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**A randomised controlled trial of fresh frozen plasma for treating
venom induced consumption coagulopathy in Australian snakebite
(ASP-18)**

Short Title: Fresh frozen plasma for snakebite coagulopathy.

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Abstract

Background: Venom induced consumption coagulopathy (VICC) is a major effect of snake envenoming.

Objectives: To investigate whether fresh frozen plasma (FFP) given post-antivenom resulted in more rapid correction of coagulation.

Patients/Methods: This was a multicentre open-label randomised controlled trial in patients with VICC of FFP versus no FFP within four hours of antivenom. Patients (>2yr) recruited to the Australian snakebite project with VICC[INR>3] were eligible. Patients were allocated in 2:1 randomisation to receive FFP or no FFP. The primary outcome was the proportion with an INR<2, six hours post-antivenom. Secondary outcomes included time from antivenom to discharge, adverse effects, major haemorrhage and death.

Results: From 70 eligible patients, 65 consented to be randomised: 41 to FFP, 24 to no FFP. Six hours post-antivenom more patients randomised to FFP had an INR<2 [30/41(73%) vs. 6/24(25%); absolute difference 48%;95%CI:23-73%;p=0.0002]. The median time from antivenom to discharge was similar [(34hr,Range:14-230h) vs. (39hr,Range:14-321h);p=0.44]. Seven patients developed systemic hypersensitivity reactions post-antivenom – two mild and one severe (FFP arm) and three mild and one severe(no FFP). One serious adverse event (intracranial haemorrhage and death) occurred in a FFP patient with pre-existing hypertension, **who was** hypertensive on admission, and developed a headache 6 hours after FFP. Post-hoc analysis found median time from bite to FFP was significantly shorter for non-responders compared to responders [(4.7h,IQR:4.2-6.7h) vs (7.3h,IQR:6.1-8h);p=0.002].

Conclusions: FFP administration post-antivenom results in more rapid restoration of clotting function in most patients, but no decrease in discharge time. Early FFP (<6-8hr) post-bite is less likely to be effective.

Keywords: Snake bites, antivenom, plasma, consumption coagulopathy

Introduction

Snake envenoming is responsible for **at least** 20,000 deaths **annually**, making it one of the world's most neglected tropical diseases according to the World Health Organisation.[1] There is an excess of 440,000 snake envenoming cases annually, with the main burden in tropical and subtropical regions of Africa, Asia, Latin America and Oceania.[1, 2] Venom induced consumption coagulopathy (VICC) is a major clinical envenoming syndrome that can result in major haemorrhage and death.[2, 3] Antivenom is the main treatment for VICC, but although it appears to neutralise procoagulant toxins, clotting factor re-synthesis and full recovery of clotting function takes 24 to 48 hours.[3] Therefore, envenomed patients remain at risk of major haemorrhagic complications such as intracranial haemorrhage for a significant time period after antivenom treatment. This has prompted the use of clotting factor replacement in VICC,[4-8] despite limited evidence for its safety and effectiveness.

VICC is the commonest manifestation of severe snake envenoming in Australia where approximately 75% of cases with VICC require antivenom.[9] A number of important snakes in Australia, including brown snakes (*Pseudonaja* spp.), tiger snakes (*Notechis* spp.) and the coastal taipan (*Oxyuranus* spp.) cause VICC due to the presence of prothrombin activators in their venoms.[3, 10, 11] These prothrombin activators activate the clotting pathway and cause a complete consumption of fibrinogen, factor V and factor VIII, resulting in unrecordable times for clotting tests (international normalised ratio [INR] and activated partial thromboplastin time [aPTT]) as well as very high D-Dimers.[3]

Factor replacement has been **suggested** for the treatment of snakebite to rapidly restore clotting factor levels and consequently reduce the risk of major haemorrhage.[6, 7] However, there is a concern that by providing clotting factors the coagulopathy will worsen since more substrate will be available for the procoagulant toxins present in the venom to activate.[12] Furthermore, there are also risks associated with the use of blood products, and there is an increasing recognition of adverse effects with fresh frozen plasma (FFP).[13] There have been only a few studies of factor replacement for VICC in humans,[4, 5, 7, 8] with two observational studies in Australia suggesting that the use of clotting factor replacement speeds the recovery of VICC.[7, 8]

We aimed to investigate whether fresh frozen plasma (FFP) administered after antivenom would lead to earlier correction of clotting function in envenomed patients with VICC.

