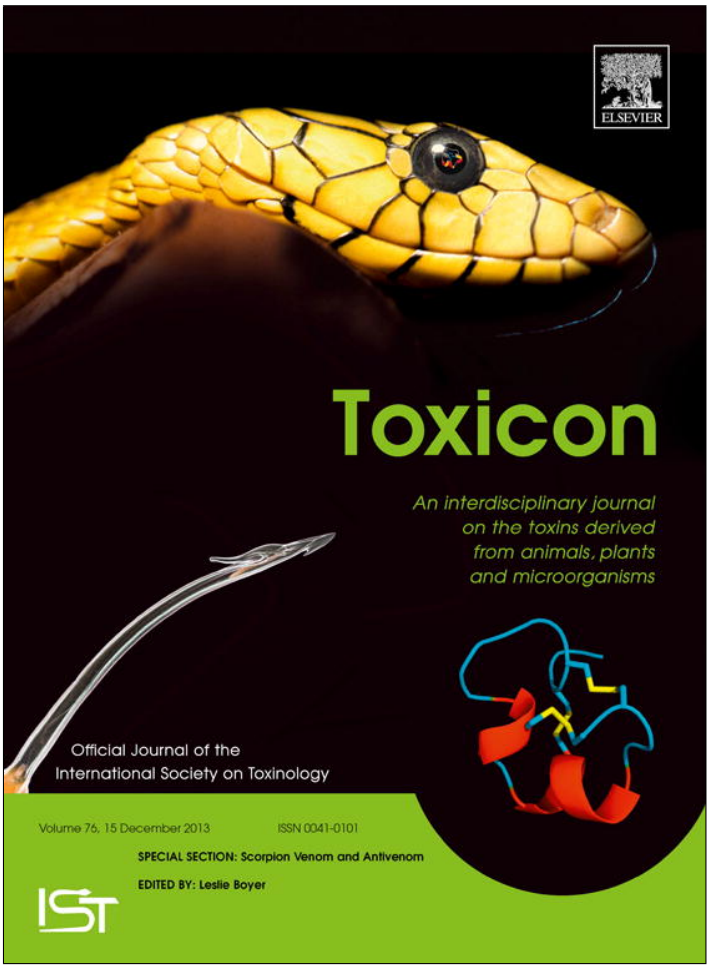

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Hemostasis dynamics during coagulopathy resulting from *Echis* envenomation

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ABSTRACT

This work provides a graphic description of the time course of hemostasis tests results during spontaneous evolution of *Echis* envenoming and correction of hemostasis disorders with antivenom therapy.

The dynamics of fibrinogenemia (g L^{-1}), prothrombin time (PT, %), activated partial thromboplastin time (aPTT, patient/normal ratio) and platelet count (Giga L^{-1}) were collected from coagulopathic envenomed patients of a 12 years prospective study in Africa. Sixty patients were included. 47 of them (78%) received an antivenom (33 ± 12 ml) and 13 did not. Thirty patients (50%) presented bleeding. Only one patient died. The time for fibrinogen to be more than 1 g L^{-1} was 181 ± 116 h (7.5 days) in the spontaneous evolution group versus 40 ± 21 h in the antivenom group ($p < 0.0001$). The times for reaching a PT above 50% were 140 ± 64 min (5.8 days) versus 25 ± 15 h ($p < 0.00001$) and for reaching an aPTT less than 1.5 times the normal values, 116 ± 76 h (4.7 days) versus 10 ± 9 h respectively ($p < 0.0002$). Thrombopenia was not a common feature of *Echis* envenomation.

This study is the first one to provide a chart of the evolution of the hemostatic tests during envenomation caused by *Echis* bites. The plots enable to estimate that, in *Echis* envenomation, in the absence of antivenom administration, hemostasis remains severely affected until the 8–10th day of evolution. On the contrary, efficient antivenom against African vipers corrects clotting functions within a few hours.

1. Introduction

Echis is a genus of venomous vipers found in the dry regions of Africa, the Middle East, Pakistan, India and Sri Lanka. The *Echis* species are among the most dangerous snakes in the world. In sub-Saharan Africa, they attribute for more than 20,000 deaths every year, since they are widespread and live in areas lacking modern medical facilities (Chippaux, 2011). *Echis* bites produce a venom-induced coagulopathy (VICC) (Isbister, 2010) resulting in a dramatic fall in fibrinogen concentration.

