

## SCORPION ENVENOMATION AND SEROTHERAPY IN MOROCCO

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**Abstract.** A clinical and biologic study was conducted in Morocco to assess the efficiency of antivenom therapy for treating victims of scorpion stings. Epidemiologic and clinical data were collected from 275 patients envenomed by *Androctonus mauretanicus mauretanicus* and *Buthus occitanus* scorpions. Patients received antivenom or other drugs. Blood samples were collected at the time of hospital admission and 1 hr and 3 hr after treatment. Serum venom levels were quantified by using an ELISA. An association was found between clinical signs of envenoming and the level of venom in serum. Patients classified as grade II (moderate envenoming) had higher serum levels of venom level than patients classified as grade I (mild envenoming). At admission to the hospital, the mean venom concentration was not significantly different between the group not treated with antivenom, the group who received 2–5 ml of antivenom, and the group who received 10 ml of antivenom. A significant decrease in serum venom levels and an improvement in the clinical conditions were observed in patients administered 10 ml of antivenom. The lower decrease in serum venom levels in patients who received 2–5 ml of antivenom was due to lower doses of antivenom. No difference in the venom concentration was observed in patients who were not treated with antivenom. The absence of administration of antivenom increased the risk of developing clinical signs at the end of the hospitalization period. However, this risk was much higher when more than 1 hr elapsed between the time of the scorpion sting and the time of hospital admission. The results demonstrate that antivenom is effective in decreasing circulating venom and morbidity. Serotherapy is more efficient when given as soon as possible after envenomation and with adequate quantities of antivenom.

In north Africa, as in numerous tropical countries, envenomation by scorpion stings is a major public health problem, particularly in children.<sup>1–4</sup> The black scorpion (*Androctonus mauretanicus mauretanicus*) and the yellow scorpion (*Buthus occitanus*) are the most dangerous scorpions and are responsible for the majority of stings in Morocco. The epidemiologic data are incomplete, but the number of scorpion stings is estimated to be 40,000 per year in Morocco.

Scorpion venoms are a complex biochemical mixture containing numerous neurotoxic polypeptides.<sup>5,6</sup> These polypeptides enhance excitability of nerve and muscle cells in scorpion sting victims and also cause death, particularly in children.<sup>7</sup> The onset of clinical symptoms is rapid (within 5–30 min) following the sting.<sup>8,9</sup> Respiratory failure and cardiovascular manifestations are the usual causes of death.<sup>9–11</sup> The toxicity of *A. mauretanicus mauretanicus* venom is due to the presence of neurotoxins that are specifically active on sodium and potassium channels.<sup>6,12</sup>

The severity of scorpion envenoming and the rapid diffusion of inoculated venom require that appropriate treatment be started as soon as possible after the sting. Most investigators consider antivenom to be the only specific treatment for envenoming by scorpion stings.<sup>1,3,13–16</sup> However, others have questioned the usefulness of antivenom in eliminating cardiovascular manifestations of scorpion stings.<sup>17–19</sup>

In Morocco, serotherapy has been one of the major therapeutic measures used in the treatment of scorpion envenoming for the last 30 years.<sup>20</sup> However, the choice of the antivenom dose and the administration protocol remain arbitrary. No clinical study has been carried out regarding the efficacy of scorpion antivenom in neutralizing circulating venom antigens or its ability to decrease morbidity. The lack of such clinical and biologic studies could be due to the absence of suitable techniques for detecting and quantifying circulating venom in envenomed patients. An ELISA that

measures venom levels after scorpion envenoming has been used for cases stung by *Androctonus australis garzonii*, *B. occitanus tunetanus*, and *Tityus serrulatus*,<sup>4,21,22</sup> but not for those stung by *A. mauretanicus mauretanicus* or *B. occitanus*.

In this study, an ELISA was developed for detecting the toxic fraction of Moroccan scorpion venom in the serum of sting victims. We conducted a prospective study in the 4 provinces of Morocco (El Kalaa, El-Jadida, Agadir, and Tan-Tan) where scorpion stings are most likely to occur, and also analyzed the kinetics of serum venom levels and the evolution of the clinical signs of envenoming after antivenom therapy to assess the efficacy of serotherapy. The epidemiologic, clinical, and biologic results of the study are reported.