Methods

This was a multicentre open-label randomised controlled trial in Australia of FFP versus no factor replacement within four hours of antivenom administration, for the treatment of snake envenoming in patients with VICC. The study was approved by fourteen Human Research Ethics Committees covering all hospitals involved in the study. The trial was registered with the Australian Clinical Trials Registry, ACTRN12607000620426.

Study recruitment and Patients

The Australian Snakebite Project (ASP) involves clinical, laboratory and academic investigators located in hospitals throughout Australia as well as the national Poisons Information Centre Network who prospectively recruit all snakebite presentations in Australia. Recruitment is done via a national free-call telephone number, whereby patients notified to ASP were assessed for suitability for the FFP trial and with those that met the inclusion criteria being invited to participate.

Patients (2 years or older) were eligible for inclusion if they had VICC and an INR greater than 3.0, antivenom had been administered for the appropriate snake (brown snake, tiger snake or taipan), there were no known allergies or objections to blood products, and four units of FFP were available and could be commenced within 4 hours of antivenom. Exclusion criteria were severe cardiac failure or significant renal failure where there is a risk of fluid overload. Patients were recruited from 28 hospitals from four States of Australia, between 1st March 2008 and 30th June 2012.

Treatment Protocol

Written informed consent was obtained from the patient or the patient's parent/guardian by the treating doctor or local site investigator with the assistance of the chief investigators. The patient was then randomly allocated to receive either FFP or nothing in a 2 to 1 ratio of active treatment to no treatment. The imbalance in allocation was to ensure that sufficient numbers of patients were administered FFP in case patients randomised to active treatment did not receive it within 4 hours due to logistic reasons and remote location.

The study used an adaptive biased coin randomisation schedule using an online data entry and randomisation system. Randomisation and allocation to FFP or not to receive FFP was done by the chief investigators (GI, SB, CP) or a research assistant (LC). This was done by accessing a password protected single web page where the inclusion criteria, exclusion criteria and consent were confirmed, before randomising to either "Give FFP" or "No FFP". Randomising the patient locked the system and the allocation was recorded on an online database which could not be changed. The online database was monitored by one chief investigator (NB) who was not involved in patient treatment, random allocation or data collection. If the allocation became unbalanced (i.e. > 40% or < 25% in the non-treatment arm or >75% or < 60% in the FFP arm), then the probability was biased to correct the imbalance (i.e. 0.75/0.25 instead of 0.67/0.33). This was done after every 20 patients recruited.

For all patients, antivenom was administered as per the treating clinician. For patients randomised to receive FFP the treating hospital cross matched and ordered the FFP. These patients received 10 to 15mL/kg up to a maximum of 4 units of FFP (\approx 1000 mL) over 30 to 60 minutes within 4 hours of anti-venom administration. Patients in

the no-FFP arm were not to receive FFP until after the 6 hour primary outcome unless there was evidence of major haemorrhage.

Data Collection

The randomised trial utilised the existing ASP infrastructure.[9, 14] The datasheets used for ASP already contained the information required for the study outcomes. All patients recruited to ASP have datasheets filled out by the local investigators or treating clinicians which are then faxed back to the chief investigator and entered into a purpose built relational database in Microsoft Access™. This includes information about the patient, bite, time and dose of antivenom, administration of factor replacement, time and results of laboratory tests including coagulation studies, adverse effects from antivenom or factor replacement and any complications (haemorrhage, death). Systemic hypersensitivity reactions to antivenom or FFP were defined as anaphylaxis if they met NIAID-FAAN consensus criteria, [15] and defined as severe according to the Brown grading system.[16]

Serum and citrate samples were collected from all recruited patients and processed according to the ASP protocol. Briefly, serum was centrifuged at 2000g for 10 minutes and citrated plasma was double centrifuged at 2000g for 10 minutes, before being stored at -80°C for the later measurement of venom concentrations and clotting factors, respectively. Snake venom concentrations were measured in serum using a previously described venom specific enzyme immunoassay (EIA).[17] Venom specific EIA was used for identification of the snake involved and included testing for brown snake, tiger snake, rough-scaled snake, taipan and Stephen's banded snake venoms. The citrated samples were used in clotting assays to measure the levels for factors I (fibrinogen), II (prothrombin), V and VIII, as well as for the measurement of

D-Dimer. All assays were performed on either a Behring Coagulation System or Sysmex CA-1500 analyzer (Dade Behring, Marburg Germany) using standard coagulometric or immunoturbidimetric methods as provided by the manufacturer.