The only efficient treatment for these hemorrhagic disorders is antivenom (Mion and Larréché, 2009). Antivenom may be unavailable in some developing countries, mainly because of elevated cost. Other limitations include decreased efficacy in some instances (Isbister et al., 2009). In addition, specific antivenom does not exist for some snake species. For these reasons, alternative treatments,

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such as plasmapheresis (Valenta et al., 2011.) or clotting factors (Brown et al., 2009) are still being investigated. In previous studies of these putative treatments, the effectiveness was assessed based on hemostasis evolution. Biological tests results describing the dynamics of hemostasis after envenoming are surprisingly scarce. A recent study precisely reporting the evolution of hemostatic factors during VICC resulting from Australian elapid envenomation (Isbister et al., 2010) resulted in features that vary significantly from viperine envenomation. Concerning *Echis* envenomation, only a few case studies have intermittently provided individual progressions of hemostatic parameters (Mion and Larréché, 2009; Christy et al., 1973; Reid, 1977). For obvious ethical reasons, antivenom trials never include a placebo group, therefore, the natural evolution of envenoming is difficult to grasp.

We undertook this study to provide a realistic description of the time factors for hemostasis disorders during both spontaneous evolutions of *Echis* envenoming and correction of hemostasis with antivenom therapy.

2. Material and methods

2.1. Data

The same investigator (SL) reviewed all statistics from the snakebite database of the French military hospital in Djibouti examining cases of spontaneous (or natural) evolution of viperine syndrome and in cases of antivenom administration. The French Military Health Service ethical commission reviewed and approved the protocol of this prospective study (1994–2006). GM, AB, FP and MP (respectively) recruited all patients for the cohort from 1994 to 2006. The figures used in this analysis were extracted by means of a standardized data collection grid for demographic (age and sex), clinical (bitten area, edema, bleeding features and clinical evolution), and biological tests (fibrinogenemia, prothrombin time, activated partial thromboplastin time and platelet count measured at predefined times). Edema was quantified as mild (local), moderate (regional) or severe (extensive).

2.2. Inclusion criteria

Only coagulopathic patients were included in the study. Coagulopathy was defined as fibrinogen <1 g L^{-1} and at least one of the following laboratory test results: prothrombin time (PT) $\leq$50%, activated partial thromboplastin time (aPTT) $\geq$1.5 times the normal values, or platelets $<$80 Giga L^{-1}. When aPTT was non-recordable (incoagulable blood), its value was arbitrarily fixed at three times the normal values for graphic representation.

2.3. Laboratory tests

Fibrinogenemia, PT, aPTT and platelet counts were measured in all patients admitted in the Intensive Care Unit for a snakebite. All the laboratory tests were performed at the hospital laboratory within one hour of blood sampling.

Fibrinogen, PT and aPTT measures were performed on START 4 semi-automated coagulation analyzer (Diagnostica Stago, France) as of 1998. Prior to this, the measures were performed on a KC4 semi-automated analyzer (Amelung, Germany) using Dade reagent (Dade Behring, The Netherlands).

The platelet count (G L^{-1}) was measured by commercially available kits on Pentra DF 120 automated analyzer (Horiba ABX, France) as of 1997. Afterward that time, it was performed by a manual visual method by Unopette (Becton Dickinson Vacutainer Systems, NJ). A study in our laboratory checked the correlation between the manual method and the automated method (unpublished data).

2.4. Antivenom administration

Clinical features consistent with *Echis* envenomation (edema and bleeding but no or limited necrosis) were described in a previous paper (Larréché et al., 2011). The snake, sometimes killed or captured, enabled the identification of *Echis pyramidum* (Fig. 1). This viper habitat expands from Algeria to Kenya and it was formerly misclassified as an *Echis carinatus* sub-species (Casewell et al., 2010).

The majority of patients admitted from 1994 to august 2001 were treated with *Echis-Bitis-Naja*® antivenom (20 ml vial, Pasteur-Mérieux, Paris, France), delivered within 20–30 min through an intravenous infusion. At the onset of the study, some patients did not receive any antivenom, because the treating physician believed it was not necessary or, in some rare occasions, it was temporarily out of stock.