### PATIENTS, MATERIALS, AND METHODS

**Patients.** A prospective study of scorpion stings was conducted in 4 provinces of Morocco (El-Kalaa, El-Jadida, Agadir, and Tan-Tan) from July to October 1997. A questionnaire was distributed to physicians in 19 hospitals to collect patient data (name, age, sex, scorpion identification, time of sting, treatment applied, clinical manifestations, and the duration of hospitalization). Scorpion identification was made by the patient or the physician. The severity of envenomation of each patient was measured by physicians according to a previously defined severity scale described by Krifi and others.<sup>4</sup> The patients were classified into 1 of 3 clinical grades. Grade I (mild envenoming) refers to patients presenting with only local symptoms, local pain, and a burning sensation. Grade II (moderate envenoming) refers to patients presenting with local and general symptoms. Grade III (severe envenoming) refers to patients presenting with local and general symptoms, together with cardiocirculatory shock, respiratory failure, acute pulmonary edema, hyperthermia, and

neurologic symptoms such as priapism, convulsions, or coma.

A total of 304 questionnaires were collected, but 29 cases were subsequently eliminated because of incomplete data. Of the 275 patients included in the study, 49% were treated with antivenom, 35% with other drugs (calcium, corticoids, antihistamines), and 16% received both treatments. All treatment decisions were made by the personal physicians of the patients. Of the 179 patients treated with antivenom, 27% received 2–5 ml and 73% received 10 ml. Antivenom was injected intramuscularly in 77.6% of the patients, subcutaneously in 6.2%, and by both of these routes in 16.2% of the patients.

Venous blood samples used as controls were obtained from healthy subjects who had never been stung by scorpions and who were undergoing routine health examinations at the Biological Center of the Pasteur Institute (Casablanca, Morocco). Blood samples of patients stung by scorpions were obtained at different hospitals in the 4 selected provinces. Samples were obtained immediately upon admission of the patients into the hospital (before the initiation of treatment with antivenom and/or other drugs). Additional samples were obtained 1 and 3 hr after the start of antivenom therapy and/or other drugs. Blood was collected into vacutainer tubes and immediately centrifuged for at  $1,500 \times g$  for 15 min at 4°C. Serum samples were kept frozen in aliquots at –20°C until tested. Informed consent was obtained from all patients who participated in this study. Venous blood samples were obtained only from subjects who agreed to participate in the study. The protocol used in this study was reviewed and approved by the Epidemiological Department of the Moroccan Ministry of Health.

**Venoms.** The venoms of scorpions (*A. mauretanicus mauretanicus* and *B. occitanus*) and snakes (*Naja haje haje*, *Cerastes cerastes*, and *Vipera lebetina*) were obtained by manual stimulation of animals in captivity at the Experimental Center of the Pasteur Institute of Morocco.

**Antivenom.** Scorpion antivenom containing F(ab')<sub>2</sub> fragments was prepared in horses hyperimmunized with crude venom of *A. mauretanicus mauretanicus*. One milliliter of scorpion antivenom will neutralize a minimum of 12.5 50% lethal doses (LD<sub>50</sub>s) in mice with a mean weight of 20 grams (1 LD<sub>50</sub> is the amount of venom that kills 50% of the mouse population). The LD<sub>50</sub> was determined as previously described.<sup>23</sup>

Microtiter plates were obtained from CML (Nemours, France). Horseradish peroxidase and *o*-phenylenediamine were obtained from Sigma (St. Louis, MO). Sephadex G-50 and CNBr-activated Sepharose 4B were obtained from Pharmacia (Uppsala, Sweden). Glutaraldehyde and all other chemical salts and solvents were obtained from Merck (Darmstadt, Germany) or Prolabo (Paris, France).

