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Review

Vipers of Major clinical relevance in Europe: Taxonomy, venom composition, toxicology and clinical management of human bites

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ABSTRACT

Snakebites in Europe are mostly due to bites from Viperidae species of the genus *Vipera.* This represents a neglected public health hazard with poorly defined incidence, morbidity and mortality. In Europe, fourteen species of "true vipers" (subfamily Viperinae) are present, eleven of which belong to the genus *Vipera*. Amongst these, the main medically relevant species due to their greater diffusion across Europe and the highest number of registered snakebites are six, namely: *Vipera ammodytes*, *V. aspis*, *V. berus, V. latastei, V. seoanei* and *V. ursinii*. Generally speaking, viper venom composition is characterised by many different toxin families, like phospholipases A2, snake venom serine proteases, snake venom metalloproteases, cysteine-rich secretory proteins, C-type lectins, disintegrins, haemorrhagic factors and coagulation inhibitors. A suspected snakebite is often associated with severe pain, erythema, oedema and, subsequently, the onset of an ecchymotic area around one or two visible fang marks. In the field, the affected limb should be immobilised and mildly compressed with a bandage, which can then be removed once the patient is being treated in hospital. The clinician should advise the patient to remain calm to reduce blood circulation and, therefore, decrease the spread of the toxins. In the case of pain, an analgesic therapy can be administered, the affected area can be treated with hydrogen peroxide or clean water. However, anti-inflammatory drugs and disinfection with alcohol or alcoholic substances should be avoided. For each patient, clinical chemistry and ECG are always a pre-requisite as well as the evaluation of the tetanus immunisation status and for which immunisation may be provided if needed.

The treatment of any clinical complication, due to the envenomation, does not differ from treatments of emergency nature. Antivenom is recommended when signs of systemic envenomation exist or in case of advanced local or systemic progressive symptoms. Recommendations for future work concludes.

The aim of this review is to support clinicians for the clinical management of viper envenomation, through taxonomic keys for main species identification, description of venom composition and mode of action of known toxins and provide a standardised clinical protocol and antivenom administration.

1. Introduction

Snakebites constitute a significant public health issue in developing and developed countries, with about 138,000 casualties registered worldwide on a yearly basis (Gutiérrez et al., 2017; Kasturiratne et al., 2008; Longbottom et al., 2018). When not lethal, snakebite outcomes often lead to the development of long-lasting disabilities, with more than 400,000 cases reported yearly worldwide. In May 2019, during the

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last World Health Assembly, the World Health Organization (WHO) stressed the importance to drastically reducing snakebite-related human deaths and disabilities by 2030 (The Lancet, 2019). Indeed, public health concern towards snakebite is relevant to both developing and developed countries, this primarily being an occupational hazard in the former, and an environmental hazard associated with outdoor activities in the latter (Alger et al., 2019; Kim et al., 2019).

In this context, Europe has a high degree of biodiversity because of its geo-morphological, climatic characteristic, and a wide range of environmental typologies. Hence, the ophidian fauna is well represented and includes different venomous snake species (Speybroeck et al., 2016). However, since the reporting of snakebites through Europe is not mandatory, many of them remain unreported and, therefore, largely underestimated (Gold et al., 2002). Only few European countries, such as France and Sweden, routinely record snakebites, which has allowed a better local clinical management and the creation of national registers, providing structured data for human snakebite occurrence (Chippaux, 2012).

Furthermore, European snakebite victims do not always seek treatment and physicians do not regularly consult poison-control centers (Gold et al., 2002). Most physicians are not trained to identify snakebites and recognise their clinical signs and treatments. This not only hampers a timely intervention and wastes medical and economical resources, but also results in a considerable underestimation of snakebites. While the WHO and other authors have published general protocols to manage snakebites, to date, standardised protocols for Europe are needed to investigate the incidence, morbidity and mortality in relation to viper bites (Mohammad Alizadeh et al., 2016; Nelson et al., 2019; Pizon and Ruha, 2015; Walter et al., 1998; WHO, 2016). This review provides keys to distinguish the main medically relevant European snakes belonging to the genus *Vipera* (*Vipera ammodytes*, *Vipera aspis*, *Vipera berus, Vipera latastei, Vipera seoanei* and *Vipera ursinii*, chosen on the basis of their greater diffusion across Europe and the highest number of registered venomous snake bites, according to Paolino et al. (2020)) from other non-dangerous species, the description of venom composition and mode of action of the main toxins and a protocol to support clinicians for the management of *Vipera* envenomations.

2. Zoological framework

2.1. Morphological distinctions between vipers and other European snakes

An important first step in the management of snakebites is the identification of the species involved in the bite. According to the recent taxonomic updates and including the main alien taxa, 57 species of snakes live in Europe, belonging to six different families: Colubridae, Erycidae, Natricidae, Psammophiidae, Typhlopidae and Viperidae (Fig. 1) (Freitas et al., 2020; Speybroeck et al., 2020; Zaher et al., 2019).

Amongst these, a limited number of taxa have been reported to be medically important (Geniez, 2018; Speybroeck et al., 2016; WHO, 2010b), and they all belong to the Viperidae family, which in Europe consists of fourteen species (see Supplementary Table 1).

Vipers can be easily identified in comparison with the other European snakes using a series of morphological characteristics as well as the symptomatology associated with their bite. Excluding Erycyidae and Typhlopidae families, which include snakes morphologically (and ecologically) very different from other European species (Fig. 1), the remaining non-viperid snakes are all colubrids *sensu lato* (Colubridae, Natricidae and Psammophiidae) (Uetz et al., 2020; Zaher et al., 2019).

The following key represents an useful tool to distinguish between vipers and colubrids *sensu lato* (Fig. 2):

- The body of vipers (Fig. 2A1) is proportionally shorter and stockier than that of the colubrids (Fig. 2B1), with a lower length/width ratio.

Fig. 1. Heads of the six snake families present in Europe: Colubridae (A), Natricidae (B), Psammophiidae (C), Viperidae (D), Erycidae (E), Typhlopidae (F). The species portrayed in the pictures are: *Zamenis lineatus* (A), *Natrix maura* (B), *Malpolon insignitus* (C), *Vipera ursinii* (D), *Eryx jaculus* (E), *Indotyphlops braminus* (F). Photo credits: Matteo R. Di Nicola.

Fig. 2. Main morphological differences between European vipers (A) and colubrids (B). The species portrayed in the pictures are: *Vipera berus* (A1, A2, A3), *Vipera aspis* (A4), *Hierophis viridiflavus* (B1, B2, B3, B4 sx), *Natrix helvetica* (B4 dx). Photo credits: Matteo R. Di Nicola.

In addition, European vipers usually reach an average size of 50−70 cm and do not exceed the meter (only *Montivipera xanthina*, limited to Greek Thrace and some Greek islands off the Turkish West coast, can exceed 100 cm). Various species of colubrids, on the other hand, abundantly exceed one meter in length.

- The pupil of vipers (Fig. 2A2) is vertical and in full light takes on a slit-like shape; the pupil of the colubrids (Fig. 2B2) is round. The only exception is *Telescopus fallax*, an (harmless to humans) opistoglyphous colubrid, present only in extreme N-E Italy, western portion of the Balkan Peninsula and Greece, which has a slit-like pupil.
- Vipers (Fig. 2A2) have at least one row of sub-ocular scales that separate the eye from the supra-labial scales; colubrids (Fig. 2B2) have the eye in contact with the supra-labial scales. The only exceptions are: *Hemorrhois hippocrepis*, from Iberian Peninsula, Mallorca, Ibiza, South Sardinia and Pantelleria; *H. algirus*, from Malta (both non-venomous colubrids).
- Vipers (Fig. 2A3) have the top of the head covered by small scales arranged irregulary or at most three shields arranged simmetrically, surrounded by smaller scales; colubrids (Fig. 2B3) have the top of the head covered by 9–11 large smooth shields symmetrically arranged. This character has no exceptions.
- Vipers (Fig. 2A4) have dorsal scales of the trunk always keeled; colubrids (Fig. 2B4) can have dorsal scales of the trunk smooth or keeled depending on the species (in Psammophiidae dorsal scales are grooved).
- -The shape of the head is not a reliable distinctive character: vipers can have a more or less triangular or a sub-oval head shape; some colubrids can have (or take, as a defense attitude) a more or less triangular head shape too.

Given that European colubrids *sensu lato* are not considered of medical relevance (WHO, 2010b), it should be mentioned that European Psammophiidae could be considered of moderate (and rare) medical importance.

Two Psammophiidae species lives in Europe: *Malpolon monspessulanus*, present in the Iberian Peninsula, Southern France and Western Liguria (Italy); *M. insignitus*, present along the Balkan coast, in Greece (including several islands), Southern Bulgaria, Turkish Thrace and on Lampedusa island (Italy) (Speybroeck et al., 2016; Uetz et al., 2020).

Malpolon spp. are opistoglyphous snakes (with grooved fangs in the rear of the upper jaw): the venom secreted by their Duvernoy's glands can produce local effects such as local pain, oedema, lymphagitis, lymphadenitis, local paresthesia or even paresis of the limb and, rarely, systemic effects such as affection of cranial nerves, eyelid ptosis, muscle weakness, dyspnea and dysphagia (Ottonello et al., 2011; Valenta,

2010).

However, because of tendency of these species to flee immediately in case of danger, the characteristics of their venom inoculation system and the moderate action of the venom itself (de facto, only an envenomation caused by a deep and persistent bite, received by an adult individual, would have real probability of provoking more relevant symptoms) do not include these snakes in those of medical relevance in Europe (Bruno and Maugeri, 1990; Geniez, 2018). For *Malpolon* species no antivenom is available (Valenta, 2010; www.toxinology.net). In case of bite from *Malpolon* spp., refer to the general indications provided in the chapter "Snakebite: Therapy and Prevention" in Valenta (2010).

2.2. Identification of European Vipera species

Among the fourteen Viperidae species present in Europe, those considered to be of major medical relevance, based on their greater diffusion and the higher number of registered snake bites, are the following six: *Vipera ammodytes*, *V. aspis*, *V. berus, V. latastei, V. seoanei* and *V. ursinii* (Fig. 3) (Paolino et al., 2020).

In case of a snakebite, correct identification of the snake species is essential to apply the most appropriate therapy. Therefore, it would be important that the bitten person takes photographs of the animal, to aid with its identification once with healthcare providers. Photographing the snake responsible for the bite is a practice recommended by the WHO (WHO, 2016), and it is becoming more and more common in countries where snakebites are significant in humans (e.g. Australia, Colombia, Malaysia; Bolon et al., 2020). Although the patient description of the biting snake can also be important for species identification, photographs are generally more reliable and can be rapidly shared with experts, thus likely allowing a faster, more accurate identification of the culprit species (Bolon et al., 2020). If the bite was accidental and the patient could not observe carefully or take a photograph of the animal, a description of the geographical area, altitude and habitat typology where the bite occurred is important to provide a basis for a tentative identification of the species.

The European vipers can be identified by morphological criteria, like pholidosis (i.e. the organisation of the body scales in number, shape, position and arrangement). Furthermore, vipers differ in ecological and distributional characteristics: there are species found only in certain areas and at certain altitudes (Geniez, 2018; Speybroeck et al., 2016). For this reason, geolocation can also help in determining the species. The reader is referred to the website of the New Atlas of Amphibians and Reptiles of Europe (NA2RE) project curated by the *Societas Europaea Herpetologica* for detailed information on the distribution of the different European viper species (http://na2re.ismai.pt/).

A simplified key for the distinction of the six main European species of the genus *Vipera* (Fig. 3) is now presented. Taxa with limited distribution near the easternmost European borders are therefore excluded for simplification.

Is it a European *Vipera*? → **1**

1a. Tip of the snout upturned or with a scaly horn; top of the head usually covered only by small scales → **2**

1b. Tip of the snout dorsally flat; top of the head usually with three main shields surrounded by smaller scales → **3**

2a. Tip of the snout more or less upturned (without a horn covered by small scales); species present in N–W Spain, France (except Corsica), Italy (except Sardinia), S and W Switzerland, extreme S–W Germany and extreme W Slovenia → ***Vipera aspis*** **(**Fig. 3A**)**

2b. Tip of the snout with a more or less evident horn covered by 4–8 small scales; rostral scale extended onto the front of the horn; species present only in Portugal and Spain (except the extreme North) → ***Vipera latastei*** **(**Fig. 3B**)**

2c. Tip of the snout with an evident horn covered by 5–20 small scales; rostral scale not extended onto the front of the horn; species present in N-E Italy, S Austria, Croatia, Bosnia & Herzegovina, Montenegro, Albania, Greece and many isles, North Macedonia, C and S

Fig. 3. Heads of the main European vipers: *Vipera aspis* (A), *Vipera latastei* (B), *Vipera ammodytes* (C), *Vipera ursinii* (D), *Vipera seoanei* (E) and *Vipera berus* (F). Photo credits: Matteo R. Di Nicola (A, C, D, F); Matthieu Berroneau (B, E).