From September 2001 on, all envenomed patients, with one exception, received two vials of the FAV-Afrique® antivenom (10 ml vials, Sanofi-Pasteur, Lyon, France), a more recent polyvalent antivenom efficient against *Echis*, *Bitis*, *Naja* and *Dendroaspis* venoms.

Whatever the antivenom, the evolution of bleeding and biology was checked every four hours during the first 12 h after being admitted. If bleeding was still going on or if hemostatic results were not getting better, additional vials were injected at H4, H8 or H12.

2.5. Graphic plots and statistical methods

Plots constructed from parameters values versus time, were expressed in days. Time of serial sampling varied

Fig. 1. *Echis pyramidum*, with the kind authorization of inf-faune (http://www.inf-faune.net/).

slightly from 1994 to 2006, but usually occurred at Hour 0 (Day 0 or D0), H4, H6, H8, H12, H16, H24 (D1) and then at D2, D3, etc. until patient's hospital discharge. H0 was the time of admission into the Intensive Care Unit. Uncertainty concerning exact time of sampling was less than 2 h from H0 to H12, 4 h from H16 to H24, and 8 h for subsequent samplings.

All patients who received antivenom therapy constituted the "antivenom group"; and all patients who did not, constituted the "spontaneous evolution group". All numeric parameters were plotted versus time (days) as mean ± 95% confidence interval for the mean (CI_{95}). Instead of a single estimate of the mean, 95% of these interval estimates, computed from samples of a given size and from Standard Error of the mean (SE), would contain the true mean. CI_{95} is a measure of precision and is itself a statistical test: if the CI_{95} of the two groups do not overlap, this means that the parameters significantly differ with a p value ≤0.05.

$$CI_{95} = m \pm 1.96\ SE = m \pm 1.96SD/\sqrt{N}$$
$$for\ N > 30 \quad (SD : Standard\ Deviation)$$

for $N < 30$, the coefficient is higher than 1.96 and read in the table of Student for $N - 1$ degrees of freedom for alpha = 0.05. The lines of best fit for the different points of the curves were plotted with the least squares method, as a polynomial function provided by the Excel™ software (Microsoft, Redmond, WA).

Continuous data presented as mean ± SD. Comparisons between the two groups used the Mann and Whitney U test for continuous variables and the CHI square test for proportions, provided by StatEL™ software for Excel™ (ad Science, Paris, France).

3. Results

Sixty envenomed patients, mostly males (66%), were included (Table 1). The average age was 28 ± 16 years (2–60 years). Among the 60 patients, 47 (78%) received an antivenom (33 ± 12 ml, extremes: 20–80 ml) and 13 did not (22%).

In some patients, antivenom administration was repeated; additional vials were injected at H4 (15 patients), H4 and H8 (11 patients) or H4, H8 and H12 (4 patients). No adverse reactions were noted.

Thirty patients (50%) presented bleeding, some with several forms of bleeding. Hemorrhagic signs were macroscopic hematuria (9 cases), hemoptysis (8 cases), bleeding gums (8 cases), local bleedings (7 cases), digestive bleedings (2 cases) and hemothorax (one case). Only one death was recorded, in a 4 years old child, who was admitted with a hemorrhagic shock and deceased upon arrival at the hospital.

There were 85 blood samples in total for spontaneous evolution group patients (6.9 ± 3.7 samples per patient) and 233 blood samples for antivenom group patients (4.7 ± 1.3 samples per patient), depending of the duration of patient's hospital stay.

3.1. Time course of fibrinogen

In the spontaneous evolution group, afibrinogenemia occurred in 12 out of the 13 patients versus 45 patients out of 47 in the antivenom group ($p = 0.53$). Fig. 2 presents the individual evolutions of fibrinogen (spaghetti plots) in the 13 patients of the spontaneous evolution group, who did not receive antivenom. It shows that, although evolution is not homogenous in all patients, fibrinogen remained less than 1 g L^{-1} from D1 to D7 after the bite. Fig. 3 presents the time course of fibrinogen in the two groups of patients. The graphic and statistical differences become obvious as of D1. The time to reach a fibrinogen above 1 g L^{-1} was 181 ± 116 h (7.5 days) in the spontaneous evolution group versus 40 ± 21 h in the antivenom group ($p < 0.0001$).

Table 1
Comparison between the spontaneous evolution and the antivenom group.