**Purification of the F(ab')<sub>2</sub> anti toxic-fraction and preparation of peroxidase-labeled F(ab')<sub>2</sub>.** The toxic fraction of *A. mauretanicus mauretanicus* crude venom was purified by Sephadex G-50 chromatography and tested for its toxic activity by subcutaneous injection into mice. Specific anti-toxic fraction F(ab')<sub>2</sub> was purified from scorpion antivenom by affinity chromatography on a toxic fraction covalently coupled to a CNBr-activated Sepharose column, as previously described.<sup>24</sup> The F(ab')<sub>2</sub>-specific toxic fraction (5 mg) was

labeled with 10 mg of peroxidase by glutaraldehyde coupling using a 2-step procedure.<sup>25</sup> Labeled F(ab')<sub>2</sub> was titrated and stored at –20°C in 50% glycerol.

**Enzyme-linked immunosorbent assay.** Microtiter plates were coated with 5 µg/ml (100 µl/well) of F(ab')<sub>2</sub> specific to the toxic fraction of *A. mauretanicus mauretanicus* venom in 0.1 M sodium carbonate buffer, pH 9.6 and incubated overnight at 4°C. The plates were washed 4 times with phosphate-buffered saline (PBS), pH 7.4, containing 0.1% Tween 20 (PBS-Tween) and coated with PBS containing 5% powdered milk and incubated for 45 min at 37°C. The plates were then washed 4 times with PBS-Tween and 100 µl of serum samples were added to each well and incubated for 1 hr at 37°C. The plates were washed with PBS-Tween, 100 µl of peroxidase-labeled F(ab')<sub>2</sub> (diluted 1,000-fold in PBS-Tween containing 5% powdered milk) were added to each well, and the plates were incubated for 45 min at 37°C. The plates were then washed with PBS-Tween and 100 µl of *o*-phenylenediamine solution (2 mg/ml in 0.01 M potassium phosphate buffer, pH 7.3, in the presence of 0.06% hydrogen peroxide) were added and the reaction was developed for 10 min at room temperature in the dark. The reaction was stopped by adding 1 M HCl per well and optical density was measured at 492 nm with a microtitration plate reader (LP400; Diagnostic Pasteur, Paris, France). Venom concentrations in samples were interpolated from a standard curve using known venom concentrations diluted in human serum from healthy donors.

The specificity of the ELISA was determined by testing 100 samples of sera from healthy non-venomated donors. The sensitivity of the assay was analyzed by the constructing calibration curves using scorpion (*A. mauretanicus mauretanicus* and *B. occitanus*) or snake (*N. haje haje*, *C. cerastes*, and *V. lebetina*) venoms. The intra-assay variability was estimated by analyzing 12 replicates of sera samples containing known venom concentrations (10, 25, and 50 ng/ml) on the same titration plate and with the same reagents. The inter-assay variability was assessed by measuring the venom concentration in the same sample for 8 consecutive days on different titration plates and with different reagents. Sera of patients were assayed 2 times in duplicate. The 3 timed collections from a given patient were assayed simultaneously in the same plate.

**Statistical analysis.** Data were analyzed with the SPSS software (SPSS Institute, Chicago, IL) using analysis of variance and appropriate measures of association (odds ratio [OR] and Pearson's chi-square). Multivariate analysis was done using hierarchic logistic regression. All tests were two-tailed. Results are expressed as the mean ± SD. Values were considered to be significantly different if  $P < 0.05$ .

## RESULTS

**Characteristics of the ELISA.** The detection limit of the assay was 0.78 ng/ml. Venom antigens were not detected in sera from healthy non-venomated donors. The assay specifically quantified *A. mauretanicus mauretanicus* venom and showed cross-reactivity of the F(ab')<sub>2</sub> fragments with the *B. occitanus* venom. There was no reactivity when snake venoms were tested (Figure 1). The intra-assay coefficients of