Serbia, Bulgaria, S and W Romania, European Turkey → ***Vipera ammodytes*** (Fig. 3C)

3a. Usually two apical scales, both in contact with the rostral scale → **4**

3b. Usually one apical scale, in contact with the rostral scale; species present in limited areas of S—E France, C Italy, Croatia, Bosnia & Herzegovina, Montenegro, Serbia, North Macedonia, Hungary, Romania, Moldova and N Albania (of the same group: *Vipera graeca* from S Albania and Greece; *V. renardi* from E Ukraine and Crimea) → ***Vipera ursinii*** (Fig. 3D)

4a. Species present only in N Spain, extreme N Portugal and extreme S–W France → ***Vipera seoanei*** (Fig. 3E)

4b. Species present in Swiss, French, Italian and Austrian Alps, C and N France, Germany, Great Britain, Belgium, Netherlands, Denmark, Norway, Sweden, Finland, Poland, Czech Republic, Slovakia, Hungary, Slovenia, Croatia, Bosnia & Herzegovina, Romania, Bulgaria, Ukraine, Belarus, Lithuania, Latvia, Estonia, Russia, Montenegro, North Macedonia, Albania, Serbia, Moldova, and extreme N Greece (of the same group: *Vipera walser*, from N-E Piedmont, Italy) → ***Vipera berus*** (Fig. 3F)

3. Venom composition of major European *Vipera* species, mechanisms of toxicity and toxicokinetic considerations

Snake venom is a mixture of proteins and peptides, organic molecules and salts in an aqueous medium (Casewell et al., 2013; Chan et al., 2016). To date, snake venom has been found to consist of 50–200 different components, generally belonging to four main toxin families: phospholipases A_2 (PLA_2s), snake venom metalloproteinases (SVMPs), snake venom serine proteases (SVSPs) and three-finger toxins (3 FTX) (Slagboom et al., 2017; Tasoulis and Isbister, 2017). Interestingly, snake venom composition varies at both interspecific and intraspecific level, depending on factors like ontogeny (Alape-Girón et al., 2008; Cipriani et al., 1996), diet (Daltry et al., 1996), sex (Menezes et al., 2006; Zelanis et al., 2016), and local-scale adaptation to the physical environment (Zancolli et al., 2019). Vipers (family Viperidae) appear to be the snake family most frequently studied in compositional venom research, with most of the investigation efforts apparently focusing on the subfamily Crotalinae (Tasoulis and Ibsister 2017). Among the subfamily Viperinae, the genus *Vipera* is the most involved one in European snakebite accidents (Paolino et al., 2020; Zanetti et al., 2018), leading to mainly hemotoxic and cytotoxic envenomation symptoms (Al-Shekhadat et al., 2019; Komori et al., 1998; Maretic et al., 2013), although neurotoxic symptoms can sometimes also occur (Ferquel et al., 2007; Luksic et al., 2006). In light of the high medical relevance of this genus, we hereby provide a comprehensive assessment of the venom components of the six *Vipera* species accountable for the highest number ophidic envenomations in Europe, namely: *Vipera ammodytes, V. aspis, V. berus, V. latastei, V. seoanei,* and *V. ursinii*. This way, we hope to provide an useful tool for understanding the venom variability present across the genus, and for developing better clinical protocols to manage snakebite caused by these species.

3.1. Venom composition

3.1.1. Vipera ammodytes

Officially listed in both Category 1 and 2 of medically important snake species of the WHO (WHO, 2010b) and traditionally considered Europe's most dangerous venomous snake (Sket and Gubensek, 1976), the nose-horned viper *Vipera ammodytes* is capable of causing life-threatening envenomations, generally characterised by local and systemic haemorrhage, tissues damage, and neurotoxicity (Luksic et al., 2006; Maretic et al., 2013; Radonic et al., 1997). In a recent study, (Hempel et al., 2018) produced comprehensive proteomes of the venoms of the two *V. ammodytes* subspecies *V. a. transcaucasiana* and *V. a. montandoni* from Turkey. In the analysed venoms, the prevalent protein groups were phospholipases A_2 (PLA_2s), vascular endothelial growth factors (VEGFs), snake venom serine proteases (SVSPs), snake venom metalloproteases (SVMPs), L-amino acid oxidases (LAAOs), cysteine-rich secretory proteins (CRISPs) and C-type lectins (CTLs). In both subspecies, PLA_2s resulted to be the most abundant protein group, highlighting the great similarity between their venoms. In a previous study by (Georgieva et al., 2008), similarities were also found between the venoms of Bulgarian specimens belonging to the two subspecies *V. a. ammodytes* and *V. a. meridionalis*, with LAAOs, PLA2s, SVMPs and SVSPs being the most abundant toxins overall. Despite this analogy, (Vasilev et al., 2014) found the lethal potential of the venoms of these two subspecies to be different. The results of this study found *V. a. meridionalis* venom to present a higher lethality compared to that from *V. a. ammodytes* venom ($LD_{50} = 0.431$ μg/g and 3.681 μg/g, respectively), likely because of the presence, in the former, of a monomeric form of phospholipase A_2. Remarkably, in all four abovementioned subspecies the neurotoxic PLA_2 vipoxin was the most abundant PLA_2, highlighting the importance of this toxin in the general venom composition of *V. ammodytes* (Georgieva et al., 2008; Hempel et al., 2018). A very recent study focusing on *V. a. ammodytes* venom of Croatian origin, and combining both proteomics and transcriptomics, found SVSPs, snaclecs (snake C-type lectin-like proteins), sPLA2s, and SVMPs to account for the vast majority of the identified venom proteins (Leonardi et al., 2019). The analysis of *V. ammodytes* venom from Serbia performed by (Gopcevic et al., 2021) resulted in the identification of 9 main toxin families, similarly to what reported in previous works (Georgieva et al., 2008; Hempel et al., 2018; Leonardi et al., 2019), thus partially confirming the general compositional pattern of this species' venom.

3.1.2. Viper aspis

Early characterisations of the venom of the asp viper *Vipera aspis* showed the presence of proteolytic enzymes, LAAOs, phospholipases, hyaluronidases, hypotensive factors, haemorrhagic factors and coagulation inhibitors (Boquet, 1967; Komori et al., 1993; Komori and Sugihara, 1990). A recent work combining transcriptomic and proteomic analyses identified a total of 64 proteins in the venom of *V. a. aspis*, with various haemotoxins (e.g. P-III snake venom metalloproteinases, C-type lectins and disintegrins) detected in considerable abundances, and phospholipases A2 being the prevalent component (Giribaldi et al., 2020). These findings are concordant with the mostly haemotoxic and cytotoxic properties of the species' venom. (Komori et al., 1998) measured the LD_{50} values of the two subspecies *V. a. aspis* and *V. a. zinnikeri*, concluding that the venom of the latter had a higher lethal potency ($LD_{50} = 0.35$ μg/g, against $LD_{50} = 0.55$ μg/g of *V. a. aspis*). The same authors suggested this difference to be attributable to the presence, in *V. a. zinnikeri* venom, of the highly lethal phospholipase A_2 PLA_2-I, which they did not find in *V. a. aspis* venom. Interestingly, neurotoxic effects of *V. aspis* venom, causing neuromuscular paralysis through selective degeneration of peripheral motor nerve terminals (Zanetti et al., 2018), have also been reported from southern France and Italy (Ferquel et al., 2007; Lonati et al., 2014). French populations of *V. aspis* have been known to produce neurotoxic symptoms for many years (de Haro et al., 1994, 2009; de Haro et al., 2002). Nevertheless, the neurotoxic component of *V. aspis* venom has been characterised only recently, with the description of two neurotoxins of the phospholipase A2 type, ammodytoxin B (also present in the venom of *V. ammodytes*) and vaspin, and with the identification of genes encoding PLA2 neurotoxins in the *V. aspis* genome (Ferquel et al., 2007; Jan et al., 2002). Intriguingly, the expression of these genes does not appear to be constant, but is instead thought to be determined by particular environmental and/or physiological stimuli. Hence the detection of different levels of PLA_2 neurotoxins in different *V. aspis* specimens, and the recommendation by some authors to consider the asp viper a "cryptoneurotoxic'' species (Ferquel et al., 2007).

3.1.3. *Vipera berus*

The adder *Vipera berus* is the most widely distributed viper in Europe and is known to cause more snakebite accidents than any other species of the genus *Vipera* (Chippaux, 2012; Reading, 1996). Because of the medical relevance of this species (WHO, 2010b), both the composition and the effects of *V. berus* venom have been thoroughly studied. The venom of this species appears to have predominantly proteolytic, haemolytic and cytotoxic properties (Hawley, 1990; Zajkowska et al., 2010). Recent studies have focused on the characterisation of the *V. berus* venom proteome, producing different results. (Latinovic et al., 2016) analysed *V. berus* venoms of Russian origin, identifying 10 different protein families: serine proteases (SVSPs), metalloproteinases (SVMPs), natriuretic peptides (NP), phospholipases A_2 (PLA_2s), aspartic proteases (AspPs), cysteine-rich secretory proteins (CRISPs), C-type lectins (snaclecs), L-amino-acid oxidases (LAAOs), disintegrins, and Kunitz-type protease inhibitors (KTPi). The produced proteome allowed to determine the relatively simple composition of *V. berus* venom, and to identify SVSPs and SVMPs as the two most abundant venom protein groups (31 % and 19 % of all identified venom proteins, respectively). This result is concordant with the clinical picture of *V. berus* envenomation being characterised by marked haemotoxic activity. More recently, (Al-Shekhadat et al., 2019) also analysed the venoms of Russian *V. berus* specimens, and identified 15 different groups of venom components, the major ones being phospholipases A2 (PLA2s), serine proteinases (SVSPs), metalloproteinases (SVMPs), bradykinin-potentiating peptides (BPPs), C-type natriuretic peptides (C-NAPs), cysteine-rich secretory proteins (CRISPs) and L-amino acid oxidases (LAAOs) Although confirming the abundance of SVSPs and SVMPs in the venom of this species (16.2 % and 17.2 % of all identified venom proteins, respectively), this study identified PLA2s as the prevalent group of venom components (25.3 % of the venom proteome). Similarly, (Bocian et al., 2016) analysed the venom of *V. berus* specimens collected from the Slovakian Republic and identified 11 different protein groups. From this analysis, the most abundant proteins resulted to be phospholipases (almost 60 % of all identified venom proteins) and serine proteases (15 % of all identified venom proteins). Other venom components were snaclecs, CRISPs, LAAOs and angiotensin-like peptides. Interestingly, SVMPs were the least abundant protein group, accounting for less than 0.15 % of all identified proteins, while in the studies from (Latinovic et al., 2016) and (Al-Shekhadat et al., 2019) they were the second most abundant class of venom components. Nevertheless, the results obtained by (Bocian et al., 2016) are concordant with the substantially haemotoxic effects of *V. berus* envenomation. The discrepancies present between the results of the abovementioned studies might be due to the application of different protein identification techniques and/or to a certain degree of venom variability within *V. berus* (Malina et al., 2017; Varga et al., 2018). Such variation has been demonstrated in the past at individual level (Malenev et al., 2007; Nedospasov and Rodina, 1992) and, more recently, also between geographically distant populations. Particularly, (Malina et al., 2017) recorded varying protease and phospholipase activity among adders of different sex and age. Furthermore, the authors detected significant differences in lethal toxicity among individual adders. Specifically, the LD_{50} values of Hungarian specimens were lower than the values recorded for an Austrian specimen used as control, and varied between 0.41 and 0.72 μg/g. These values are partially concordant with the median LD_{50} traditionally reported for *V. berus* venom in mice, being 0.55 μg/g (Minton, 1974), but not with the median LD_{50} reported by (Al-Shekhadat et al., 2019) for Russian adders, being 19.8 μg/mouse in 18−20 g mice. This discordance, however, is likely attributable to the different venom injection modes applied in the considered studies (i.e. intraperitoneal in (Al-Shekhadat et al., 2019), intravenous in (Malina et al., 2017). Finally, (Malina et al., 2017) reported for the first time predominantly neurotoxic neuromuscular activity in *V. berus* venom collected from Hungarian specimens, furtherly highlighting the presence of geographic venom variation in the species.