	Spontaneous evolution	Antivenom	All
N (%)	13 (22%)	47 (78%)	60 (100%)
Delay of hospitalization (hours)	56 ± 58	28 ± 27	34 ± 36
Age (years)	31 ± 14	28 ± 16	28 ± 16
Children <14	2 (15%)	9 (19%)	11 (18%)
Sex ratio (M/F)	12/1	36/11	48/12
Bitten area			
Lower limb	6 (46%)	29 (62%)	35 (58%)
Upper limb	7 (54%)	17 (36%)	24 (40%)
Face	0	1 (2%)	1 (2%)
Edema	13 (100%)	40 (85%)	53 (88%)
Mild	3	4	
Moderate	4	21	
Severe	6	15	
Bleeding	4 (31%)	26 (55%)	30 (50%)
Transfusion (PRBC)[a]	1	5	6
Evolution			
Hospital stay (days)	8 ± 4 (1–14)	4 ± 2 (2–43)**	
Death	1	0	

**$p < 0.002$ (spontaneous evolution vs antivenom).
[a] PRBC: Packed red blood cells.

3.2. Time course of PT

Fig. 4 presents the time course of PT. The statistical difference arises by the 24th hour. In the spontaneous evolution group, the time to reach a PT above 50% was 140 ± 64 min (5.8 days) versus only 25 ± 15 h in the treated group ($p < 0.00001$).

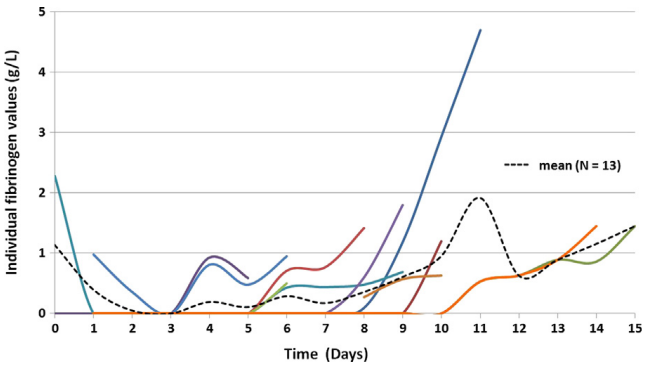

Fig. 2. Individual evolution of fibrinogen (g L^{-1}) in the 13 envenomed patients of the spontaneous evolution group (spaghetti plots) who did not receive antivenom. J0 is here the time of the bite. Evolution is very variable, but fibrinogen remains under 1 g L^{-1} in all patients from D1 to D7.

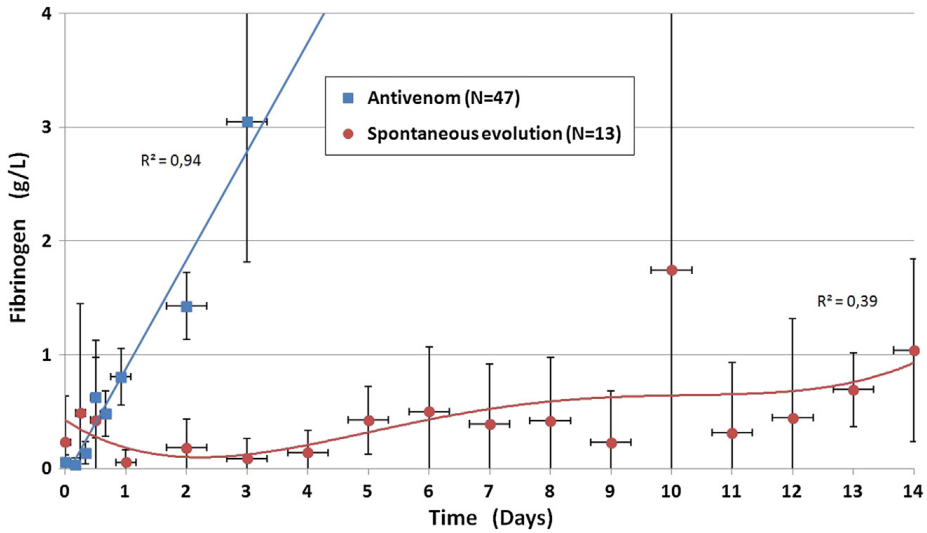

Fig. 3. Time course of fibrinogen in the two groups of patients. The graphic and statistical differences become obvious as of D1. In the spontaneous evolution group, fibrinogen exceeds 1 g L^{-1} only as of D10. On the contrary, patients of the antivenom group had a fibrinogen above 1 g L^{-1} at around the end of D1. The CI_{95} at D10 is large because there were only two patients left. There was only one patient for the four subsequent measures. For the first sampling times, some CI_{95} are nil, because the occurrence of afibrinogenemia in all patients.