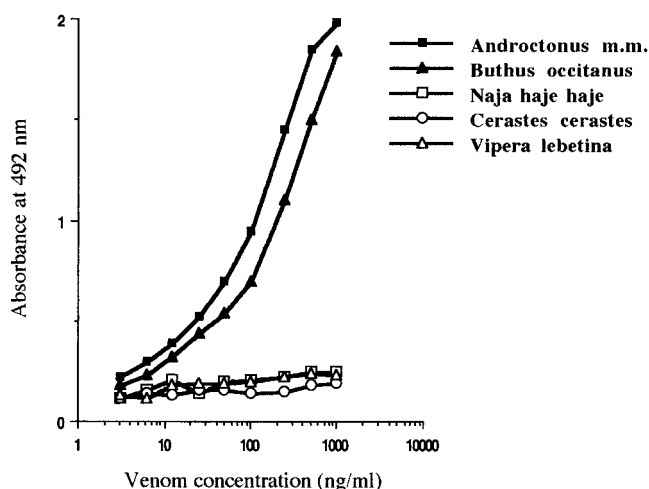


FIGURE 1. Specificity of the ELISA used in this study. Values are the means of 2 assays performed in duplicate.

variation ranged between 1.4% and 4%. The inter-assay coefficient of variation was 6.3%.

#### Epidemiologic and clinical features of envenomation.

Epidemiologic data and clinical features of 275 envenomed patients are shown in Table 1. The distribution of scorpion stings indicates that stings occur more often in the first half of the evening (46.9%) when scorpions search for prey. The distribution of scorpion stings by geographic origin of the patients indicates that most scorpion stings occurred in rural areas (73.8%). The clinical manifestations were extremely diverse, with mainly local signs (local pain and a burning sensation) and rarely general symptoms (sweating, hyperthermia, and shivering).

**Influence of the delay between the sting and hospital admission on the severity of envenomation.** The delay between the sting and the hospital admission ranged from 5 min to 16 hr ( $1.27 \pm 1.6$  hr): it was 5 to >30 min in 33.8% of the patients, 30 min to >3 hr in 57.4%, and 3 to 16 hr in 8.8%. The percentage of patients living in the city and arriving at a hospital within 30 min of being stung was significantly higher than that of patients living in rural areas (42% versus 32%;  $P < 0.05$ ). Patients from rural areas were 1.57 times more likely to take more than 30 min to get to a hospital after the scorpion sting than urban patients ( $OR = 1.57$ ,  $P < 0.05$ ). The time elapsed after the sting was significantly associated with the geographic origin of the patient ( $P < 0.05$ ).

When the delay in arriving at the hospital was more than 30 min, the percentage of patients diagnosed as grade II was higher than patients diagnosed as grade I (36% versus 14.8%). Patients who arrived at the hospital within 30 min after being stung were 3.2 times more likely to be diagnosed as grade I than those taking more than 30 min ( $OR = 3.2$ ,  $P < 0.05$ ). The severity of clinical grading at hospital admission was influenced by the time elapsed after the sting. Consequently, the delay between being stung and the hospital admission may be considered a factor of prognosis.

**Association between venom levels in serum and severity of envenomation.** Patients classified as grade II had a significantly higher venom concentrations in their serum ( $38.86 \pm 10.8$  ng/ml) than patients classified as grade I

TABLE 1  
Epidemiologic features of envenomed patients

Parameters	Patients (%) (n = 275)
Sex	
Male	134 (48.7)
Female	141 (51.3)
Age range (years)	
0–14	75 (27.3)
15–29	91 (33.1)
30–44	60 (21.8)
>44	49 (17.8)
Scorpion species	
<i>Androctonus mauretanicus</i>	229 (83.3)
<i>Buthus occitanus</i>	38 (13.8)
Not specified	8 (2.9)
Sting site	
Upper limbs	142 (51.6)
Lower limbs	119 (43.3)
Other parts of the body	14 (5.1)
Time	
Midnight to before 6:00 AM	22 (8)
6:00 AM to before noon	82 (29.8)
Noon to before 6:00 PM	34 (12.4)
6:00 PM to before midnight	129 (46.9)
Not specified	8 (2.9)
Geographic origin of patients	
Rural	203 (73.8)
Urban	69 (25.1)
Not specified	3 (1.1)
Clinical signs	
Pain (n = 217)	185 (85.3)
Burning sensation (n = 192)	93 (48.4)
Sweating (n = 256)	30 (11.7)
Shivering (n = 258)	11 (4.3)
Hyperthermia (n = 249)	8 (3.2)
Clinical grade	
Grade I	247 (90)
Grade II	28 (10)

