COMMENTARY

Snakebites and microvesicles: Popping bubbles

Mettine H.A. Bos PhD 🔟 | Henri H. Versteeg PhD 🔟

Department of Internal Medicine, Division of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands
This is a commentary on *Enjeti et al* [2018]: https://doi.org/10.1002/rth2.12164

Correspondence
Mettine H.A. Bos, Department of Internal Medicine, Division of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands.
Email: M.H.A.Bos@lumc.nl

Snakebite envenoming is estimated to cause 95 000-125 000 deaths and up to 400 000 permanent injuries or disabilities worldwide on an annual basis. This serious health threat has been recognized by the World Health Organization, and snakebite envenoming is classified as a category A neglected tropical disease.[1] In May of 2018, the World Health Assembly adopted a resolution on snakebite envenoming,[2] thereby providing a mandate to develop a comprehensive plan to effectively control the burden of envenoming and reduce its debilitating impact. The multisectoral strategy that is required to achieve this will address socioeconomic, environmental, and healthcare challenges. Targeted actions to raise awareness have culminated in, among others, a recent international scientific conference where many essential stakeholders united in the fight against snakebite envenoming.[3] While the highest health burden from venomous snakebites falls on south Asia and sub-Saharan Africa,[4] exact information on the number of envenomings, clinical effects of snakebites, and effective treatments are lacking on a global scale. In an effort to address this, the Australian Snakebite Project registry has compiled information on a substantial proportion of all Australian snakebites since 2005.[5,6] This study clarified that the most commonly observed clinical envenoming phenotype in Australia is venom-induced consumptive coagulopathy (VICC),[5] an unbridled activation and consumption of the coagulation system. This is due to the venom composition of the snakes that are most commonly involved in these envenomings[5]; *Pseudonaja* species contain procoagulant factor Xa-Va-like and *Notechis* species factor Xa-like enzymes.[7-9] Once injected into the blood stream of the prey, the *Notechis* factor Xa-like protease interacts with prey factor V(a),[10] and the circulating venom factor Xa-Va-like enzymes are capable of converting prothrombin of the prey into thrombin.[10,11] Action of thrombin induces VICC, which is characterized by a (complete) consumption of fibrinogen and factors V and VIII, elevated D-dimer levels, and an international normalized ratio >3.[12,13]

From the envenomed individuals that developed VICC as reported in the Australian Snakebite Project, approximately 10% presented with microangiopathic hemolytic anemia (MAHA),[5] resulting from intravascular red cell fragmentation with schistocytosis and thrombocytopenia. Most MAHA cases were observed following taipan (*Oxyuranus* species, 26% MAHA cases) and brown snake (*Pseudonaja* species, 14% MAHA cases) envenoming, while bites from *Notechis* and *Hoplocephalus* species each led to MAHA in 7% of envenomed individuals. Interestingly, this correlates with the venom composition of these snakes, with both *Oxyuranus* and *Pseudonaja* venom comprising factor Xa-Va-like prothrombin activators, while a factor Xa-like protease is found in the venom of *Notechis* and *Hoplocephalus* snakes.[7-9] Also, the onset of VICC appears to proceed more rapidly following envenoming by *Pseudonaja* snake bites relative to those of *Notechis* snakes,[14] and MAHA only seems to occur following VICC-associated snakebites.[5] Despite these indirect observations, the exact pathophysiology of venom-associated MAHA remains unclear.

To gain insight into the venom-mediated damage to the endovascular system, in their recent article in *Research and Practice in Thrombosis and Haemostasis*, Enjeti and colleagues have focused on the role of microvesicles (MVs) in snakebite patients.[15] Microvesicles are shed by outward blebbing of the plasma membrane and typically function to transfer cargo, such as proteins and ribonucleic acids, from a donor cell to an acceptor cell, leading to behavioral changes in the acceptor cell. Microvesicles may be shed by platelets, endothelial cells, megakaryocytes, and tumor cells, and the diameter of these vesicles ranges from 30 to 1000 nm.[16,17] Elevated levels of MVs in the circulation are often associated with pathophysiological conditions such as sepsis, diabetes mellitus, cardiovascular diseases, and cancer,[18-22] and MVs may even impact progression of disease.

In their study, Enjeti and colleagues primarily studied MVs generated by platelets, red blood cells, and endothelial cells. Compared to control subjects, platelet MVs were significantly higher in the blood of snakebite patients that did not suffer from MAHA, while paradoxically red blood cell–derived MV levels were

enhanced in patients experiencing MAHA.[15] In snakebite patients either suffering from MAHA or not, endothelial MVs were decreased. These observations led the authors to speculate on the usefulness of red blood cell MVs as a surrogate marker for MAHA. While the results of the current study are certainly of interest, a number of drawbacks may be identified. First of all, characterization of the MVs in this study could have been more extensive; flow cytometry was applied to detect MVs, and this method only detects larger MVs. Thus, the majority of MVs (with a diameter of <400 nmol L^{-1}) may have been missed. It remains unclear how many of the red blood cell MVs were actually red cell fragments, with fragmentation being a hallmark of MAHA. Second, while the diagnostic use of MVs in snakebite patients sounds attractive, equipment allowing for high throughput analysis of MVs in snakebite patients is not readily available. More rapid and precise diagnostic strategies to identify envenomed patients with MAHA could lead to a faster administration of specific treatment modalities. Current therapy of envenomed patients consists of specific antivenom administration, with early treatment (preferably within the hour post-snakebite) being most effective, as VICC develops very rapidly.[14] However, VICC has also been reported to resolve within 24-48 hours without treatment,[10] which is likely due to re-synthesis of the depleted coagulation proteins. While the reports are scarce with respect to the treatment of MAHA, this syndrome seems to resolve following toxin neutralization by antivenom administration.[10]