3.3. Time course of aPTT

Fig. 5 shows aPTT. Despite large IC_{95}, the difference was significant as soon as D1. On the average, aPTT remained above 1.5 times the normal values during 113 ± 76 h (4.7 days) without antivenom, versus 10 ± 9 h in the treated group ($p < 0.0002$).

3.4. Time course of platelets

Fig. 6 shows that thrombocytopenia is not a common feature of *Echis* envenomation. There were no differences between the groups.

4. Discussion

Our study shows the evolution of the most clinically important hemostatic tests during the envenomation provoked by *Echis* bites in West Africa. Viper venom is a complex mixture of glycoproteins, including many enzymes interfering with primary hemostasis and hemostatic cascade (Larréché et al., 2008). Metalloproteinases, the so-called hemorrhagins, induce endothelial lesions responsible for edema and mucosal bleedings. Proteins impairing primary hemostasis, particularly disintegrins, can inhibit or activate platelets which infrequently results in thrombocytopenia. Proteins interfering with coagulation are a mixture of procoagulant proteases (prothrombin activators like ecarin or carinactivase in *Echis* venom, thrombin-like enzymes, and factor X or factor V activators) and anticoagulant proteases (factor IX and X inhibitors, protein C activator, anticoagulant phospholipases A2). The venom may sometimes contain fibrinolytic enzymes and plasminogen activators. VICC definitely differs from disseminated intravascular coagulation (DIC). It is characterized by a potentially lethal hemorrhagic syndrome rather than

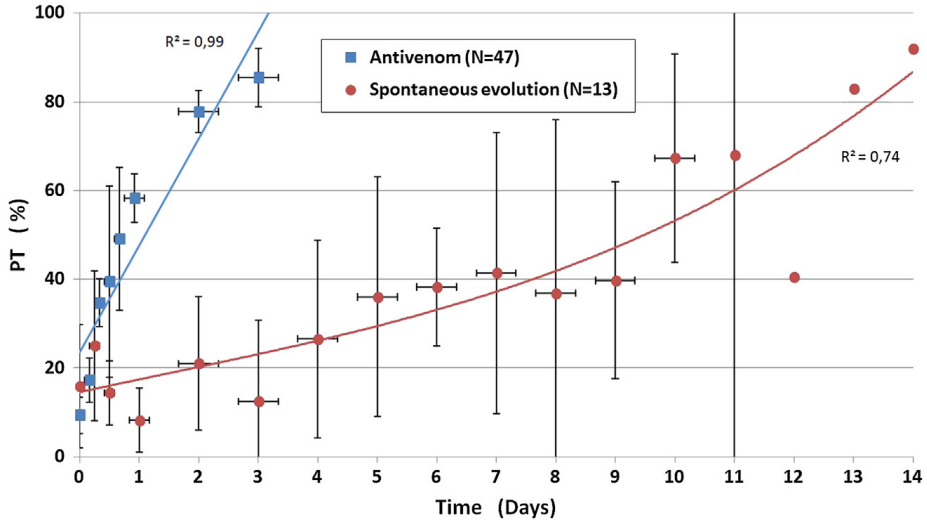

Fig. 4. Time course of PT. The statistical difference arises here by the 24th hour. Same remarks as for Fig. 3.