Data Analysis

The primary outcome was the proportion of patients with a significant return of coagulation function defined by an INR < 2.0 , six hours after antivenom treatment was commenced. Secondary outcomes included time from antivenom to discharge, adverse effects from FFP (transfusion related acute lung injury, systemic hypersensitivity reactions), major haemorrhage as defined by the International Society on Thrombosis and Haemostasis,[18] and death prior to hospital discharge. Clotting factor levels post-antivenom were a pre-defined secondary outcome but citrate samples were only available in less than half of patients due to problems with immediate processing and freezing in some regional laboratories.

The sample size was based on previous studies from ASP which suggested that approximately 20% of patients not given FFP recovered to an INR <2 after 6 hours.[7, 8] We considered an absolute increase of 30% of patients recovering to an INR <2 in 6 hours to be clinically important. To detect this absolute increase with a significance level (alpha) of 5% and a power of 80%, a minimum of 34 and 68 patients would need to be recruited to each arm of the trial respectively (i.e. a total of 102 patients). To allow for protocol non-compliance (failure to deliver FFP within 4 hours) we initially planned to recruit 120 patients over 3 years for the duration of funding. There was no plan for interim analyses. Compliance with FFP allocation was higher than expected, but enrolment rates were lower than expected and after 4 years the study had to be

stopped after randomising only 65 patients because the grant funding for the study had finished.

At the completion of the study, one chief investigator (GI) who was blinded to the treatment allocation extracted the data from the ASP outcomes database. Cases were only identifiable by study numbers. The classification of primary and secondary outcomes, including the presence or absence of adverse reactions or complications were then determined (GI) and reviewed by two investigators (CP, SB). Finally the fourth investigator who was not involved with the day-to-day conduct of the study (NB) checked for missing or inconsistent data and then unblinded treatment allocations using information from the online randomisation database.

Statistical analysis

All continuous variables were summarised as medians and interquartile ranges (IQR) and proportions were presented with 95% confidence intervals (CIs) to make the interpretation easier. The dichotomous primary outcome was analysed by intention-to-treat using Fisher's exact test. A 'per protocol analysis' was also done comparing the group randomised to FFP who actually received a full dose of FFP within 4 hours with those randomised to no-FFP who did not receive it prior to the 6 hour INR. For secondary outcomes appropriate statistical tests based on data distribution were used for continuous outcomes and proportions with 95% CIs were calculated for dichotomous outcomes. All analyses and graphs were done with GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

Results

There were 322 patients recruited to the ASP cohort study during the period March 2008 until June 2012. Seventy patients with **VICC and an INR > 3.0** were eligible for the FFP study, and 65 patients from 28 hospitals consented and were randomised (Figure 1). Forty-one patients were randomised to receive FFP and 24 to not receive FFP. The two study arms had similar baseline characteristics including patient demographics and type of snake (Table 1).

Primary Outcome

Six hours after antivenom administration, 30 of 41 (73%) patients randomised to receive FFP had an $INR < 2$, compared to only 6 of 24 (25%) patients not given FFP (absolute difference of 48%; 95% CI: 23% to 73%; $p=0.0002$) [Figure 2]. Supplementary figure 1 shows the time course of the INR for patients in each arm. The median time to recovery post-antivenom of the INR to less than 2 for patients randomised to FFP was 6 hours (IQR: 3.8 to 9.1hr) compared to 14 hours (IQR: 7.9 to 21.9hr; $p=0.0005$) for those patients who did not receive FFP (Figure 3). There was only one patient not receiving the assigned treatment (randomised to the FFP arm but not given FFP) and the 'per-protocol analysis' was not different from the intention-to-treat analysis results (data not shown). Of the 47 patients with brown snake envenoming, 21 of 30 (70%) randomised to FFP had an $INR < 2$ at 6 h, compared to 5 of 17 (29%) not given FFP ($p=0.0136$).