3.1.4. *Vipera latastei*

Despite the acknowledged medical importance of this species (WHO, 2010b), to date a comprehensive assessment of *V. latastei* venom composition has yet to be produced. In a study aiming at characterising the toxic activity of *V. latastei* venom, (Arez et al., 1993) venom samples collected from only two specimens, one male and one female, from northwest Portugal. The performed analyses showed a certain degree of intersexual variation, with the male's venom apparently presenting a more complex composition than the venom produced by the female. The venom profiles also seemed to differ from the ones produced by (Saint-Girons and Detrait, 1992), obtained from animals collected in Spain, suggesting the presence of geographic variation. In the same work from 1993, Arez et al. performed LD_{50} tests on 18−20 g mice, obtaining a LD_{50} of 14.43 g/mouse for the male specimen, and of 27.30 g/mouse for the female. These results are partially concordant with the LD_{50}s measured by (Detrait et al., 1983) for *V. latastei* (LD_{50} = 25 g/20 g mouse) and for the subspecies *V. latastei gaditana* (LD_{50} = 35.3 g/20 g mouse). Moreover, in the venoms of the two *V. latastei* specimens considered, the authors identified components determining haemorrhagic activity, which they speculated to be haemorrhagins, likely corresponding to haemorrhagic snake venom metalloproteinases (SVMPs). Interestingly, the antibodies present in ViperaTAb® antivenom, produced using *V. berus* venom, appear to very effectively recognise and neutralise the toxic components present in the venom of other European vipers, including *V. latastei* (Casewell et al., 2014). Although these findings could possibly suggest the presence of similarities between the venoms of *V. latastei* and *V. berus*, the lack of knowledge about *V. latastei* venom components prevents any actual comparison.

3.1.5. *Vipera seoanei*

As already stated for *V. latastei*, currently no exhaustive information about the composition of *V. seoanei* venom is available, despite the recognised medical relevance of this species (WHO, 2010b). Nevertheless, the toxicity of *V. seoanei* venom has been studied in the past. (Detrait et al., 1990) performed LD_{50} tests on 20 g mice using venoms gathered from different *V. seoanei* populations from Spain. Interestingly, while the obtained proteinograms showed very limited levels of compositional divergence among the investigated populations, the toxicity appeared to vary geographically. Specifically, while venoms from populations from the Basque Country and the Cantabrian coastal areas showed very limited lethality (LD_{50} = 23,1–23,6 μg/mouse), the venoms gathered from the westernmost populations (i.e. Galicia and North of León) appeared to have a consistently higher toxicity (LD_{50} = 6,9−9,9 μg/mouse). Similar levels of toxicity have been reported for *V. seoanei* specimens from Portugal, showing an average LD_{50} of 9,7 μg per 18−20 g mouse (Archundia et al., 2011), potentially supporting the presence of a West-East toxicity gradient. However, the lack of detailed information about the components present in *V. seoanei* venom and their effects doesn't allow to go beyond mere speculation about what could cause these differences.

3.1.6. *Vipera ursinii*

Vipera ursinii is generally considered to be the least medically significant species of the genus *Vipera*, mainly because of the limited amount of venom it can inject and the typically very mild and local envenomation symptoms it can cause (Dely and Joger, 2005; Krecsak et al., 2011). The venom of this species has been reported to cause haemorrhagic effects in mice, but no myotoxicity (Mebs and Lange-luddeke, 1992). (Balija et al., 2020) assessed the lethal toxicity of *V. ursinii* venoms collected from Croatian specimens by performing LD_{50} tests on both rats and crickets, Orthoptera composing almost the totality of the species' diet (Dely and Joger, 2005; Nilson and Andren, 2001). The results of these tests showed higher toxicity of *V. ursinii* venom in crickets compared to that in mice (i.e. mass normalised LD_{50}s being 9.8 μg/g and 1.94 μg/g, respectively), suggesting strong specificity of the venom for the insect prey. Interestingly, the injected mice and

crickets showed similar modes of dying, presenting symptoms suggesting neurotoxicity, independently from the amount of venom injected (Balija et al., 2020). Proteomic analyses of *V. ursinii* venom performed by the same authors allowed the identification of 25 different proteins belonging to seven main protein families: snake venom metalloproteinases (SVMPs), snake venom serine proteases (SVSPs), secreted phospholipases A2 (PLA2s), cysteine-rich secretory proteins (CRISPs), snake C-type lectin-like proteins (snaclecs), Kunitz-type protease inhibitors (KTPis), and venom nerve growth factors (VNGFs). SVMPs resulted to be the most abundant toxin family in *V. ursinii* venom, representing 55 % of all detected venom proteins (Balija et al., 2020). The predominance of P-III SVMPs, known to have high haemorrhagic potential, very likely explains the haemorrhagic effects of this species' venom (Mebs and Langeluddeke, 1992). These effects might furtherly be exacerbated by the action of another protein family also detected in *V. ursinii* venom, the snake venom serine proteases (SVSPs), which although less abundant than SVMPs, are known to affect haemostasis and cause coagulopathy (Sajevic et al., 2011).

3.2. Mode of action of major Vipera toxins and mechanistic link with their toxicity

3.2.1. Phospholipase A_2

Snake phospholipases A_2 belong to the superfamily of secreted PLA_2s. PLA_2 enzymes are commonly found in mammalian tissues as well as arachnid, insect, and snake venom. This group of enzymes catalyses the hydrolysis of the sn-2 acyl bond in membrane phospholipids in erythrocytes and various cells. The consequence of this biochemical reaction is not only membrane lesion leading to direct haemolysis but also a disproportionate release of arachidonic acid. The subsequent formation of anti-inflammatory and inflammatory mediators such as prostaglandins and leukotrienes from arachidonic acid through the action of cyclooxygenases or lipoxygenases also lead to the spectrum of toxic effects from snakebite, including inflammation and pain occur at the site. Many of the snake PLA_2 enzymes produce a wide spectrum of toxic effects including neurotoxicity, myotoxicity, cardiotoxicity, cytotoxicity, anticoagulation, convulsions, hypotension, and inflammation (Rouault et al., 2006). For example, ammodytin L (AtnL), a well-known myotoxic protein with a secreted phospholipase A2 (sPLA2) structure, is the main cardiotoxic molecule of the *V. a. ammodytes* venom (Karabuva et al., 2017).

3.2.2. Snake venom serine proteases (SVSPs) and metalloproteinases (SVMPs)

SVSPs and SVMPs exert their procoagulant activity by targeting one or more coagulation factors in the blood coagulation cascade (Serrano, 2013; Takeda et al., 2012). Snake venom serine proteinases (SVSPs) target the body's haemostatic system and cause systemic haemodynamic disturbances by specifically activating key proteins of the blood coagulation cascade and by affecting kallikrein–kinin, fibrinolytic or complement systems, as well as by interfering with endothelial cells and platelets (Serrano, 2013). Depending on their principal target in the coagulation system, they are usually thrombin-like enzymes, activators of prothrombin, factor V and factor X. TLE and FV activators are exclusively SVSPs, while activators of factor X and prothrombin can be either SVSPs or SVMPs. SVMPs are zinc-dependent endopeptidases varying in size from 20 to 100 kDa and are grouped into several subclasses according to their domain organisation (Gutierrez et al., 2016; Takeda et al., 2012). SVMPs are phylogenetically most closely related to the mammalian ADAM (a disintegrin and metalloproteinase) family of proteins and belong to the M12B family of metalloproteinases. SVMPs are the primary factors responsible for local and systemic haemorrhage. The haemorrhagic activity of SVMPs results from the disruption of vessel walls and allowing the escape of the blood contents into the stroma following the cleavage of basal membrane components underlying capillary endothelial cells. Not all SVMPs possess haemorrhagic activity but target the body's haemostatic system through pro- or anti-coagulant effects (e.g. fibrinogenase, fibrolase, prothrombin activating activities), platelet aggregation inhibitor and apoptotic or pro-inflammatory activities (Takeda et al., 2012).

3.2.3. Snake C-type lectin-like proteins (snaclecs)

Snaclecs are the largest nonenzymatic group of proteins in the *Vipera ammodytes* venom (Leonardi et al., 2019). These proteins have a variety of biological activities, including anticoagulant- and platelet-modulating activities but have no lectin activity (Clemetson, 2010; Morita, 2005). Snaclecs bind to receptors on platelets, inducing either inhibition or activation of their aggregation. The nonenzymatic mechanism of platelet-related snaclecs toxicity is thought responsible for the thrombocytopenia observed in *Vipera ammodytes* envenomed patients (Al et al., 2010; Frangides et al., 2006b; Luksic et al., 2006). Snaclecs also potentiate the haemorrhagic activity of SVMPs (Rucavado et al., 2005).

3.2.4. Disintegrins

Disintegrins comprise another family of nonenzymatic dimeric toxins present in the *Vipera ammodytes* venom. Disintegrins are a group of low molecular weight polypeptides that modulate cell adhesion, migration, apoptosis, platelet aggregation, and angiogenesis (Lazarovici et al., 2019). They are naturally derived by proteolytic processing from metalloproteinase precursors and carrying the integrins' recognition motifs RGD, KGD, WGD, VGD, MGD, RTS, KTS and hence can target different types of cellular receptors.

3.3. Toxicokinetic considerations

In addition to differences in venom composition between the different *Vipera* species, inter-species differences in toxicokinetics in the context of human envenomisations need to be considered. Toxicokinetics of a range of venoms (whole or individual components) from Elapidae and Viperidae species have been studied in experimental animals as well as limited human data for a number of European viper species from envenomation cases. A systematic analysis of the literature allowed to fit the toxicokinetics of venom concentrations from animal studies to a two-compartment model consisting of a rapid distribution phase and a slow elimination phase following intravenous injection or intramuscular or subcutaneous administration of the whole venoms or purified toxins (Sanhajariya et al., 2018). The bioavailability of venoms or toxins ranged from 4 to 81.5 % following intramuscular administration and around 60 % following subcutaneous administration. The volume of distribution and the clearance varied across species (Sanhajariya et al., 2018). Human data on Elapidae and Viperidae were much scarcer and a one-compartment model provided the best fit, with an elimination half-life of 9.71 ± 1.29 h (Sanhajariya et al., 2018). Another factor further complicates toxicokinetic modelling of snake venom and toxins is the existence of two venom entry pathways (lymphatic and vascular) (Helden et al., 2019). In addition, differences in toxicodynamic properties of viper toxins have been identified. For instance, enzymatically active PLA_2 is present in the venom of both *V. aspis* and *V. berus*, but only the PLA_2 of *V. aspis* has been shown to be neurotoxic (Zanetti et al., 2018). Overall, the venom composition of *Vipera* spp. in Europe is not only variable across species and their respective geographical distributions (Abdelhamid et al., 2016; Ferquel et al., 2007) but the toxicokinetic and toxicodynamic properties may also vary. Hence, it is difficult to identify the most venomous viper species without considering the medical relevance and significance of the envenomation as reported recently (Paolino et al., 2020).

4. Clinical management of viper envenomation

4.1. First aid in the field

After being bitten by a suspected viper, the most critical aspect for the patient is to remain calm, and immediately call an emergency number. This is particularly relevant because any agitation and movement will worsen the envenomation through an increase in blood flow and consequent accelerated circulation of toxins in the blood system. Fig. 4 illustrates the first aid procedure to support patients in the field.

Once the patient has reached an emergency team, the next step is the immobilisation of the affected area. For bites in the lower limbs, it can be applied a bandage of at least 10 cm wide while pulling and exerting a moderate compression. The bandage width should be extended as much as possible, preferably below the area of the bite. Finally, a rigid splint should be applied so that it remains fixed. If the bandage and the rigid splint steps have been placed correctly, the compression exercised around the area of the bite will not be discomforting for the patient and, above all, it can be kept in place for several hours. The immobilisation bandage will be then removed once the patient gets to the hospital. For bites in the upper limbs, a compression bandage is also applicable (Fig. 5), with a recommended width of about 7 cm high from a bandage roll of about 6 m long.