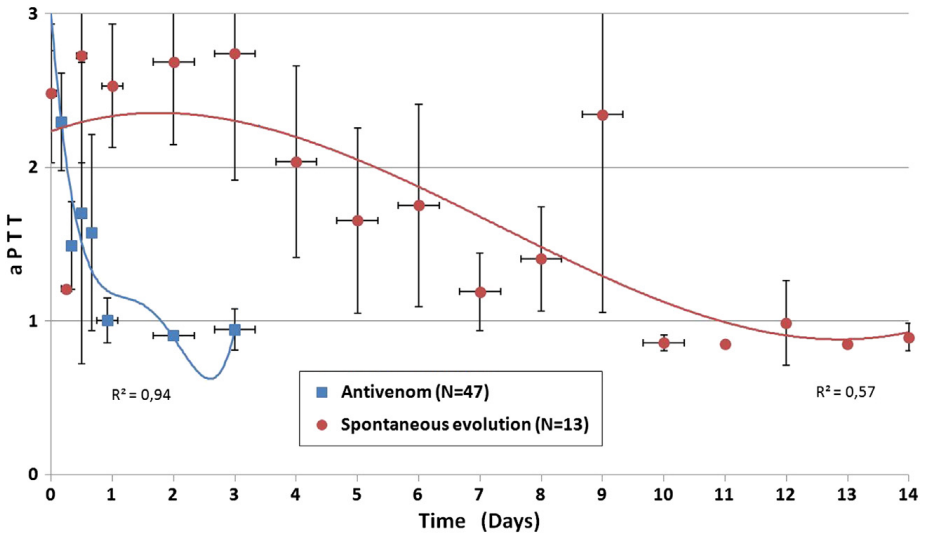

Fig. 5. Time course of aPTT. Despite large IC_{95}, the difference is significant as early as day 1. Again, it takes more than one week in the spontaneous evolution group to observe an aPTT less than 1.5 times the normal values. Same remarks as for Fig. 3.

fibrin deposition (Isbister, 2010). Fibrinogen consumption results from the direct and abnormal degradation of fibrinogen by thrombin-like enzymes or by meizothrombin formed under the action of prothrombin activators. Fig. 6 confirms that platelets are rarely implicated in the hemostatic disorders. Platelet abnormalities are more likely initiated by disintegrins (like echistatin) and other related molecules, than consumption.

Despite the medical importance of *Echis*, most published studies are of epidemiological nature or antivenom trials reporting the evolution of clinical bleeding (Manent et al., 1992). Because of common paucity in laboratory tests in developing countries, clotting disorders have often been explored by means of a whole blood coagulation test (Chippaux et al., 2007). This is clinically useful but cannot acutely analyze hemostasis, because it is of binary nature.

In our previous investigation, envenomation occurred in 87% of the bitten patients, 93% of which exhibited a coagulopathy (Larréché et al., 2011). In the present series, the proportion of treated patients does not differ from that observed in developed countries (Isbister et al., 2009). Similarly as in other studies, VICC developed early, usually between the second and the12th hour after the bite (Ireland et al., 2010).

All the curves in the two groups statistically and visually diverge before the 24th hour. The plots show that during *Echis* envenomation, in the absence of antivenom administration, hemostasis remains severely affected until the 8–10th day. As early as 1977, studies indicated that clotting defects showed a mean duration of 10 days. In Reid's unusual observation (himself having been bitten by an *Echis*), clotting defects persisted for as long as 20 days (Reid, 1977). In contrast, efficient antivenom was able to correct clotting defects within hours; and in most patients, the beginning of recovery starts within two hours (Chippaux et al., 2007).

Antivenom effectiveness in *Echis* envenomation contradicts with the Australian findings. According to one study group, antivenom must be administrated within one