( $17.82 \pm 1.9$  ng/ml) ( $F = 7.30$ ,  $P = 0.007$ ). These results indicate that the clinical severity of envenomations by *A. mauretanicus mauretanicus* and *B. occitanus* is closely related to the concentration of venom in serum of the patient.

#### Efficacy of antivenom in neutralizing venom antigens.

The kinetics of venom concentrations in serum during the hospitalization of grade I patients who were treated compared with those not treated with antivenom (but treated with other drugs) are shown in Figure 2. Grade I patients were divided into 3 groups: those not treated with antivenom, those treated with 2–5 ml of antivenom, and those treated with 10 ml of antivenom. At admission, the venom concentration was not significantly different ( $F = 0.96$ ,  $P = 0.38$ ) between the group not treated with antivenom ( $18.72 \pm 24.28$  ng/ml), the group who received 2–5 ml of antivenom ( $12.10 \pm 15.70$  ng/ml), and the group who received 10 ml of antivenom ( $19.35 \pm 36.07$  ng/ml). Thus, all patients showed higher levels of venom antigens at the hospital admission. The venom concentrations were not modified either 1 or 3 hr later in patients not treated with antivenom. However, 1 hr after administration of antivenom, venom concen-

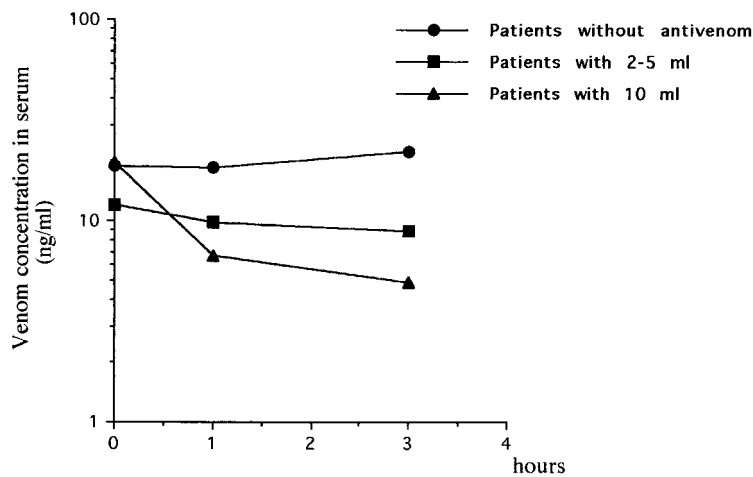


FIGURE 2. Time course of serum venom levels in grade I (mild envenoming) patients. Semi-logarithmic plots of serum venom concentrations over time are shown. Serum samples from grade I patients were collected at 3 time intervals: upon admission to the hospital (time = 0) and 1 and 3 hr later, as described in the Materials and Methods. The mean serum venom concentration was measured in patients treated with 10 ml of antivenom ( $n = 113$ ), in patients treated with 2–5 ml ( $n = 42$ ), and in patients not treated with antivenom ( $n = 76$ ).

trations decreased significantly in patients who received antivenom. When compared with the group not receiving antivenom ( $F = 9.45$ ,  $P = 0.0001$ ), the decrease in venom concentration was greater in patients treated with 10 ml of antivenom ( $6.64 \pm 11.12$  ng/ml) than in patients receiving 2–5 ml ( $9.86 \pm 14.86$  ng/ml). Three hours after serotherapy, the venom concentration was  $4.98 \pm 15.25$  ng/ml in the group who received 10 ml of antivenom and  $8.81 \pm 15.63$  ng/ml in the group who received 2–5 ml of antivenom compared with  $22.20 \pm 27.04$  ng/ml in the group not receiving antivenom ( $F = 8.49$ ,  $P = 0.004$ ). The kinetics of venom concentrations in serum of grade II patients treated or not treated with antivenom was not determined because of the small number of grade II patients and because the number of patients decreased over time.