The clinician should start from the fingertips, reaching up to the elbow making sure that the bandage does not prevent arterial circulation through checking that the pulse is perceptible. For snake bites that are near or even above the elbow, the entire arm up to the shoulder should be bandaged. Subsequently, the immobilisation of the bite area will be completed with a splint, blocking the arm against the trunk. In the rare cases of bites in the head and neck region, it is advisable to apply a rigid pad over the bitten area, keeping it compressed with an adhesive elastic patch. Any commonly known techniques such as arterial compressions, tourniquets, cutting and open the wound, sucking the venom out with the mouth, burning, use of oxidising agents, phenol, potassium permanganate are ineffective and may only increase the risk of clinical complications. The use of stun guns or other sources of high voltage, low amperage direct current electric shocks to treat venomous bites and stings is also not supported by the literature (Ben Welch, 2001; Dart and Gustafson, 1991; Panfoli et al., 2010). Sucking the wound using extractors, seems to be ineffective, but will not cause any additional major damage to the tissue compared to other methods. In any case, the authors do not recommend any type of self-treatments. As reported above, after the bite, it is important to remain calm and reduce the movements to slow down the circulation and the distribution of the toxins in the body. It is not recommended to prescribe any pharmaceutical drugs outside the hospital or without a medical doctor's prescription. However, in the case of pain, an analgesic therapy can be performed using for example paracetamol (adult dose maximum 3 g in 24 h; children 10−15 mg/kg/day maximum 100 mg/kg/day). At the same time, it is important avoiding anti-inflammatory drugs, such as acetyl salicylic acid, to avoid a blood circulation thinning and a greater risk of bleeding. If there is no evidence of neurotoxic symptoms, common sedatives such as low doses of benzodiazepines (diazepam 2 mg–4 mg [10−20 drops]) or of sleeping tablets (lorazepam 1 mg [10 drops]) may be administered to re-assure patients in the case of anxiety. Transport into a hospital should be facilitated by emergency services, by car or helicopter for the most remote areas. In all these situations, the patient must make as little movements as possible and must be constantly re-assured. The neck should be supported in a neutral position and the victim should be seated, as risk of suffocation due to the possibility of regurgitation of material coming from the stomach is always plausible particularly because vomiting is often one of the first symptoms of envenomation.

4.2. Laboratory and clinical investigations

After a snakebite, limited localised signs may be caused by a small quantity of venom. However, clinicians should remain on alert since the development of symptoms and complications can arise within hours. The patient should remain in observation for a day (24 h). Once the patient has been hospitalised, the local or-regional poison control center (PCC) should be contacted as soon as possible, to reassure family members, to deal with any rapid worsening of symptoms, and to access a dose of antivenom for administration to the patient. For each patient, baseline laboratory investigations should be always performed including coagulation tests (e.g. prothrombin time, thrombin time, partial thromboplastin time, fibrinogen, platelet count, international

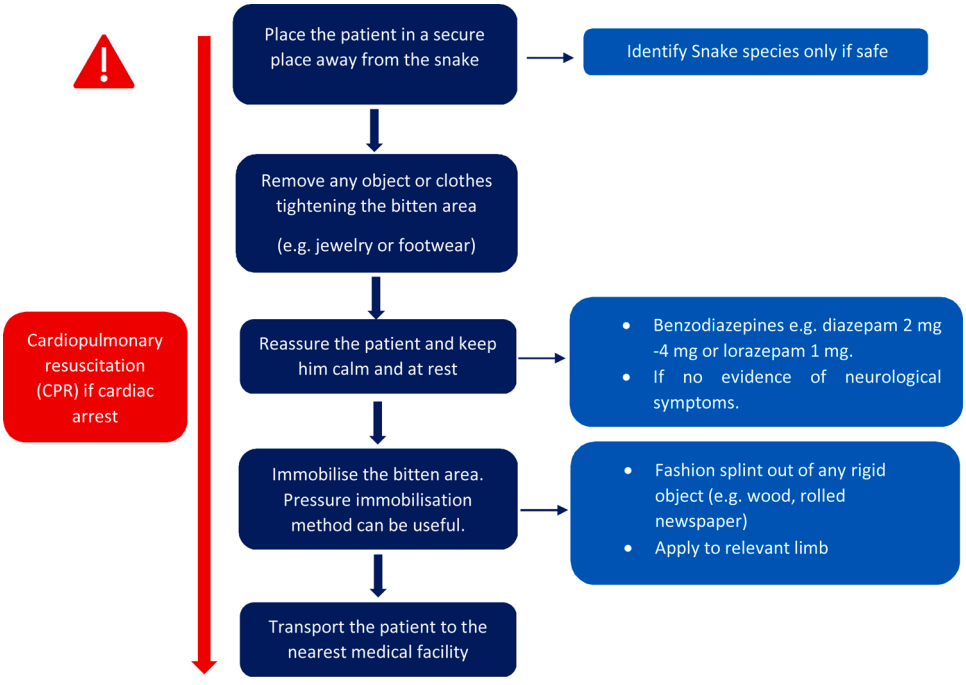

Fig. 4. First aid in the field after viper envenomation.

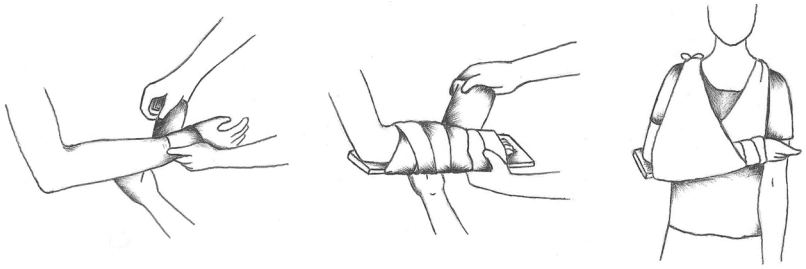

Fig. 5. Bandage during first aid in the field after viper envenomation.

normalised ratio, whole blood clotting test), blood count, urinalysis, clinical chemistry for liver function (bilirubin, alanine Aminotransferase, aspartate aminotransferase, Gamma-glutamyl-transpeptidase, lactate dehydrogenase, creatine phosphokinase), glycaemia, renal function (urea, creatinine, electrolytes). All laboratory investigations should be all performed at Time 0 and every 6 h for 24 h, together with digital oximetry and electrocardiogram (ECG). Close monitoring of glycaemic control should be considered, especially in critically ill patients. Together with such analyses, others may be conducted based on the patient's medical history. If no clinical symptoms of envenomation occur within 24 h and laboratory investigations are satisfactory, the patient can be discharged.

4.3. Treatment of local and systemic symptoms

The highest percentage of bites are mostly localised in the upper and lower extremities and are mainly characterised by local and regional symptoms. However, systemic symptoms may arise and can become life-threatening (Nelson et al., 2019). The following section provides recommendations for treatment of local and systemic symptoms.

4.3.1. Treatment of local symptoms

Local symptoms that are subsequent to a viper's bite can range from mild to severe and generally speaking, the bitten area is mostly characterised by the presence of one or two visible fang marks (approximately 6 mm–8 mm apart each) which is associated with a typical blood drip. However, fang marks may be immediately visible and could result in a diagnostic delay that may be fatal for the patient (Beer and Putorti, 1998). On the other hand, "dry bites" characterised by the presence of fang and tooth marks, are not associated with venom inoculation and therefore do not result in local or systemic symptoms after 24 h (American Academy of Orthopaedic Surgeons (AAOS) et al., 2016; Nelson et al., 2019). Despite this, patients who have suffered a dry bite may experience symptoms such as arrhythmia, dyspnea, anxiety, vaso-vagal shock, increase in blood pressure, sweating and tremor, which are often associated with an understandable fear of the consequences from venomous bites (although no venom has been injected) and this might mislead the clinician (WHO, 2010a). Soon after a bite, the bitten area generally becomes swollen and painful. Nevertheless, in the lower limbs the swelling can arise hours after (Nelson et al., 2019). The sequence of events involves a local oedema which then may extend to the whole limb within hours as well as a concurrent onset of ecchymosis located and delimited around the fang marks with the potential of becoming widely extended. Lymphangitis and loco-regional lympho-adenopathy may be present in some cases which is indicative of the spread of the venom into the lymphatic system. Haemorrhagic vesicles and blisters may eventually arise in the bitten area most often after 12 h. It is important to perform an accurate clinical evaluation of vesicles and blisters, since their extension can be synonymous of an underlying necrosis which arises in 5.5 % of envenomation cases due to European *Vipera* bites (Luksic et al., 2006; Nelson et al., 2019).

For local treatment, any jewellery or watches should be removed, as these could hinder local disinfection and in case of oedema, they could tear the skin apart accelerating the necrosis process. The bitten area should be examined with caution and any residual dirt should be removed. The affected area can be treated with hydrogen peroxide or with plain water since viper venom is water soluble. Disinfection with alcohol or other chemicals substances should be avoided since toxic compounds can be generated (WHO, 2010a). The swollen area must be marked with a dermographic pen and monitored every hour to record the unit of swelling, bruising and/or necrosis. The measurements are initially performed every 1–2 hours, but may be more frequent in case of rapid progression of local symptoms. In this case, the affected extremity may be slightly raised (Anz et al., 2010), taking care not to reduce arterial perfusion pressure in the tensely swollen limb which may potentially increase the risk of intra-compartmental ischaemia (WHO, 2010a). Although the teeth and fangs of the viper can be pathogen carriers, the prophylactic administration of antibiotics is not generally advised. However, in the case of risk of secondary bacterial infections and acute infection (e.g. erysipelas), broad spectrum antibiotics (e.g. amoxicillin, cephalosporin, azithromycin, metronidazole, trimethoprim + sulfamethoxazole) may be prescribed. Overall, it is proposed to initiate a prophylactic antibiotic treatment only in evident cases of symptoms pointing out a local infection (e.g. impetigo, erysipelas), heavily soiled skin area, use of inappropriate and non-sterile local technical manoeuvres or in cases of underlying diseases increasing the risk of secondary infections (e.g. diabetic patients or immunosuppressed patients) (WHO, 2010a). The tetanus immunisation status of the patient should always be assessed and immunisation should be provided when appropriate. From historical data, it is noted that the percentage of ulceration and massive necrosis is extremely rare in cases of bites from European vipers compared to bites from other Viperidae as for example Central and Southern American *Bothrops* spp. or Asian *Daboia* spp. (Luksic et al., 2006; Nelson et al., 2019). Such ulcerations or necrosis most often arise within 24−72 hours and a swab of the affected area is recommended for diagnostic purposes. The most common pathogens that have been isolated in patients after snakebites include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Proteus* sp. and *Enterococcus faecalis* (Wagener et al., 2017). While ciprofloxacin and amikacin have proven to be the most effective systemic treatments against the first two pathogens, *Enterobacteriaceae* are mostly sensitive to ampicillin, amoxicillin/clavulanic acid, cefuroxime, ceftriaxone, ciprofloxacin, gentamicin and amikacin (Nagoba et al., 2011; Wagener et al., 2017). In addition to systemic treatments, local treatment, particularly under important localised cutaneous necrosis, involves local wash with a solution of undecyl-starch propyl betaine or a topical collagenase ointment together with chlortetracycline cream. Debridement and subsequent wound management play a pivotal role in these cases (Nagoba et al., 2011), while flaps and full-thickness skin graft should be considered only when soft tissue defect has been diagnosed (Bozkurt et al., 2008). Once the skin ulcer has healed, an annual dermatological follow-up should be performed in the patient to prevent a possible scar-related cutaneous degeneration which can also occur in other dermatological conditions (WHO, 2010a; Mercuri et al., 2018, 2020). In extremely rare cases, a compartment syndrome may arise, it is characterised by an excessive pressure inside an enclosed muscle space in the

body reducing blood flow to the tissues; it may induce a massive and rapid necrosis as a potential life threatening situation (Supplementary Fig. 1). In physiological conditions, pressure within the compartments is between 0 mmHg–8 mmHg (Torlincasi et al., 2020). The intra-compartment pressure should be measured every 4 h for a minimum of 24 h (Kakagia, 2015; Wall et al., 2010). An intra-compartmental pressure greater than 30 mmHg associated with clinical symptoms known as the Five "Ps", i.e. Pain, Pulselessness, Paraesthesia, Paralysis and Pallor, indicates a compartment syndrome and a need for fasciotomy (Kim et al., 2019; Torlincasi et al., 2020). In children, compartment pressure cannot be easily measured at the bedside and in this case, fasciotomy should not be performed without adequate diagnosis of the compartment syndrome (Laohawiriyakamol et al., 2011). The fasciotomy should be performed ideally within six hours after the diagnosis of a snakebite but it is not recommended after 36 h (Torlincasi et al., 2020). The main differential diagnosis for compartment syndromes includes deep vein thrombosis, cellulitis, gas gangrene, phlegmasia cerulea dolens, rhabdomyolysis and peripheral vascular injuries (Torlincasi et al., 2020).

Finger bites deserve a separate discussion since an excessive swelling in the digits can be observed and pressure within the compartment may exceed the vascular capacity and compromise the integrity of small vessels and nerves (Bozkurt et al., 2008). In such cases, the literature has demonstrated that dermotomy and decompression of the entire finger must be performed as soon as possible, in order to provide sound functional and cosmetic results (Bozkurt et al., 2008; Watt, 1985). Other supportive treatments include mannitol (e.g. 25 g intravenous bolus followed by 5−10 g intravenous/h), as a useful adjunct in ischaemic-reperfusion injuries and may reduce intra-compartmental pressure (Shah et al., 1996) or hyperbaric oxygen (Fitzpatrick et al., 1998; Watt, 1985). Systemic steroids have also been used and have been shown to provide positive results (Watt, 1985).