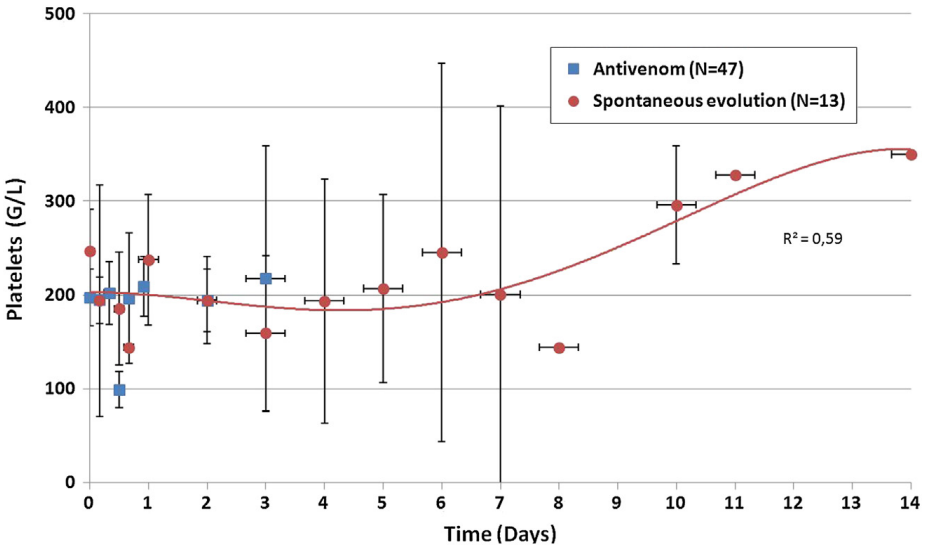

Fig. 6. Time course of platelet counts. Thrombopenia is not a common feature of *Echis* envenomation. There are no differences between the two groups.

hour after an Australian elapid bite in order to significantly impact recovery time (Isbister et al., 2009). We previously demonstrated, however, that *Echis* antivenom corrects hemostatic disturbances even in case of late administration (e.g. several days after the bite) (Larréché et al., 2011).

Variable degrees of biological alterations and normalization time can be attributed to variability in venom composition and inoculated quantity. There are often discrepancies between laboratory and clinical parameters (Ireland et al., 2010). In a study carried out in Cameroun, 30% of snakebites involved hemostasis disorders without clinical signs (Singletary et al., 2005). In our series, we frequently observed that bleeding ceased before hemostatic parameters were restored. This attributes that immunoglobulin fragments had neutralized venom compounds that were not quantified by routine laboratory tests, such as hemorragins or disintegrins.

For a peculiar patient, our data might help to discuss the efficacy of an alternative treatment or the possibility of a simple spontaneous evolution. For example, in light with our charts, the interesting case of an *E. pyramidum* bite reported by Valenta et al., in 2011 could have been differently discussed (Valenta et al., 2011). An initial antivenom (elaborated from Asian *E. carinatus* venoms) was clearly ineffective. While a second antivenom administered on day 7 (elaborated from *E. leucogaster* venoms) was considered effective by the authors. Fibrinogen began to rise on the 5th day. The recovery of clotting disorders may thus have been the result of the natural evolution. For the same reason, we should not agree with the authors, who stated that plasmapheresis carried out on day 4, was efficient.

The present study has some limitations. Hemostasis parameters were not measured on a single analyzer throughout the study period, meaning there may have been some variations in the results. Both coagulation analyzers used a chronometric technique and the small patient variations indicate no clinical significance between them. SD, SE and CI_{95} were rather important at some sampling times and give an uncertainty for the real location of the curves, especially for spontaneous evolution group ($N = 13$) and aPTT. Indeed, patients who do not need antivenom are scarce. Among the 13 patients of the spontaneous evolution group, four were bleeding and should have received antivenom, but we explained the reasons why they were not treated. As one can observe in Fig. 2, the variability of curves also reflects the true variability of envenomation, depending on many factors, like the size of the snake, and the injected amount of venom, which may largely vary. Variations of the same amplitude were also observed in the large series of Isbister et al. (2010). Another fact could mitigate the comparability of the two groups: patients of the spontaneous evolution group had been bitten 56 ± 58 h before admission at ICU, versus 28 ± 27 h for patients of the antivenom group. This modest difference (compared to the 8–10 days of spontaneous evolution) was neither clinically nor statistically significant.

In fact, these limitations do not impede a clinically useful interpretation of the best–fit curves, which deviate early in the evolution. IC_{95} has the interest of taking into account the variation of the number of included patients at each sampling time and provides a “zone” of probability around the least square curves. Another uncertainty arises from the fact that different practitioners supervised the cohort, thus introducing a bias regarding the exact hours of the sampling times. We considered this by providing a horizontal confidence interval. Finally, Bouffard hospital’s laboratory was not able to provide a quantitative measurement of D-dimers, which are an interesting part of the VICC process (Isbister et al., 2010).

5. Conclusion

This is the first large study of timed hemostatic tests during *Echis* envenomation. *Echis* bites induce a prolonged and severe hemostatic disorder, with a non-clotting blood lasting more than one week. With antivenom, we observed a constant and rapid improvement with a significant and obvious graphically clear-cut between treated and non-treated patients. The curves we provide may help as comparisons for other research, to decide whether a treatment is effective or if the observed evolution of hemostatic parameters can attribute to the spontaneous recovery of envenoming.

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Conflict of interest

None.

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