**Effectiveness of antivenom according to the sting-admission interval.** Table 2 shows that the patients who arrived at the hospital no more than 1 hr after being stung and did not receive antivenom had a 2.67 times greater risk of developing signs of severe envenoming signs than patients who received antivenom ( $OR = 2.67$ ,  $P < 0.005$ ). When the delay was more than 1 hr, the risk was 3.67 times higher ( $OR = 3.67$ ,  $P < 0.005$ ). These observations indicate that antivenom therapy is more efficient when given as soon as possible after the envenomation.

**Effect of antivenom on clinical signs.** The evolution of

clinical features after antivenom treatment is shown in Figure 3. Local pain and a burning sensation decreased significantly within 3 hr in patients treated with antivenom and were lowest at 3 hr. General symptoms (sweating, shivering, and hyperthermia) also decreased after antivenom therapy with time. Patients not treated with antivenom and hospitalized no more than 4 hr had a 2.7 times greater risk of developing severe envenoming than those treated with antivenom ( $OR = 2.7$ ,  $P < 0.001$ ).

**Comparison between the effect of antivenom and symptomatic treatment on the evolution of clinical signs at the end of hospitalization.** At the end of their hospitalization, most (62.8%) of the patients not treated with antivenom still showed symptoms of envenoming. The absence of administration of antivenom increased the risk of developing signs of envenomation at the end of the stay in the hospital ( $OR = 2.2$ ,  $P < 0.05$ ).

**Factors influencing the evolution of clinical signs at the end of hospitalization.** Table 3 shows that the geographic origin of patients and the clinical grade at hospital admission were prognosis factors that influenced the evolution of clinical signs of envenomation. The antivenom dose was significantly associated with a decrease in morbidity.

## DISCUSSION

The serotherapy approach for scorpion envenoming is generally based on clinical observations.<sup>14</sup> Other than the work of de Rezende and others<sup>26</sup> in patients envenomed by the scorpion *T. serrulatus*, no quantitative clinical studies have been carried out to determine the role of antivenom in neutralization of venom in patients stung by scorpions. We report the first clinical study in Morocco designed to assess venom kinetics in patients envenomed by *A. mauretanicus mauretanicus* and *B. occitanus* and to study the evolution of venom concentration and clinical signs after serotherapy. In this context, we developed a noncompetitive ELISA to quantify toxic antigens from Moroccan scorpion venom in the serum of patients stung by scorpions.

TABLE 2

Effectiveness of antivenom according to the sting admission interval

	% of patients with symptoms	% of patients without symptoms	OR* $P < 0.005$
Sting-admission interval $\leq 1$ hr			
With antivenom	27	73	2.67 (1.36–5.25)
Without antivenom	50	50	
Sting-admission interval $> 1$ hr			
With antivenom	24	76	3.67 (1.55–8.66)
Without antivenom	54	46	

\* OR = odds ratio. Values in parenthesis are 95% confidence intervals.