Finally, one of the most severe consequences of the bite is the amputation of a finger or of a limb, with a serious impact on the patient's quality of life, as well as psychological implications. The amputation may only be required in the case of massive and non-responsive necrosis involving a bone or based on a clinical and radiographic diagnosis.

4.3.2. Treatments of systemic symptoms

Once the patient has arrived in the hospital, a general physical examination is required after monitoring the local site of the bite. Blood pressure and heart rate should be monitored and electrocardiogram (ECG) and pulse oximetry should be performed. The skin and mucous membranes (mouth, anorectal and genital tract) should be assessed for any evidence of petechiae, purpura, discoid haemorrhages and ecchymoses. In addition, the conjunctive should be checked for eventual signs of haemorrhage, as well as the gingiva and nose for any signs of bleeding and epistaxis. Abdominal tenderness may suggest gastrointestinal or retroperitoneal bleeding. Intra-cranial haemorrhage is suggested by lateralising neurological signs, asymmetrical pupils, convulsions or impaired consciousness (WHO, 2010a). All these findings are discussed in the following paragraphs. Finally, the clinician should ask the patient for any known allergies particularly regarding food, pharmaceuticals or insect's bites) to plan the most appropriate therapy. Indeed, patients with an anaphylactic reaction to European viper bites may be likewise allergic to stings from *Hymenoptera* (insects like bees, wasps and hornets) and other allergens (Valenta, 2010).

4.3.3. Acute and severe cases

In acute and severe cases resulting from viper bites, patients may develop circulatory shock and angioedema a few minutes after the actual bite mostly because of an anaphylactic shock. It is important to highlight that such an anaphylactic or anaphylactoid reactions to the venom may appear in previously exposed and unexposed patients (Beer and Putorti, 1998; The European Resuscitation Council, 2020). Airway patency, respiratory movements, arterial pulse and level of consciousness must be monitored instantly (WHO, 2010a). In these cases, rapid primary clinical assessment and resuscitation using the *ABCDE* approach are needed: *A*irway; Breathing [respiratory movements]; *C*irculation [arterial pulse]; *D*isability of the nervous system [level of consciousness]; *E*xposure and environmental control [protect from heat, cold, etc…]). After the anaphylactic reaction has been diagnosed, epinephrine should be quickly administered intramuscularly (0.3−0.5 ml as 0.3−0.5 mg in adults, 1:1000 dilution or 0.01 mL/kg body weight in children), although repeat doses may be given within five minutes intervals until symptoms improve. Subsequently, when the patient is being rescued, it is important to improve the stabilisation of the airways, administer high flow oxygen, fluid bolus (500−1000 ml in adults and 20 mL kg^{-1} in children), chlorphenamine (adults and children $\geq$ 13 years: 10 mg IM or intravenous [EV]; children between 6–12 years 5 mg; infant between 6 months and 6 years 2.5 mg; newborn $\leq$ 5 months 250 mcg Kg^{-1}) and hydrocortisone (adults and children $\geq$ 13 years: 200 mg IM or EV; children between 6–12 years 100 mg; infant between 6 months and 6 years 50 mg; newborn $\leq$ 5 months 25 mg). Pulse oximetry, blood pressure and electrocardiogram should always be performed (The European Resuscitation Council, 2020). Finally, in the case of loss of consciousness due to neurological, cardiovascular or haematological causes, the rescue techniques do not differ from those for other aetiologies and advanced life support methods are applied according to the guidelines of the European Resuscitation Council (The European Resuscitation Council, 2020).

4.3.4. Psychiatric condition: anxiety and the use of benzodiazepines

When acute severe reactions are absent upon arrival at the hospital, various clinical and instrumental procedures are performed to provide the appropriate follow-up and therapy. First, the patient should stay calm, since a normal/low heartbeat helps to reduce venom circulation in the blood stream. Accordingly, in absence of neurotoxic clinical signs, it is useful to administer low doses of benzodiazepines, such as diazepam 2 mg–4 mg (10−20 drops) or lorazepam 1 mg (10 drops). As stated, these compounds should be avoided and/or administered carefully in suspected cases of neurotoxic symptoms, that can occur after envenomation from *Vipera aspis* and *V. ammodytes'* bites (Giribaldi et al., 2020). In any case, any deep sedation is generally not desirable after a snakebite (WHO, 2010a). If deep sedation is necessary, the essential life functions of the patient must be monitored with care and respiratory airways should be protected from aspiration of the stomach content.

4.3.5. Hypotension and hypertension

Patients suffering from hypotension, with vasodilation and extravasation should be treated with catecholamines and plasma expansion. In case of severe hypotension, additional application of colloids infusions is required (Valenta, 2010). Administration of fresh frozen plasma (FFP) (at least 10 mL/kg of body weight) will support plasma volume-expansion as well as normalisation of potential disseminated intravascular coagulation (DIC)-like disorder.Thus, applying the plasma will be the most helpful solution in cases of hypotension and haemostasis disorder. However, if the blood pressure continues to decrease, catecholamines (norepinephrine, 0.1 μg/kg/min achieved by diluting 4 mg in 50 mL of volume and applied at a speed of 5 mL per hour) must be taken into consideration. When envenomation symptoms are also associated with signs of myocardial toxicity and arrhythmias, the infusion of catecholamines with a β-mimetic effect (e.g. dobutamine with a mean dosage of 5 μg/kg/min) should be considered (Valenta, 2010). In contrast, hypertension after a *Vipera*'s bite is not a common symptom (Malina et al., 2008). Neuromuscular blockade at bulbar level, neurotoxin venom–induced release of catecholamines, decreased parasympathetic stimulation, dysautonomia and renal injury are rarely causes of high blood pressure after *Vipera*'s bite (Senthilkumaran et al., 2014). With regards to treatment, it is important to highlight the usefulness of angiotensin-converting-enzyme (ACE) inhibitors and toavoid beta-blockers which are likely to increase severe alpha-agonist effects

via an antagonistic effect on beta-receptors (Senthilkumaran et al., 2014).

4.3.6. Gastrointestinal symptoms

Gastrointestinal symptoms are also part of common early symptoms after systemic envenoming and include vomiting, nausea, abdominal pain and diarrhoea (Valenta, 2010; WHO, 2010a). Chlorpromazine 25–50 mg in adults, and 1 mg/kg of body weight in children is recommended as an anti-emetic in the case of vomiting (Valenta, 2010). Those symptoms can be mild in healthy adults and do not provide a direct indication for the need to administer an antivenom. In contrast, such symptoms in children should be evaluated carefully, because of the potential greater distribution of the venom in relation to body weight with increased risks of complications and it can provide a sufficient basis to start an anti-venom treatment (Valenta, 2010). Diarrhoea may arise and is usually caused by the release of kinines, in which case adequate hydration should be provided, and electrolytes should be assessed.

4.3.7. Neurotoxicity

Signs of neurotoxicity may arise and oral intake of drugs is contraindicated because of the potential risk of aspiration caused by loss of the swallowing reflex. If respiratory failure occurs, intubation and mechanical ventilation should be considered. Neurotoxicity is mostly due to the presynaptic acting neurotoxic PLA_2 from the venom of *Vipera ammodytes* and in some cases *V. aspis* (Valenta, 2010). Despite neurotoxic symptoms are rarely seen in *Vipera* bites, a substantial quantity of neurotoxins can cause fatal paralysis and for this reason, must be treated rapidly. Neurotoxicity often arises through the involvement of cranial nerves inducing ptosis, ophthalmoplegia with double or fuzzy vision, dysphagia with increased salivation, dysarthria, a variable degree of visible facial muscle paralysis, but also through general muscular weakness. An important symptom of acute and severe neurotoxicity is the "broken neck sign" for which the muscles flexing the neck are paralysed, as well as "paradoxical respiration" for which the abdomen expands rather than the chest on attempted inspiration (WHO, 2010a). In these serious clinical conditions, the patient may experience loss of consciousness and death from respiratory failure within a few minutes. Symptomatic treatment of such a status involves securing the patient's respiratory airways, which can be performed through tracheal intubation, and mechanical ventilation.

In the presence of neurotoxic signs and absence of antivenom, ventilation with air or oxygen is needed. When mechanical ventilation is not available, manual ventilation can be performed (anaesthetic bag). Anti-cholinesterase drugs can be very useful in patients with neurotoxic symptoms, above all in patients bitten by *Vipera ammodytes and V. aspis*. In these patients, the Tensillon test, to differentiate between myasthenia gravis and other conditions, should be performed. The Tensillon test involves intravenous injection of 2 mg edrophonium, and after 30 s another 8 mg. The patient is observed for 10−20 min and is monitored for an improvement of ptosis and ventilation capacity. When edrophonium is not available, intravenous atropine sulphate (0.6 mg for adults and 50 μg/kg for children) followed by an intramuscular injection of neostigmine bromide or methyl-sulphate (0.02 mg/kg for adults and 0.04 mg/kg for children) are good alternatives for the same purpose. Under a positive Tensillon test, it is important administer atropine and neostigmine, for patients who are capable of swallowing, doses of atropine 0.6 mg (twice a day) and neostigmine (15 mg four times a day) or pyridostigmine (60 mg four times a day) is recommended (WHO, 2010a). Patients who are unable to swallow can be maintained on neostigmine methyl-sulphate (0.5–2.5 mg every 1–3 hours up to 10 mg/24 h maximum for adults or 0.01−0.04 mg/kg every 2-4 h for children) by intramuscular, intravenous or subcutaneous injection together with atropine to block muscarinic side effects (WHO, 2010a). The Tensillon test should be performed by a neurologist and particular attention must be taken for individuals over 50 years old, for patients under corticosteroids or pro-cholinergic drugs (Pascuzzi, 2003). There may be some instances under which the Tensillon test cannot be performed and an alternative is the ice test. The ice test aims to check the presence of neurotoxic symptoms and consists of applying an ice-filled plastic glove to one eye for 2 min to assess improvement of ptosis, possibly due to inhibition of anticholinesterase (WHO, 2010a) Symptomatic treatments may prove ineffective particularly when envenomation is associated with neurotoxins combined with presynaptic toxins that may block the neuromuscular junction at the presynaptic level (Georgieva et al., 2008; Giribaldi et al., 2020; Pungercar et al., 1999), such as ammodytoxins A, B and C in *Vipera aspis* and *V. ammodytes*. Accordingly, nerve ending damage can be only prevented by immunologically unbinding through early administration of a relevant antivenom (Valenta, 2010). Overall, the authors highlight that successful symptomatic treatments reported above should not be considered an alternative to the use of viper antivenom, but constitute a rather useful complementary therapy to immunotherapy, which should be administered, as soon as possible unless contraindicated (Valenta, 2010).

4.3.8. Cardiotoxicity and arrhythmias

Adverse Cardiovascular Events after a Venomous Snakebite (ACVE) is defined as the occurrence of at least one of the following conditions: myocardial injury [based on troponine I elevation within 48 h of presentation or ECG evidence of ischemic changes, such as ST elevation, ST depression, or T wave inversion], shock (defined as hypotension requiring vasopressors), ventricular dysrhythmias (ventricular tachycardia, ventricular fibrillation, torsades de pointes) and cardiac arrest. Elevation of the ST segment in II, III, AVF leads, transient horizontal ST depression in V5-V6, peaked T-waves, intermittent 2:1 s degree heart block and transitional junctional escape rhythm are the most common ECG alterations after *Vipera*'s bites (Frangides et al., 2006a; Kurtovic et al., 2016; Moore, 1988; Varga et al., 2018). Any myocardial and electrocardiographic changes must be treated specifically and in combination with antivenom administration.

4.3.9. Haematological alterations

Coagulopathy and haemorrhagic effects are common symptoms of envenomations caused by most snakes of the family Viperidae (Gutiérrez et al., 2005a, b, 2016). Minimal signs of coagulopathy can be detected only after laboratory investigations, while more severe haematological symptoms are mostly detectable with clinical symptoms, such as bleeding from wounds, mucous membrane tissues, nose, mouth, gums, conjunctiva, as well as internal haemorrhages (gastro-intestinal tract, brain, body cavities and hematuria). Pro-coagulative clinical complications may also occur, causing microembolisation or thrombo-embolism in the early or late phases, leading to DIC-like consumption disorders and eventually to organ dysfunction syndrome. Prognosis for these clinical manifestations involve the presence of micro-thrombotisation and interstitial oedema when the capillary integrity is damaged. Symptoms include different levels of respiratory distress syndrome, anuria and alterations of blood chemistry (i.e. increase in bilirubin and hepatic enzymes), while clinical hypofibrinogenemia or defibrination may be observed and associated with blood levels decreasing next to null; in the absence of bleeding, application of a fibrinogen concentrate is not recommended (Valenta, 2010). In contrast, when bleeding is observed, fibrinogen is replenished with at least 0.5–1.0 g/l using fibrinogen concentrates or fresh-frozen plasma (10−20 ml/kg body weight). However, in all these situations, the first choice remains antivenom administration and symptomatic treatments should be performed if the antivenom administration has been contraindicated or unavailable on a temporary or long-term basis.

