# Discovering innovative drugs and diagnostic tools to control haemostasis by venomics of the *Vipera a. ammodytes* snake

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Snake venoms are a rich source of proteins acting on the haemostatic system, *i.e.* platelet aggregation, coagulation cascade and fibrinolysis. Many of these components have already been proved as useful for developing novel agents for treatment of vascular thromboembolic disorders (1). The venom of *Vipera a. ammodytes* (*Vaa*) snake causes different clinical disorders in man, the most pronounced of which are clotting disorders and haemorrhage. We have used a systematic proteomic approach to explore haemostatic activity of *Vaa* venom. With a two dimensional gel electrophoresis of the raw venom we obtained 208 discrete protein spots of molecular masses ranging from 10 to 70 kDa. Spots were excised from the gel and tryptic digests of proteins analysed by mass spectrometry. Identified proteins belong to 13 different families, including those that have a high potential to affect haemostasis, *i.e.* serine and metalloproteinases, phospholipases, C-type lectins and disintegrins. Our intent has however not been only to detect venom components acting on haemostasis but also to find components affecting particular parts of haemostatic system critical in some pathologies. In this way we have been targeting novel-pharmacological leads to develop pathology-specific therapeutics. For this reason in the first step the whole venom was fractionated by gel filtration and fractions were tested *in vitro* for their effect on different blood clotting factors, blood coagulation, and platelet aggregation and agglutination. In this screen we have detected several inhibitors of platelet aggregation and agglutination, probably acting on different platelet receptors and/or their ligands. Other components affected the process of blood coagulation, either activating different steps of this process or inhibiting it. In the second step, we have purified components in the venom fractions with haemostatic activity to homogeneity using various chromatographic technics in order to identify those responsible for effects at the molecular level. Some of them were present in rather small amounts. Thus the venom gland cDNA library from *Vipera a. ammodytes* was constructed, that will enable isolation of specific nucleotide sequences and synthesis of proteins using recombinant production.

References:

1. T. Sajevic, A. Leonardi, I. Križaj, *Toxicon.* **2011**, *57*, 627-645.