Of the 11 patients who received FFP but did not respond to the treatment, one was given FFP greater than 6 hours post-antivenom, two received reduced doses of FFP (2 and 3 units) and two had an early recovery at 3 hours post-antivenom (INR 1.8 and 2.0) and then a rebound to higher INRs, although not to the levels seen prior to

treatment (refer to Supplementary Figure 1). Table 2 provides a comparison of FFP patients who did and did not respond to treatment. Non-responders were given antivenom and therefore FFP much earlier than the responders and a poor response to FFP was not associated with age, sex or snake type. There was evidence of further clotting factor consumption in patients who did not respond to FFP with a more prolonged decrease in fibrinogen associated with the administration of FFP (Figure 4). This is supported by a much higher ratio of D-Dimer to fibrinogen in non-responders compared to responders (Figure 5). A receiver operating characteristic (ROC) curve was created to explore the overall effect of the time to FFP post-bite in predicting non-response to FFP treatment (Figure 6; AUC = 0.84; p=0.001) and also the threshold value that best predicted non-response. FFP given earlier than 5.8 hours post-bite was the optimal cut-point based on Youden's index; and the most sensitive cut-point was 8 hours (suggesting the risk of non-response after 8 hours is very low).

Secondary Outcomes

There was no significant difference between patients receiving FFP compared to those who did not in any of the secondary outcomes which are summarised in table 3. In 49 patients who were discharged with a normal INR (< 1.3), the time to reach this complete recovery was significantly shorter in the FFP patients (Table 3). Sixteen patients were discharged with the coagulopathy largely recovered but without a normal INR being recorded (Last INR ranged from 1.3 to 1.9).

Seven patients developed systemic hypersensitivity reactions after antivenom but before FFP – two mild and one severe in the FFP arm and three mild and one severe in the no FFP arm. One patient in the FFP arm had a severe reaction with urticaria, angioedema and hypoxia, to a second FFP treatment given 24 hours after antivenom.

One serious adverse event occurred in the form of an intracranial haemorrhage in a patient with brown snake envenoming in the FFP arm. She had pre-existing hypertension and was hypertensive on admission, but did not develop a headache until 6 hours after FFP when the INR was still >12. She died one day later.

Discussion

This study has shown that the administration of FFP within four hours of antivenom results in more rapid restoration of clotting function in the majority of patients with VICC. Almost three-quarters of patients receiving FFP had an INR of less than 2, six hours post-antivenom and this was also associated with a more rapid complete recovery to a normal INR. However, this was not associated with a more rapid hospital discharge of patients and the numbers were too small to determine if the administration of FFP could reduce the risk of major haemorrhage. An interesting finding was that non-responders to FFP treatment were administered FFP earlier after the bite than those who responded well to FFP.

There are a number of limitations to this trial including the small size and the unblinded allocation of FFP. While the study was stopped before it reached the intended sample size, the observed effect of FFP on the INR was much greater than anticipated in the power calculation and major clinical outcomes (death and major haemorrhage) were very uncommon so that there was little further information likely to be gained from continuing the study. The trial was of a sufficient size to demonstrate that the use of FFP rapidly corrects the INR and therefore provides the basis for larger studies to investigate clinical outcomes that are uncommon and investigate the safety of FFP. The trial could not be blinded because of the impossibility of providing a convincing placebo for factor replacement. However, the primary outcome was an objectively measured laboratory parameter and there was concealed allocation to FFP, so the potential for bias was low.[19]

The dose of FFP in this study was 10 to 15 ml/kg up to a maximum of 4 units. A retrospective review of FFP use suggested that 2 or 3 units of FFP was insufficient,

with recovery in less than half of patients in that study receiving 2 or 3 units compared to all patients receiving 4 units.[7] Insufficient FFP (2) or late administration (1) may explain the lack of response in 3 of the 11 FFP patients who did not have an INR less than 2 at 6 hours (Table 2). Two further patients had a rebound in the INR (Table 2; Supplementary Figure 1; thick line). The remaining six patients with a treatment failure appeared to recover at the same rate as the non FFP patients. This suggests that although the majority of patients improve rapidly with FFP there is a small subgroup of patients who do not respond. This subgroup of patients received FFP significantly earlier after the time of bite.

This raises the possibility that FFP can be consumed in the first few hours of envenoming despite antivenom treatment. Figure 4 shows that in the FFP non-responders the fibrinogen remains low compared to patients not receiving FFP and that responders recover more rapidly. In addition, the D-Dimer to fibrinogen ratio **in Figure 5** shows that this slow recovery is likely to be due to further consumption. It is unlikely that this consumption of FFP administered early is due to active venom since antivenom was administered in all patients and previous *in vitro* data has confirmed the efficacy of antivenom for neutralising the procoagulant effect of venom.[20] Ongoing consumption may be due to the presence of active clotting factors in the initial period of resolution of VICC. Until this issue is resolved, FFP administration within 6 to 8 hours of a bite should only be given if there are compelling reasons for administration such as active major bleeding. **The use of alternative therapies such as specific factor concentrates requires further investigation in this setting.**