Administration of coagulation factor concentrates is indicated if a critical decrease in these factors has been evidenced, with indications including persistent obstinate bleeding. Heparin treatment has been recommended in situations such as DIC-like disorder following the snakebite, reduction of the incidence of thrombocytopenia and DIC, even in case of hypotension and acute renal failure. Specifically, 5000

units and a further 2500 units every 8 h have been found to be effective (Valenta, 2010). It should be mentioned that WHO generally does not recommend the use of heparin in the case of snakebite, since it is ineffective against venom-induced thrombi and may cause bleeding because of its anti-coagulant properties (WHO, 2010a). However, if a DIC diagnosis has been performed, the patient is stabilised and bleeding has stopped, minimal doses of non-fractionated heparin or more preferably low molecular heparin are recommended until laboratory tests are fully normalised (Valenta, 2010). Anti-fibrinolytics, such as tranexamic acid, can be effective if the bleeding has been caused by an increase in plasminogen activation. However, caution is needed since antifibrinolytics inhibit the degradation of microembolisation; moreover, they may increase the risk of organ failure as part of the DIC-like disorder, and they should be avoided in the case of snake-bites (Valenta, 2010; WHO, 2010a). It is important to substitute platelets if bleeding continues, in decline under 20,000–50,000/mm^3. The same protocol should be followed in case of a decrease in erythrocyte concentrations particularly below 25%–30% of the physiological values (Valenta, 2010). In the case of severe bleeding or need of urgent surgery, once specific antivenom has been given to neutralise venom pro-coagulants and other anti-haemostatic toxins; restoration of blood coagulation and platelet function can be accelerated through the adminstration of fresh frozen plasma, cryoprecipitate (fibrinogen, factor VIII), fresh whole blood or platelet concentrates (WHO, 2010a).

4.3.10. Myotoxicity

Myonecrosis or acute muscle damage, is a very common outcome of snakebite envenomation. Viperid venom is well recognised to induce local myonecrosis, particularly prominent in envenomations caused by members of the subfamily Crotalinae (Gutiérrez et al., 2008, 2009). The myotoxic effects of viper venom is mostly due to non-enzymatic toxins and enzymes of the PLA2 type which causes destruction of striated muscle cells known as rhabdomyolysis and resulting in minor or even major myonecrosis (Lomonte and Gutiérrez, 2011; Valenta, 2010). Rhabdomyolysis is characterised by the disruption of skeletal muscle integrity, leading to the direct release of intracellular muscle components, including myoglobin, creatine kinase (CK), aldolase, and lactate dehydrogenase, as well as electrolytes, into the bloodstream and extracellular compartment (Lomonte and Gutiérrez, 2011; Torres et al., 2015). Rhabdomyolysis can range from an asymptomatic illness with elevation in the CK levels to a life-threatening condition associated with extreme elevations in CK, electrolyte imbalances, acute renal failure where myoglobin will cause obstruction in glomerular capillaries and renal tubules and DIC (Torres et al., 2015). The classic triad of symptoms of rhabdomyolysis consists of myalgia, weakness, and tea-coloured urine (Torres et al., 2015). Systemic manifestations may include tachycardia, general malaise, fever, nausea and vomiting. The clinical manifestations of DIC and multi-organ failure may subsequently appear (Torres et al., 2015). The main complications of rhabdomyolysis are: acute kidney injury, compartment syndrome, hypovolemia, late hypercalcemia, hypocalcaemia, hypophosphatemia, hyperkalaemia and DIC (Torres et al., 2015). The main treatment for snake-bite related rhabdomyolysis is antivenom therapy. Symptomatic treatment of rhabdomyolysis is limited to an adequate parenteral supply of liquids to prevent a decrease in perfusion pressure in the glomeruli and facilitate production of a sufficient quantity of primary urine (Nelson et al., 2019). Besides, the fluid supplements are also important to prevent hypotension which is often common in *Vipera* envenomation (Valenta, 2010).

4.3.11. Renal damage and failure

As reported in the previous paragraphs, oliguria in association with acute renal failure may be caused by several mechanisms, above all induced by myoglobinuria and haemoglobinuria. Oliguria is defined as a urine output of less than 400 mL/day or less than 20 mL/hour (WHO, 2010a). Besides, the reduced blood pressure may induce an acute renal failure of the pre-renal type due to reduced perfusion. The development of microthrombi in instances of DIC-like coagulation disorders can also induce an acute renal failure (Valenta, 2010). To minimise the risk of renal damage from excreted myoglobin and/or haemoglobin, it is important to correct hypovolemia, maintain saline diuresis, correct severe acidosis with bicarbonate through a single infusion of mannitol (200 mL of 20 % solution over 20 min) (WHO, 2010a). Finally, several nephrotoxic elements present in snake venom can directly produce renal damage (Nelson et al., 2019). Accordingly, to protect kidneys from such damage, secure adequate perfusion pressure and sufficient renal blood flow must be ensured. If systemic pressure cannot be sustained by increasing intravascular volume, vasopressor therapy is indicated. In addition, in order to maintain sufficient renal blood flow, if circulatory conditions permit, catecholamines are indicated, but not before intravascular volume is restored (Valenta, 2010). Accordingly, if the patient experiences intravascular volume depletion fluids should be administered. As a rule of thumb, two litres of isotonic saline can be administered to an adult patient over an hour bearing in mind that it should be suspended if the patient experiences pulmonary oedema. The stimulation of diuresis is often managed through a slow injection of 100 mg of furosemide (4−5 mg/minute); it is possible to administer a second dose of furosemide of 200 mg if urine output of 40 mL/hour is still not restored, at this point. .if the urine output is still below the required baseline the patient should be transferred to a Renal Unit. Notwithstanding, ECG, serum troponin, potassium, urea, creatinine, pH, bicarbonate, calcium and phosphate should be monitored frequently. The patient's diet should be bland, high in calories (1700 kcal/day) and low in proteins (less than 40 g/day) (WHO, 2010a). In some cases, a severe acidosis may arise with symptoms including hypotension, deep sighing "Kussmaul" breathing, very low plasma bicarbonate concentration or very low pH (<7.10) and treatment with sodium bicarbonate should be performed as soon as possible (WHO, 2010a). Based on the volume of distribution of bicarbonate (40 % of body weight), bicarbonate deficit can be calculated. Usually 2–3 ampoules (40 mL of 8.4 % sodium bicarbonate equivalent to 1 mmol/mL) in 5% dextrose water, can be administered every 3−4 hours (WHO, 2010a). In the case of a renal failure, treatment does not differ from that of other organ failure from different etiologies, with the exception of antivenom administration (Valenta, 2010). If the renal insufficiency is too severe and characterised by clinical uraemia; fluid overload; changes in blood biochemistry (including one or more of the following: creatinine >4 mg/dl [500 μmol/l]; urea >130 mg/dl [27 mmol/l], potassium >7 mmol/l [or hyperkalaemic ECG changes]) as well as symptomatic acidosis, alternative treatments such as hemofiltration or haemodialysis, or intermittent haemodialysis are indicated depending on the patient's status. The majority of acute renal failure cases often have a reversible course, however chronic complications, such as chronic renal failure can still occur (Valenta, 2010).

4.3.12. Hyperglycaemia

Since the nineteenth century, stress hyperglycaemia (SH) has been described and relates to inflammatory and/or stressful conditions secondary to acute injury or stress responses in patients with severe diseases (e.g., sepsis, trauma, poisonings) (Abdelhamid et al., 2016). Snakebite envenomation is a condition that may potentially induce SH. In relation to inflammatory and stress condition that are secondary to severe envenomation, adrenergic hyperstimulation, is probably the main factor causing hyperglycaemia and this mechanism has been evidenced in other envenomations (e.g. scorpion envenomation). Hyperglycaemia can also be induced by pain, acidosis, alteration in intravascular volume and hypoxia. Claudet et al. (2016) highlighted that hyperglycaemia is a potential risk factor for high-grade envenomation after European *Vipera* bites in children (Claudet et al., 2016). The presence of hyperglycaemia in association with other factors that may increase the progression of symptoms to high-grade envenomation, may represent an indication to transfer paediatric patients to a tertiary level hospital where the antivenom is available (Claudet et al., 2012).

Evidence from a study has shown that in critically ill patients from intensive care settings, hyperglycaemia is associated with increased morbidity and mortality, regardless of the reason for admission, even though the sample size was low (Viana et al., 2014). Additionally, blood sugar level is an extremely variable parameter which is influenced by a number of factors (food intake, time of blood glucose monitoring, underlying medical conditions, treatment settings). Overall, hyperglycaemia should be cautiously considered as a risk factor for progression to high-grade envenomation and further studies are required to refine such assessment.

4.4. Antivenom therapy

Specific immunotherapy for snakebites involves the administration of snake antivenom, which can be monovalent or polyvalent. The former should be preferred when available, because of its species-specificity. Nevertheless, the latter, although potentially effective against the venom of more species (Gutiérrez et al., 2005a, b, 2014), tends to be used more frequently as it is not always possible to identify the species responsible for the bite (Valenta, 2010).

Antivenom administration must always be considered with caution because of the severe potential side-effects it can trigger (León et al., 2013). Accordingly, these are recommended when signs of systemic envenomation exist or in the case of locally advanced or progressive symptoms (Valenta, 2010). However, the possible onset of side effects should not delay or negate the administration of the antivenom. This point is of particular relevance in reference to *Vipera* antivenoms currently available in Europe, since a recent review concluded adverse reactions attributable to them have been rarely reported and appear to be treatable with standard therapy (Lamb et al., 2017).

4.4.1. When should antivenom be administered?

In some cases, the response to the antivenom occurs immediately and its prompt use allows to neutralise the toxins and other components of the venom, therefore preventing irreversible organ damage. Accordingly, the administration of antivenom within 1–2 hours following a snakebite is essential. Therefore, indication for the need of antivenom administration should be assessed rapidly, although always based on the presence of systemic and/or local envenomation symptoms. The clinical, laboratory and instrumental alterations that are commonly considered to require antivenom infusion are synthesised in the Stockholm criteria, although the degree of envenomation (G) is more commonly used to assess situations under which antivenom is needed.

Table 1
Clinical gradation of viper envenomation, Boels et al. (2012).

Grade	Envenomation	Clinical features
0	No envenomation	Fang marks No oedema No local reaction
1	Minimal envenomation	Local oedema around the site of bite No systemic symptoms
2	Moderate envenomation	2a Regional oedema (most of the bitten limb) and/or haematoma 2b Grade 2a + moderate general symptoms: mild hypotension, vomiting, diarrhoea, neurotoxic signs and/or biological criteria for severity: Platelets < 150 G/L Leukocytes > 15 G/L INR > 1.5 Fibrinogen < 2 g/L
3	Severe envenomation	Extensive oedema spreading to the trunk and/or severe general symptoms (severe hypotension < 80 mmHg systolic, shock, bleeding)

Boels, D., Hamel, J.F., Bretaudeau, M., Harry, P., 2012. European viper envenomings: assessment of Viperfav and other symptomatic treatments. Clin. Toxicol. 50.189–196.

The Stockholm criteria states that the antivenom should be administered when the following symptoms arise: hypotension and circulatory shock, protracted severe gastrointestinal symptomatology, mucous membrane oedema with a risk of bronchial obstruction, rapid extension of oedema to an entire limb and/or to the torso, neurological symptomatology with depressed CNS and peripheral and central paresis. In extreme cases of insufficient clinical signs, the antivenom may be administered when any of the following conditions are fulfilled: leukocytosis exceeding $15-20 \times 10^9$/l, elevation of AST, ALT, CK or other enzymes, metabolic acidosis, hemolysis, ECG changes, coagulation disorders.

While the Stockholm criteria are less known, the grading of snake envenomation (G) is the most commonly reported score in the scientific literature, although it has modified over the years. The authors recommend in this paper the grading proposed by Boels et al. (2012) where the envenomation grades are divided into 4 main groups: G0-G1-G2 and G3 (Table 1) and for which the application of the antivenom is recommended from the G2 stage onwards (Kang et al., 2016). Development of an enlarged tender lymph node draining the bitten limb is another condition that justifies the use of an antivenom (WHO, 2010a).