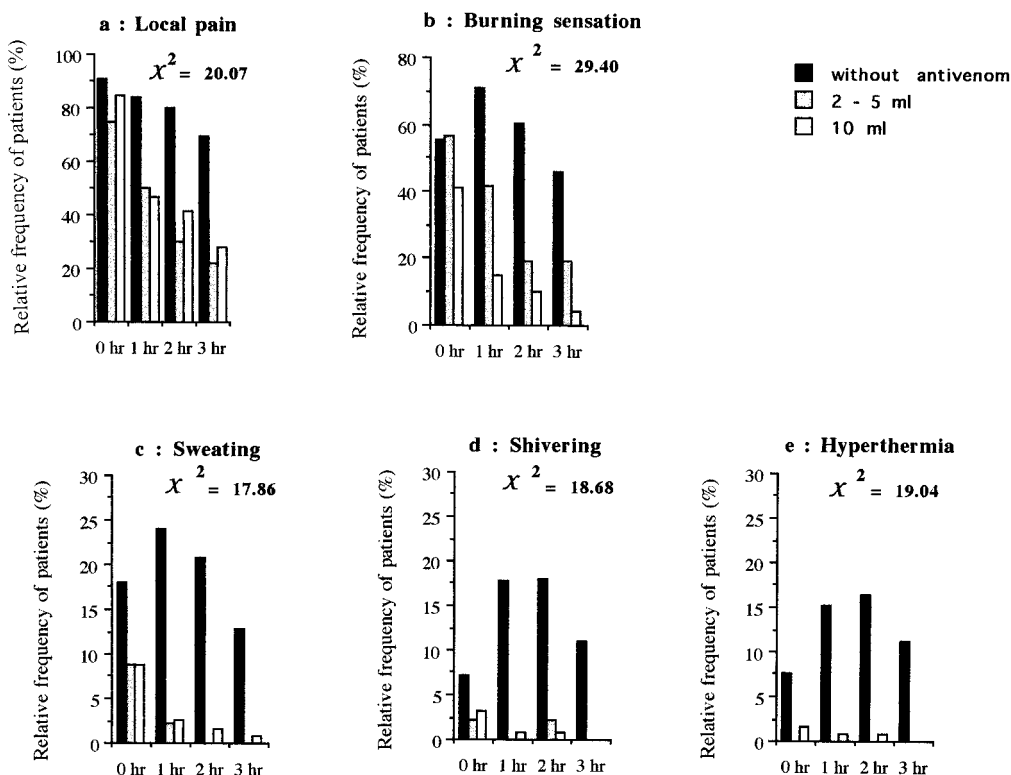


FIGURE 3. Effect of antivenom on the evolution of clinical signs. The relative frequency of patients for each clinical sign over time (upon admission to the hospital and 1, 2, and 3 hr later) is shown. The number of patients in each treatment group is local pain ( $n = 217$ ), burning sensation ( $n = 192$ ), sweating ( $n = 256$ ), shivering ( $n = 258$ ), and hyperthermia ( $n = 249$ ). The statistical significance of each treatment group is  $P < 0.001$ .

The ELISA we developed was simple, reproducible, and very sensitive. This assay showed that antivenom produced against *A. mauretanicus mauretanicus* specifically recognizes the venom of this scorpion and cross-reacts with the venom of another scorpion (*B. occitanus*). This result confirms previous findings that showed that the antivenom produced by Institute Pasteur of Morocco is able to neutralize almost all venoms of dangerous north African scorpions.<sup>27</sup>

To examine the relationship between the severity of scorpion envenoming and serum venom levels, we measured the concentration of venom in serum samples of patients upon hospital admission. The results showed a correlation between the severity of envenoming and serum venom levels. In envenomed patients classified as grade II, venom concentrations were higher than in envenomed patients classified as grade I. Our results confirmed those of others reported.<sup>4,21,28</sup> Indeed, it has been shown that Brazilian patients with systemic manifestations after *T. serrulatus* envenoming had significantly higher plasma venom concentrations than patients with only local pain at the site of the sting.<sup>21,28</sup> In envenomed Tunisian patients, a correlation between clinical symptoms of envenoming and the level of scorpion venom antigens in serum was reported.<sup>4</sup> Therefore, the quantification of circulating toxin concentrations should be of clinical interest in assessing the severity of envenomation and, consequently, improving the management of patients stung by scorpions.

In this study, in which an antivenom composed of  $F(ab')_2$  fragments was used, we did not observe any allergic reactions or anaphylactic shock after administration of antivenom.

