

## Insights of *Bothrops atrox* envenoming through label-free proteomics

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**Introduction:** Snakebite envenoming is a neglected tropical disease affecting over 2 million people worldwide. In Brazil, the Amazonas state has an 80% incidence rate of snakebite accidents involving *Bothrops atrox*. Understanding its venom protein mechanism is incomplete, offering an opportunity to uncover biochemical alterations induced by the venom. **Objective:** Characterize the protein profile of *B. atrox* envenoming victim's urine. **Methodology:** We collected the urine of 10 snakebite patients (envenomed) and 10 of healthy individuals (control). Protein extraction was made with methanol precipitation followed by Urea/Thiourea, reduction with dithiothreitol (DTT), alkylation with iodoacetamide (IAA), incubation with DTT and then digestion with trypsin. The peptide mixture was desalted, concentrated, resuspended in acid formic, and analyzed using an Ultimate 3000 Basic online with a Fusion Lumos Orbitrap mass spectrometer set in data-dependent acquisition mode (DDA). Data analysis was performed using the software Patternlab for Proteomics V (PLV) applying a peptide spectrum match for identification, and XIC for protein relative quantification. The biological processes associated with the proteins found were investigated using UNIPROT, Human Protein Atlas, and Reactome databases. **Results:** We identified 16,015 peptides, 1,909 proteins with redundancy and quantified 1,082 proteins with at least two unique peptides. Comparing both conditions, we found 32 proteins exclusively identified in the snakebite envenoming patients' urine. From these, we highlight Carboxypeptidase B2 (CPB2) which reduces the fibrinolysis process prolonging blood clots and may act to avoid mucous membrane bleeding. Also, Apolipoprotein C-II (APOC2), involved in the catabolism of extracellular lipids, and Myocilin (MYOC) which is associated with cell growth regulation. We suggest these might be residues of cells and tissues affected by the venom, or even of a "push-pull" mechanism to control, eliminate, and regenerate the biochemical homeostasis. Furthermore, Dermcidin is associated with antimicrobial activity during early bacterial colonization, and its presence might occur due to microbial exposure by the snakebite tissue rupture. Regarding the shared proteins, we found 29 proteins more abundant in the envenomed group. Using the STRING database, we found a network of proteins interacting related to fibrin formation, infection defense, and lipid regulation. We highlight the proteins Fibrinogen beta chain (FGB), Fibrinogen alpha chain (FGA), Fibrinogen gamma chain (FGG), and Apolipoprotein C-III (APOC3) that form a backbone of the blood clot process formation associated with infection-stimulated blood loss prevention. In addition, the protein Proenkephalin (PENK) is a neuropeptide that plays a role in pain perception, stress response, and cell cycle regulation in kidney regeneration, the latter being associated with renal failure, a major complication in *Bothrops* envenoming. **Conclusion:** Our study revealed protein alterations in the urine of *B. atrox* snakebite victims linked to coagulation, lipid metabolism, and antimicrobial activity during or as a byproduct of envenomation, offering insights for further

research and potential improvements in diagnosis and treatment.

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