4.4.2. Types of antivenom

The seven antivenom currently available in Europe are showed in Table 2. Overall, five antivenoms are polyvalent: *Viperfav (Sanofi-Pasteur®)* against *V. aspis*, *V. berus* and *V. ammodytes* venoms; *Polisera (Vetal Serum®)* against *V. ammodytes* (as well as *Macrovipera* spp.); *European Viper Venom Antiserum (Institute of Immunology Inc®)* against *V. ammodytes, V.aspis, V. berus, V.ursini* (as well as *Macrovipera* spp.); *Viekvin (Institute of virology, vaccines and sera, Torlak®)* against *V. berus* and *V. ammodytes; ViperaTAb (MicroPharm Ltd®) against V. berus, V. aspis, V. ammodytes, V. latastei.* In contrast, *Viper Venom Antitoxin (Biomed®)* and *Snake Venom Antiserum (Bul Bio®)* are specific to *V. berus* and *V. ammodytes*, respectively. In a preclinical assessment another new polyvalent antivenom (*Inoserp Europe*, Latoxan S.A.S. and Alphabiotoxine Laboratory) has recently showed appropriate neutralizing potency against the venoms of several *Vipera* species (*V. ammodytes*, *V. aspis*, *V. berus*, *V. latastei*), *Montivipera* and *Macrovipera* (García-Arredondo et al., 2019).

4.4.3. Administration modality and doses

Although intra-muscle administration is also recommended by some manufacturers, the intravenous route should be the preferred route of administration since it allows a greater bioavailability of the antivenom (Figs. 6 and 7). Indeed, antivenoms are large molecules (Fab fragments or sometimes whole IgG) which, after intramuscular injection, are absorbed slowly via the lymphatic system and associated with a poor bioavailability. Indeed, the site of inoculation of the venom is reached in 2 h by 1.4–6 % of the antivenom when administered intramuscularly or subcutaneously and by 85 % when administered intravenously (WHO, 2010a).

Intramuscular administration of antivenom should be taken into consideration when the distance between the site of the viper bite and the hospital is large and requires hours of travel or in patients with no intravenous access (WHO, 2010a). In these exceptional circumstances, the dose of antivenom should be divided between a number of sites in the upper anterolateral region of both thighs, followed by a massage to aid absorption. Antivenom should not be injected into the gluteal region as absorption is exceptionally slow and unreliable with the risk of sciatic nerve damage when the injection is given by an inexperienced operator. In any case, it is important to point out that the use of anti-ophidian serum must be prioritised in the hospitals particularly because its use outside the hospital is poorly effective (most often administered intramuscularly or subcutaneously) and would expose the patient to risks of adverse or serious reactions (WHO, 2010a).

Regarding the storage pending infusions, lyophilised antivenoms should be stored at < 25 °C and liquid antivenoms should be stored at

Table 2
Characteristics of the seven antivenoms currently available in Europe from safety data sheets.

Antivenom name	Marketing Authorization Holder (MAH)	Source	Type	*Vipera* spp raised against / neutralizing activity against other viper venoms	Recognized by WHO [63]	Antibody concentration	Affinity purified	Formulation	Recommended dose and route of administration by MAH	Total amount of protein per dose	Storage instructions	Shelf-life of the antivenom
Viper Venom Antitoxin	Biomed Sera and Vaccines Manufacturing Company, Warsaw, Poland	Equine	F $(ab')_2$	*V.berus*	Yes	NR	No	Injectable solution, ampoule of 5 mL	5 mL IM	450−850 mg	Refrigerator (+2 °C ÷ +8 °C)	36 months
European viper venom antiserum	Institute of Immunology, Zagreb, Croatia	Equine	F $(ab')_2$	*V.ammodytes /V. aspis, V. berus, ursini, Macrovipera lebetina*	Yes	25 mg/mL	No	Injectable solution, ampoule of 10 mL	10 mL IM Can use up to 40 mL IV in very severe cases	1000 mg	Refrigerator (+2 °C ÷ +8 °C)	36 months
ViperaTAb	MicroPharm Ltd UK	Ovine	Fab	*V.berus /V. aspis, V. ammodytes, V. latastei*	No	100 mg/mL	Yes	Injectable solution, bottle of 100 mg/ 4 mL	8 mL IV	200 mg	Refrigerator (+2 °C ÷ +8 °C)	24 months
Viekvin	Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia	Equine	F $(ab')_2$	*V.ammodytes /V. berus*	Yes	NR	No	Injectable solution, ampoule of 5 mL	5−10 ml IM	NR	Refrigerator (+2 °C ÷ +8 °C)	36 months
Viperfav	Sanofi Pasteur, France	Equine	F $(ab')_2$	*V. berus, V. aspis, V. ammodytes*	Yes	99−116 mg/ mL	No	Injectable solution, bottle of 4 mL	4 mL IV	396−468 mg	Refrigerator (+2 °C ÷ +8 °C)	36 months
Snake Venom Antiserum	Bul Bio NCIPD Ltd, Sofia, Bulgaria	Equine	F $(ab')_2$	*V.ammodytes*	No	NR	No	Injectable solution, bottle 100 AU	10 mL SC and 10 mL IM	900−1700 mg	Refrigerator (+2 °C ÷ +8 °C)	24 months
Vetel polysera	Vetal Serum and Biological Products Manufacturig Industry and Commerce, Andiyaman, Turkey	Equine	F $(ab')_2$	*V.ammodytes, Macrovipera lebetina, Montivipera xanthina*	No	NR	No	Injectable solution, ampoule of 10 mL	10−50 ml IM or IV in severe envenoming	NR	Refrigerator (+2 °C ÷ +8 °C)	24 months

IM: intramuscular; IV: intravenous; NK: not known; NR: not reported; SC: subcutaneous; WHO: World Health Organization.

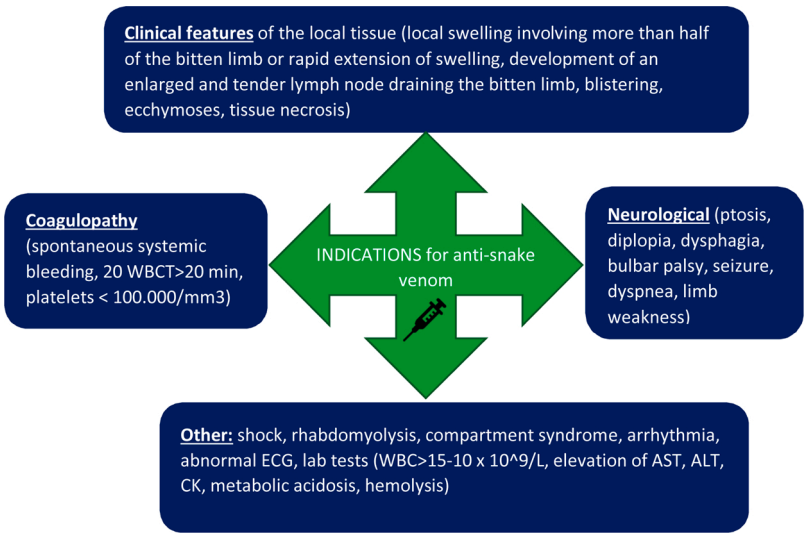

Fig. 6. Indications for antivenom administration after viper envenomation.

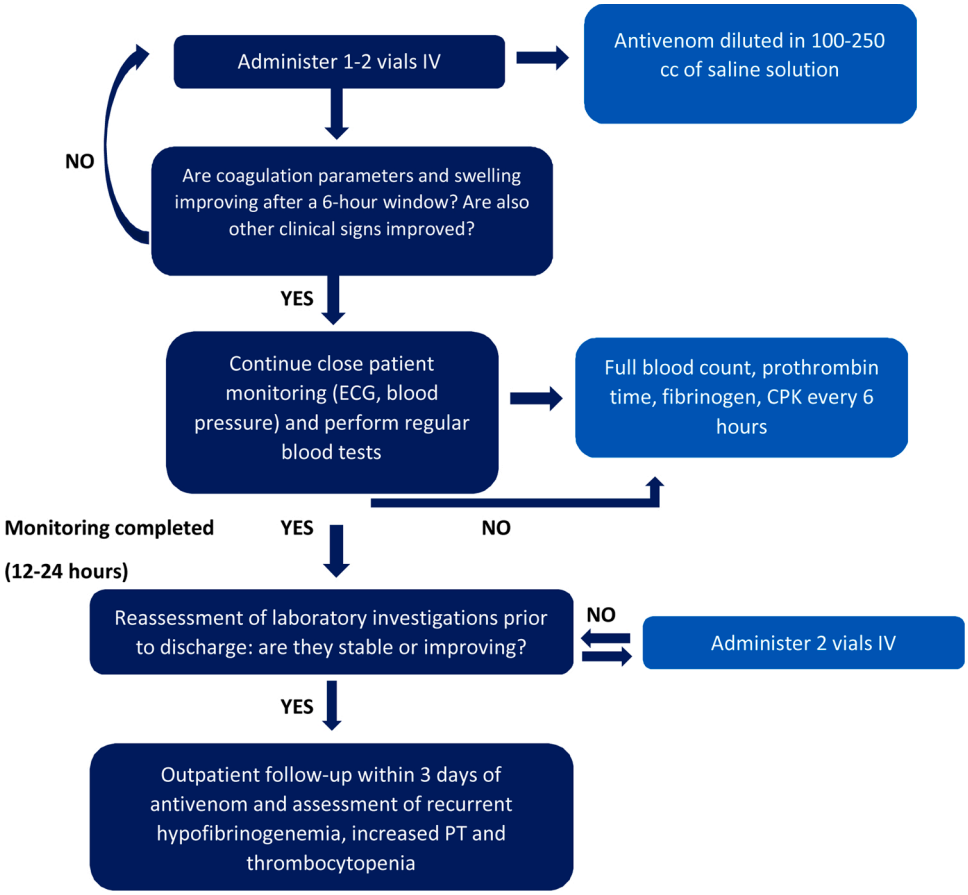

Fig. 7. Antivenom administration modified according to Goldfrank's Toxicologic Emergencies textbook.

2–8 °C and not frozen (Valenta, 2010). The WHO guidance proposes two major methods of administration of the antivenom: I) reconstituted freeze-dried antivenom or neat liquid antivenom diluted in approximately 5–10 ml of isotonic fluid per kg body weight and it is infused at a constant rate over a period of about one hour; II) reconstituted freeze-dried antivenom or neat liquid antivenom is given by slow intravenous injection (not more than 2 mL/minute) (WHO, 2010a). It is important to highlight that since snake injects the same amount of venom in both adults and children, the dosage of the antivenoms is the same independently from the patient's age and usually ranges between 4 ml–50 ml, based on the type of the antivenom used (WHO, 2010a). In any case, the manufacturers always report the recommended amount of serum. However, a formal contact with a poison control center is always recommended.

Currently, there is no standardised and harmonised protocol for antivenom administration across Europe and variation across country is

wide but, most often, 1−2 ampoules of antivenom are administered (Kang et al., 2016; Lamb et al., 2017). The peer reviewed literature reports that in 15.4 % of cases, additional administration of antivenom after the initial dose may be needed, because of poor therapeutic efficiency of the antivenom or recurrence of clinical manifestations. These include persistence or recurrence of poor blood coagulation after 6 h, bleeding after 1−2 hours and an increase in the clinical symptoms of neurotoxicity or cardiovascular toxicity after 1−2 hours (Nelson et al., 2019; WHO, 2010a). The antivenom should be administered within a certain time window to optimise its effectiveness and when after an initial dose the clinical situation is worsening (Kang et al., 2016). Above all, envenomation by *Vipera* species may result in signs of systemic envenoming and may recur within 24−48 hours after the snakebite in patients with an initial response to the antivenom showing cessation of bleeding and restoration of blood coagulability, although cases of recurrence have also been shown after 3–14 days the administration of antivenom (Valenta, 2010; WHO, 2010a). The recurrence of envenomation symptoms has been discussed to involve two key aspects namely the continued release of venom from the pool at the site of the bite and the dissociation of the antigen-toxin complex associated with faster antitoxin clearance. Such recurrence of symptoms in patients is more common following injection of antivenom containing antigens with a shorter elimination half-life, such as the Fab-fragment (Valenta, 2010). Plasmapheresis can also constitute a complementary strategy which can be combined with the administration of antivenom when recurrence of symptoms occurs and in such circumstances administration should alternate plasmapheresis (Valenta, 2010).

4.4.4. Antivenom adverse reactions and prevention

According to a recent review, adverse reactions reported after antivenom administration are rare (Lamb et al., 2017). Among the three most widely used antivenoms, namely Zagreb (not produced anymore), Viperfav, and ViperaTAb, the last one showed the lowest incidence of adverse reactions, probably due to reduced protein load and shorter elimination half-life (Lamb et al., 2017). However, clinicians should monitor any adverse reactions on a case by case basis since they may occur with any antivenom and at any time particularly because of inter-individual differences in the toxicokinetics and toxicodynamics of the antivenom. On a general basis, the frequency of adverse reactions after the antivenom administration all over the world has been reported to be around 10 %, which is either an early adverse reaction (within a few hours) or a late one (five days or more). The risk of adverse reaction is dose-related, except in rare cases for which IgE-mediated Type I hypersensitivity results in sensitisation (WHO, 2010a, 2016).

