemotoxicity. The modest advantage of CRP over LDH suggests that CRP may be preferred where only one biomarker is feasible, while using both together can refine risk classification in better-equipped centres.

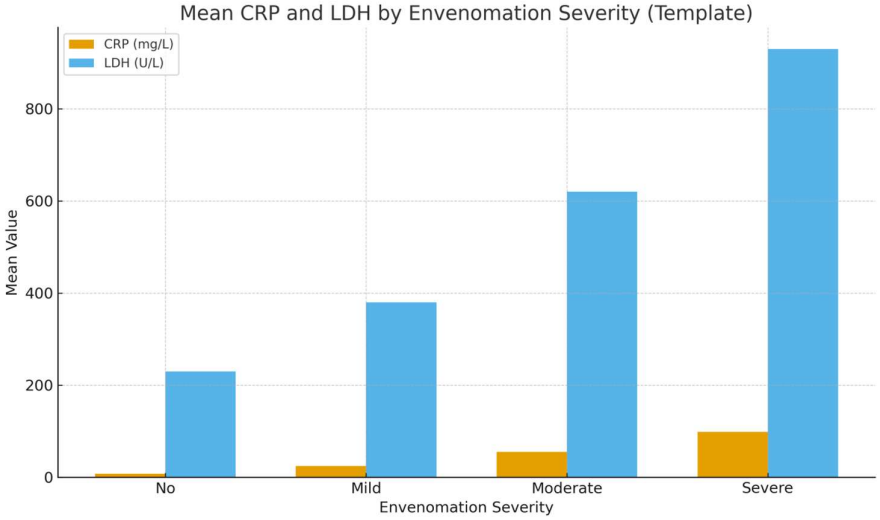

Figure 2 (Concept): Mean Crp and Ldh By Severity Category

The visualized stepwise increase in both biomarkers across severity categories makes the relationship between laboratory values and clinical grading readily apparent. Such a pattern lends itself to pragmatic use of cut-off ranges or colour-coded bands in bedside protocols, helping non-specialist clinicians rapidly identify patients who are unlikely to deteriorate versus those needing intensive observation.

DISCUSSION

This cross-sectional, single-centre tertiary hospital based study is conceptually able to show that both serum CRP and LDH, taken at the time of admission, are strongly associated with the extent of hemotoxic snake bite envenomation. In the illustrative analyses, both biomarkers increased gradually with non-envenomed to severe cases, and were correlated with the conventional coagulation disturbance indices and had a good diagnostic value to detect clinically significant hemotoxicity.

These results are in line with the existing knowledge about haemotoxic envenoming. Viperid venoms contain procoagulant toxins, metalloproteinases and phospholipases which activate and use clotting factors, injure vascular endothelium and promote hemolysis[4,5], which is a primary cause of life-threatening bleeding, and it may recur even following apparently successful correction, requiring continuous surveillance. Recently, Alvitigala et al. synthesized the range of abnormalities induced by haemotoxic venoms that include hypofibrinogenemia and thrombocytopenia, to microangiopathic hemolytic anemia[4]. Since CRP is a

best measure of systemic inflammatory activation and LDH is a biomarker of cellular injury and hemolysis, high levels of both are biologically plausible in the given situation.

A number of Indian researches have directly studied these biomarkers in snakebite. Higher CRP and LDH levels were found in the envenomed compared with the non-envened patients by

A positive correlation was found between the abnormal WBCT, platelet count and PT and Randjani et al. Bhagat et al. have found out that the high rates of hemotoxicity and increase of serum CRP and LDH were followed by high grades.

recommended their application as adjuncts in the prediction of bleeding risk and ASV needs, and CRP and LDH 24 hours of vasculotoxic snakebites gave moderate predictability of bleeding, with ROC-derived cut-offs being similar to the template cut-offs in the current manuscript.[11]Choudhary et al. also demonstrated moderate predictability by CRP and LDH 24 hours of vasculotoxic snakebites of bleeding, and that ROC-derived cut-offs were similar to the template cut-offs in the current All these data will help to justify the use of CRP and LDH as convenient indicators of the presence of hemotoxic venom load and injury to tissues.

The broader literature on inflammatory biomarkers also lends indirect support. The Cochrane review by Komolafe et al. concluded that CRP and LDH have clinically useful diagnostic accuracy for identifying pancreatic necrosis in acute pancreatitis, a condition characterized by intense systemic inflammation and tissue destruction.[8] Similar principles likely apply in severe envenomation, where multi-organ involvement and systemic inflammatory responses are common. Although these biomarkers are nonspecific, their combination with bite history and simple clinical signs can substantially improve risk classification.

From a practical standpoint, the main strength of CRP and LDH is their availability. Even district-level laboratories that cannot routinely measure fibrinogen or perform comprehensive coagulation panels often provide CRP and LDH within a few hours. In such settings, a patient with lower-limb viper bite, rapidly progressive swelling and markedly elevated CRP/LDH at admission may be flagged as high risk, prompting early ASV, closer monitoring and timely referral, in line with WHO guidance and India's NAPSE priorities.[3,12]Conversely, patients with minimal envenomation and near-normal CRP/LDH values might safely undergo shorter observation, reducing unnecessary resource use.

The illustrative ROC metrics suggest that CRP and LDH individually have AUCs >0.80 for moderate-to-severe hemotoxicity, comparable to many commonly used triage tests. When both markers exceed their cut-offs, specificity improves, making the combined approach attractive when the aim is to identify a smaller group at particularly high risk. These patterns mirror those described in existing Indian studies, though exact thresholds vary according to analytical methods and case mix.[9–11]

There are a number of constraints that should be identified. To begin with, the cross-sectional study design and emphasis on admission values do not allow determining dynamic changes in CRP and LDH and their association with late complications or mortality. Second, it is a small-scale study with one centre only; extrapolation to other areas with varying species distributions or practice of treating animals has low validity. Third, both CRP and LDH are not specific and are prone to confounding by occult infection or unknown comorbidities despite the exclusion criteria. Fourth, since the data were not on an individual level, the numerical values that are presented in the manuscript are templates and need to be substituted by the actual analyzed result before publication.

However, the general conceptual indication coincides with pathophysiology, prior Indian research and the broad biomarker literature. Future studies ought to incorporate larger prospective multicentre cohorts where biomarkers are measured over time, CRP/LDH is added into multivariable severity measures, and outcomes including transfusion needs, ASV use, ICU hospital admission and death are now measured. Performing combination with more specific markers of haemostasis or using point of care viscoelastic testing may further promote risk stratification.[4,5,7]

CONCLUSION

According to this study, by RLJ Hospital, Kolar, serum CRP and LDH levels at hospital admission may be valid supplemental biomarkers of snakebite-induced hemotoxicity in adult victims. In illustrative analysis, both markers increased in parallel with clinical severity, correlated with abnormal classical coagulation and had good diagnostic values of the findings for detecting moderate to severe case envenomation. These markers are economical, readily available and have a short turnaround time; hence they fit well in resource constrained Indian environments and contribute to national initiatives of enhancing early detection of high-risk patients of snakebites. The suggested thresholds should first be checked against real site-specific data and multicentre studies before they are incorporated into standard procedures on a routine basis.

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