It has been reported that acute allergic reactions together with anaphylactic shock may be caused by crude antivenom preparations.<sup>29</sup> The reduction of adverse reactions due to antivenom has been shown to be due to deletion of the Fc part of the antivenom immunoglobulin.<sup>30</sup>

This study showed that antivenom was efficient in reducing the levels of circulating venom antigens and decreasing clinical symptoms. Similarly, the efficacy of antivenom in neutralizing circulating venom and systemic symptoms was reported in patients stung by *T. serrulatus*.<sup>26,31</sup>

The efficacy of serotherapy is probably dose-dependent. Indeed, the decrease in the circulating venom concentration was significant after treatment with 10 ml of antivenom. Doses less than 10 ml were insufficient for producing good clinical improvement. Similar results were reported in rabbits; these showed that low doses of antivenom could not completely negate the electrocardiographic effects caused by the venom.<sup>9</sup> Thus, administration of high doses of antivenom was recommended in some countries.<sup>15,16</sup> For example, the recommended dose in Brazil is 20 ml.<sup>16</sup> In Saudi Arabia, higher doses (5–20 ml compared with 0.5–1 ml) reduced the mortality rate from an 4–6.8% to less than 0.05%.<sup>3,7,15</sup> Thus, the ineffectiveness of serotherapy reported by some researchers<sup>18,19,32</sup> could be partially explained by the use of low potency and insufficient doses of antivenom. The use of high potency and adequate doses of antivenom is an important factor to be considered.

The clinical grade of the patients at hospital admission was influenced by the sting-admission delay, which was sig-

TABLE 3

Factors influencing the evolution of clinical signs at the end of hospitalization

	% of patients with symptoms	% of patients without symptoms	Odds ratio	P
Geographic origin of patient				
Urban	50.8	49.2	8.80	0.003
Rural	30.5	69.5		
Scorpion*				
A.m.m.	36.4	63.6	0.62	0.73
B.o.	29.7	70.3		
Sex				
Female	39.8	60.2	2.20	0.13
Male	31.1	68.9		
Age				
Children	38.4	61.6	0.36	0.54
Adults	34.4	65.6		
Sting site				
Upper pelvis	28.2	71.8	8.00	0.004
Lower pelvis	45	55		
Clinical grade at admission				
Grade I	30.5	69.5	25.84	0.0000
Grade II	80.8	19.2		
Sting admission interval				
≤1 hr	35.8	64.2	0.02	0.88
>1 hr	35	65		
Antivenom dose				
10 ml	20.5	79.5	23.29	0.0001
2–5 ml	40.4	59.6		
No antivenom	51.6	48.4		

\* A.m.m. = *Androctonus mauretanicus mauretanicus*; B.o. = *Buthus occitanus*.

nificantly associated with the geographic origin of the patient. When the time elapsed between sting and arrival at the hospital increased, the risk of being classified as a severe grade(s) also increases. Thus, the elapsed time is a prognosis factor. The risk of developing signs of envenoming at the end of hospitalization is high when patients arrived later at the hospital. This risk exists also in patients treated with serotherapy. Therefore, the effectiveness of serotherapy depends not only on the antivenom dose, but also on the time elapsed between scorpion sting and administration of antivenom. This is consistent with results reported in Saudi Arabian patients.<sup>3</sup> Similar results were reported in mice by Revelo and others.<sup>33</sup> These investigators showed that serotherapy is particularly effective when antivenom is administered immediately after venom injection, and that the serotherapy is less effective when administered hours after envenomation. However, antivenom must be administered despite the long delay between the sting and administration of serotherapy. Despite the decrease in the curative effect of antivenom when the sting-serotherapy delay increases, its preventive effect must still be considered in the treatment of envenomed patients. Indeed, free  $F(ab')_2$  not complexed to venom removes toxins fixed on their receptors and prevents their binding.<sup>34</sup> In this context, it has been suggested to use a large quantity of antivenom when patients arrive later at a hospital after being stung.<sup>2</sup>

In conclusion, this study shows that serotherapy is the most important therapeutic measure to use after scorpion en-

venoming, particularly in rural areas, where the means to treat victims in intensive care units are absent. Neutralization of venom by antivenom is intimately dependent on both dose and time of administration of  $F(ab')_2$ .

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