In order to predict an eventual anaphylactic reaction, a small dose of intramuscular or conjunctiva venom have been reported from the literature, however such practices are not recommended (Valenta, 2010). Generally speaking, an anaphylactic reaction may appear within 10−180 min after the administration of the antivenom. There are no specific contraindications for the antivenom. Notwithstanding, patients that have developed reactions to horse (equine) or sheep (ovine) serum in the past and patients with a personal medical history indicating the presence of atopy (atopic dermatitis and asthma) have an increased risk to develop anaphylaxis. Those patients who have anaphylactic reactions may be managed with the administration of subcutaneous epinephrine prior to antivenom treatment (dose of 0.25 mL/0.25 mg of epinephrine and dilution of 1:1000 [0.1 %]) (Valenta, 2010). Hydrocortisone can also be administered at the same time (dose 2−4 mg/kg body weight) together with anti-H1 antihistamines (cimetidine or chlorphenamine) or anti-H2 antihistamines (cimetidine, cetirizine). If an acute and true anaphylactic reaction arises, antivenom administration must temporarily be suspended, and the therapy of the anaphylactic reaction does not differ from that of the same response from different etiologies (Valenta, 2010). Pyrogenic reactions may also arise usually within 60–120 minutes after the treatment and are characterised by shaking chills, fever, vasodilatation and a fall in blood pressure. The patient's body temperature must be decreased using antipyretics (e.g. for paracetamol) and intravenous fluids should be given to correct an eventual hypovolaemia. Serum sickness syndrome may also occur as a delayed hypersensitivity reaction, triggered by the snake anti-venom, equine and rabbit anti-thymocyte globulin (ATG) (Pascuzzi, 2003). Such syndrome may arise with a median time of 7 days after the administration of the antivenom (range between 1–12 days) and it is mainly characterised by cutaneous rashes, itching, arthralgia, fever (also high as 40 °C), lymphadenopathy (swelling of lymph nodes), head and neck malaise, hypotension, splenomegaly, glomerulonephritis, proteinuria and haemoglobinuria. Treatment for serum sickness syndrome includes chlorphenamine (dose of 2 mg for adults six hourly, and 0.25 mg/kg/day in children in divided doses) as well as prednisolone (dose 5 mg for adults six hourly, and 0.7 mg/kg/day in children in divided doses for 5–7 days) (WHO, 2010a)

4.4.5. Unavailability of antivenom

The antivenin should always be available from local or regional poison control centers and if not contacting national poison centers could be an option. However, in very rare occasions, antivenom may not be available and related therapeutic measures must be implemented. In any case, the ABCDE rescue techniques do not differ from those applied during other life-threatening conditions. These are based upon the life-saving protocols and skills that are carried out in the so-called "advanced life support" according to the guidelines of European Resuscitation Council (The European Resuscitation Council, 2020). The organ damage can be treated as other aetiologies. Plasmapheresis can be a satisfactory alternative to specific therapy by antivenoms whenever an antivenom is not available. This procedure can be applied even when the affected individual suffers from an allergic reaction to the antivenom (Valenta, 2010).

5. Conclusion and future perspectives

This paper provided an account of the taxonomy of European *Vipera* species involved in human envenomation, the venom composition of the six species of major clinical relevance and their key mechanisms of toxicity, together with protocols for the clinical management of *Vipera* bites in Europe (which is summarised in Fig. 8).

In order to further characterise the variability of viper toxins in Europe and improve the clinical management of viper bites, future work is recommended. Here are some examples of topics of increasing interest or to look into:

- Further characterisation of viper taxonomy at the sub-species level using modern molecular tools (next generation sequencing) as well as viper toxins using proteomics approaches.
- From a taxonomical perspective, many subspecies are still described only on a morphological basis, without being supported by molecular evidence. Of interest is the recently described *Vipera walser* from northern Italy, for which the taxonomic status is debated: further molecular analysis would allow to define differences from *V. berus* (Speybroeck et al., 2020).
- With regards to venom composition, proteomic characterisation of the venom toxins has not yet been performed for *V. seoanei*, *V. latastei* and *V. walser* and would be of great interest.
- *in vitro* methods using human cells to investigate: a) the molecular basis of viper toxins' toxicity (this would also support to move away from using laboratory animals to determine LD_{50}s), b) the toxicokinetics of viper toxins and develop physiologically-based kinetic models that can be applied to all European viper species.
- Stimulate clinicians to record case reports on viper bite cases in a structured database to provide sound statistics of the frequency of viper bites and associated clinical features across Europe.

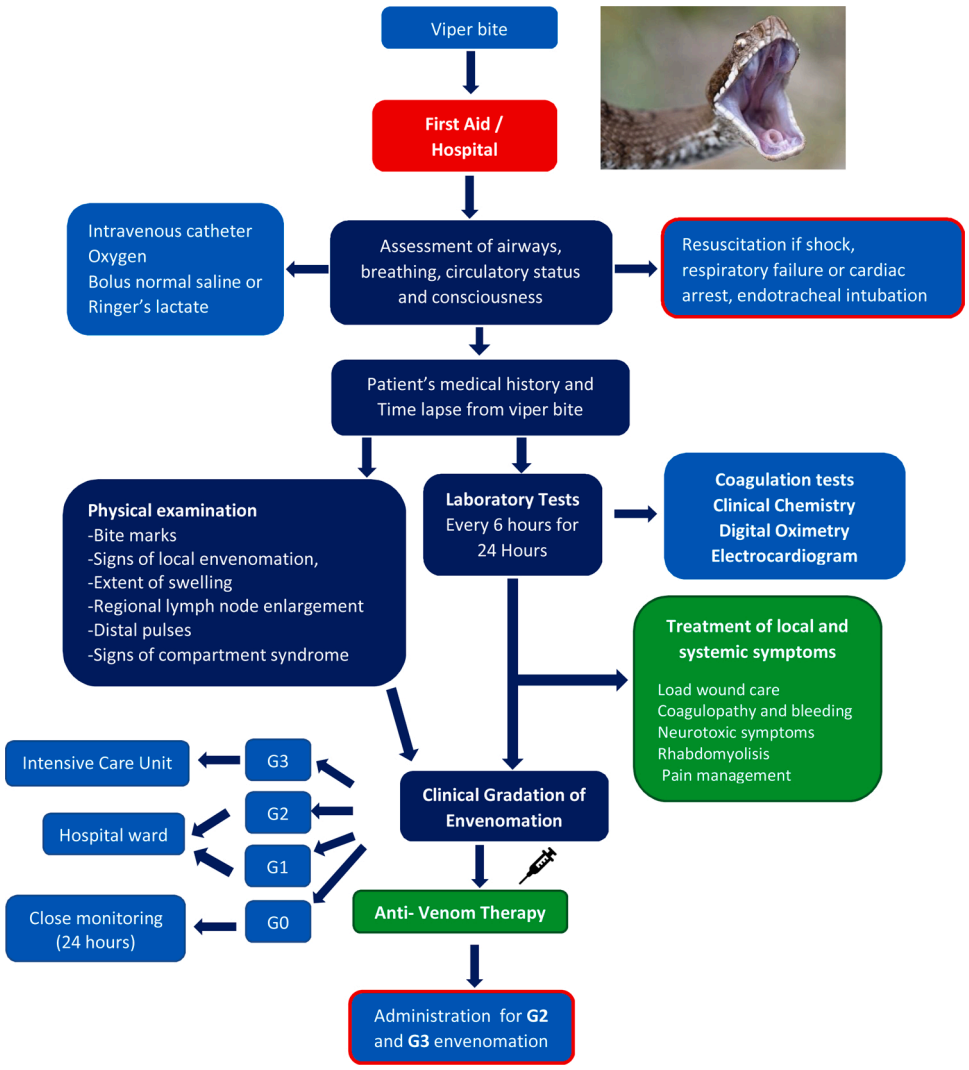

Fig. 8. Clinical management of viper envenomation.

CRediT authorship contribution statement

Matteo R. Di Nicola: Conceptualization, Writing - original draft, Writing - review & editing. **Andrea Pontara:** Writing - original draft, Writing - review & editing. **George E.N. Kass:** Writing - original draft, Writing - review & editing. **Nynke I. Kramer:** Writing - review & editing. **Ignazio Avella:** Writing - original draft. **Riccardo Pampena:** Writing - review & editing. **Santo Raffaele Mercuri:** Writing - review & editing. **Jean Lou C.M. Dorne:** Writing - original draft, Writing - review & editing. **Giovanni Paolino:** Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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The views expressed in this paper belong to the authors only and do not reflect the views of the European Food Safety Authority.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tox.2021.152724.

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Supplementary Table 1. European species and subspecies of the family Viperidae*

Suborder: Serpentes Linnaeus, 1758

Superfamily: Colubroidea Oppel, 1811

Family: Viperidae Oppel, 1811

Subfamily: Viperinae Oppel, 1811

Genus: *Macrovipera* Reuss, 1927

Species: *Macrovipera lebetinus* (Linnaeus, 1758)

Subspecies: *Macrovipera lebetinus lebetinus* (Linnaeus, 1758)

Species: *Macrovipera schweizeri* (Werner, 1935) [monotypic]**

Genus: *Montivipera* Nilson, Tuniyev, Andrén, Orlov, Joger and Herrmann, 1999

Species: *Montivipera xanthina* (Gray, 1849)

Subspecies: *Montivipera xanthina xanthina* (Gray, 1849)

Subspecies: *Montivipera xanthina dianae* Cattaneo, 2014

Subspecies: *Montivipera xanthina nilsoni* Cattaneo, 2014

Subspecies: *Montivipera xanthina occidentalis* Cattaneo, 2017

Genus: *Vipera* Laurenti, 1768

Species: *Vipera ammodytes* (Linnaeus, 1758)

Subspecies: *Vipera ammodytes ammodytes* (Linnaeus, 1758)

Subspecies: *Vipera ammodytes meridionalis* Boulenger, 1903

Subspecies: *Vipera ammodytes montandoni* Boulenger, 1904

Species: *Vipera aspis* (Linnaeus, 1758)

Subspecies: *Vipera aspis aspis* (Linnaeus, 1758)

Subspecies: *Vipera aspis francisciredi* Laurenti, 1768

Subspecies: *Vipera aspis hugyi* Schinz, 1833

Subspecies: *Vipera aspis zinnikeri* Kramer, 1958

Species: *Vipera berus* (Linnaeus, 1758)

Subspecies: *Vipera berus berus* (Linnaeus, 1758)

Subspecies: *Vipera berus bosniensis* (Boettger, 1889)

Subspecies: *Vipera berus nikolskii* Vedmederya, Grubant & Rudajewa, 1986

Species: *Vipera dinniki* Nikolsky, 1913 [monotypic]**

Species: *Vipera graeca* (Nilson & Andrén, 1988) [monotypic]***

Species: *Vipera kaznakovi* Nikolsky, 1909 [monotypic]***

Species: *Vipera latastei* Boscá, 1878

Subspecies: *Vipera latastei latastei* Boscá, 1878

Subspecies: *Vipera latastei gaditana* Saint Girons, 1977

Species: *Vipera renardi* (Christoph, 1861)

Subspecies: *Vipera renardi renardi* (Christoph, 1861)

Subspecies: *Vipera renardi bashkirovi* Garanin, Pavlov & Bakiev in Bakiev et al., 2004

Subspecies: *Vipera renardi puzanovi* Kukuskin, 2009

Species: *Vipera seoanei* Lataste, 1879

Subspecies: *Vipera seoanei seoanei* Lataste, 1879

Subspecies: *Vipera seoanei cantabrica* Braña & Bas, 1983

Species: *Vipera ursinii* (Bonaparte, 1835)

Subspecies: *Vipera ursinii ursinii* (Bonaparte, 1835)

Subspecies: *Vipera ursinii macrops* Méhely, 1911

Subspecies: *Vipera ursinii moldavica* Nilson, Andrén & Joger, 1993

Subspecies: *Vipera ursinii rakosiensis* Méhely, 1893

Species: *Vipera walser* Ghielmi, Menegon, Marsden, Laddaga & Ursenbacher, 2016 [monotypic] ****

* Considered geographic area according to Speybroeck et al. (2020). The validity of the presented taxa and their attribution to specific or subspecific rank can vary according to the different authors. The descriptions of many subspecies have been made only on a morphological basis and require confirmation on a molecular basis.

** Species considered of doubtful validity according to Freitas et al. (2020).

*** Species that need further studies to assess taxonomic status according to Freitas et al. (2020).

**** Taxon not to be considered at specific level according to Speybroeck et al. (2020).

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Supplementary Figure 1. Evaluation and management of compartment syndrome