Finally, the current study failed to answer why envenomation leads to MV generation and if these MVs play a role in MAHA and/or the coagulopathy often seen in snakebite patients. Furthermore, it is ambiguous whether MAHA is secondary to VICC, and if so, whether fibrin deposition in the microvasculature could contribute to the pathophysiology of MAHA. While this would be consistent with the formation of schistocytes and the observed thrombocytopenia, the correlation with both platelet and endothelial MVs remains to be identified. Until further work is performed on the link between MVs and venom-associated MAHA in snakebite patients, a causal role for such MVs in these pathological processes may just be a popping bubble.

RELATIONSHIP DISCLOSURE

H.H. Versteeg reports nothing to disclose; M.H.A. Bos reports participation on an advisory board for uniQure.

AUTHOR CONTRIBUTIONS

Both authors co-wrote the manuscript.

ORCID

Mettine H.A. Bos https://orcid.org/0000-0002-8164-817X
Henri H. Versteeg https://orcid.org/0000-0003-4294-175X

REFERENCES

1. Chippaux J-P. Snakebite envenomation turns again into a neglected tropical disease! J Venom Anim Toxins Incl Trop Dis. 2017;23:38–9.
2. Burki T. Resolution on snakebite envenoming adopted at the WHA. Lancet. 2018;391:2311.
3. Snakebite – from science to society. Jun 21-22, 2018; Naturalis, Leiden, The Netherlands. [Accessed 2019 January 10] Available from https://snakebite.naturalis.nl/.
4. Longbottom J, Shearer FM, Devine M, et al. Vulnerability to snakebite envenoming: a global mapping of hotspots. Lancet. 2018;392:673–84.
5. Johnston CI, Ryan NM, Page CB, et al. The Australian Snakebite Project, 2005-2015 (ASP-20). Med J Aust. 2017;203(3):119–25.
6. Warrell DA. Snakebites: reducing their international impact. Med J Aust. 2017;203:112–3.
7. Rao VS, Joseph JS, Kini RM. Homologues of mammalian blood coagulation factor Xa. Biochem J. 2003;369:635–42.
8. Bos MHA, Camire RM. Procoagulant adaptation of a blood coagulation prothrombinase-like enzyme complex in Australian Elapid venom. Toxins. 2010;2:1554–67.
9. Lister C, Arbuckle K, Jackson TNW, et al. Catch a tiger snake by its tail: differential toxicity, co-factor dependence and antivenom efficacy in a procoagulant clade of Australian venomous snakes. Toxins. 2017;202:39–54.
10. Newell-Caito JL, Laha M, Tharp AC, et al. Notecarin D binds human factor V and factor Va with high affinity in the absence of membranes. J Biol Chem. 2011;286:38286–97.
11. Bos MHA, Boltz M, Pierre LS, Masci PP, Lavin MF, Camire RM. Venom factor V from the common brown snake escapes hemostatic regulation through procoagulant adaptations. Blood. 2009;114:686–92.
12. Masci PP, Rowe EA, Whitaker AN, de Jersey J. Fibrinolysis as a feature of disseminated intravascular coagulation (DIC) after Pseudonaja textilis textilis envenomation. Thromb Res. 1990;59:859–70.
13. Isbister GK. Procoagulant snake toxins: laboratory studies, diagnosis, and understanding snakebite coagulopathy. Semin Thromb Hemost. 2009;35:93–103.
14. Isbister GK, Scorgie FE, O'Leary MA, Seldon M, Brown SGA, Lincz LF. Factor deficiencies in venom-induced consumption coagulopathy resulting from Australian elapid envenomation: Australian Snakebite Project (ASP-10). J Thromb Haemost. 2010;8:2504–13.
15. Enjeti AK, Lincz LF, Seldon M, Isbister GK. Circulating microvesicles in snakebite patients with microangiopathy. Res Pract Thromb Haemost. 2018;3:121–5.
16. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373–83.
17. Herring JM, McMichael MA, Smith SA. Microparticles in health and disease. J Vet Intern Med. 2013;27:1020–33.
18. Matsumoto H, Yamakawa K, Ogura H, et al. Enhanced expression of cell-specific surface antigens on endothelial microparticles in sepsis-induced disseminated intravascular coagulation. Shock. 2015;43:443–9.
19. Li S, Wei J, Zhang C, et al. Cell-derived microparticles in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Cell Physiol Biochem. 2016;39:2439–245.
20. Zaldivia MTK, McFadyen JD, Lim B, Wang X, Peter K. Platelet-derived microvesicles in cardiovascular diseases. Front Cardiovasc Med. 2017;4:74.
21. Campello E, Spiezia L, Radu CM, et al. Endothelial, platelet, and tissue factor-bearing microparticles in cancer patients with and without venous thromboembolism. Thromb Res. 2011;127:473–7.
22. Toth B, Liebhardt S, Steinig K, et al. Platelet-derived microparticles and coagulation activation in breast cancer patients. Thromb Haemost. 2008;100:663–9.