Factor V and factor VIII in FFP are affected by the timing of storage, thawing and subsequent delays to administration.[21] Previous studies have demonstrated that

deficiencies of factor V and VIII are the most important in Australian elapid VICC, and the recovery of these factors correlates best with the recovery of the INR.[3] It is therefore reasonable to consider that the patients who did not respond to FFP may have been given FFP that had low activity of Factor V and VIII; either due to poor storage or premature thawing (>24 h) prior to administration. Unfortunately this information was not collected during our study and should be recorded in future studies. The measurement of factor activity in FFP prior to administration would also assist in determining if this is the cause of non-response in some patients.

VICC is caused by a number of important groups of snakes around the world[10], including *Echis* spp. (saw-scaled and carpet vipers)[5], *Daboia russelli* (Russell's viper)[22], *Calloselasma rhodostoma* (Malaysian pit viper)[23] and *Atrox* spp. and *Bothrops* spp. (Rattlesnakes from the Americas)[24]. Although this study of Australian elapids cannot be immediately translated to other snakes, the study provides support for the use of FFP after sufficient time and antivenom has been given. **In addition, FFP is cheap and readily available through-out the world and in many resource poor countries may be more easily accessible than antivenom.**

Further and larger studies are required with snakes in other parts of the world.

Author contributions

G.Isbister^{*,†}, S.G.Brown^{**}, N.A.Buckley^{†,‡} designed the study in consultation with M.Seldon[¶]; G.Isbister^{*,†}, S.G.Brown^{**} and C.B.Page[§] coordinated recruitment; G.Isbister^{*,†} and S.G.Brown^{**} undertook data collection; F.E.Scorgie[¶] undertook laboratory studies under the supervision of L.F.Lincz[¶]; G.Isbister^{*,†}, S.G.Brown^{**}, N.A.Buckley^{†,‡} undertook the analysis; G.Isbister^{*,†} wrote the paper N.A.Buckley^{†,‡}, C.B.Page[§], F.E.Scorgie[¶], L.F.Lincz[¶], M.Seldon[¶] and S.G.Brown^{**} reviewed drafts. G.Isbister^{*,†} takes responsibility for the study.

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Figure Legends:

Figure 1: Consort diagram showing all patients notified to ASP, exclusions and outcomes in each of the study arms. Exclusion criteria were age < 2 years, previous adverse reactions to fresh frozen plasma (FFP) and FFP not being available.

Figure 2: Scatter diagram of the INR at 6 hours post-antivenom comparing those receiving FFP and those not receiving FFP. The shaded area represents cases when the INR is unrecordable (ie the blood does not clot at all).

Figure 3: Scatter plot (top panel) comparing the time for the INR to be less than 2 for patients given FFP versus those not receiving FFP, and the cumulative frequency (bottom panel) of the time for the INR to be less than 2 comparing patients given FFP (thick line) to patients not receiving FFP (dashed line).

Figure 4: Comparison between FFP patients responding to treatment (Panel A) and FFP patients not responding to treatment (Panel B) for fibrinogen concentrations versus time (dashed line). The closed circles indicate the time FFP was commenced and the open circles the time that FFP was finished. The non-dashed part of the fibrinogen concentration line for each patient connects the fibrinogen concentrations immediately before and immediately after FFP administration. The thick black line is the median fibrinogen concentration versus time for 112 patients with VICC from a previous study who did not receive FFP.[3]

Figure 5: Comparison of plots of the ratio of D-Dimer:fibrinogen versus time for FFP patients responding to treatment (grey non-dashed line) and FFP patients not responding to treatment (black dashed line). The thick black line is the median D-

Dimer:fibrinogen ratio for 112 patients with VICC from a previous study who did not receive FFP.[3]

Figure 6: A receiver operating characteristic (ROC) curve assessing the predictive value of the time to FFP post-bite for patients not responding to FFP treatment (AUC = 0.84; p=0.001). The optimal cut-point based on Youden's index for giving FFP is indicated at 5.8 hours post-bite and the most sensitive cut-point was 8 hours.

Supplementary Figure 1: Plots of the INR versus time for patients receiving FFP (top panel; thin dashed line) compared to patients not receiving FFP (bottom panel; thin dashed line). In the FFP group three patients were not given FFP or a lower dose (thick dashed line) and two had a rebound in their INR (thick line).

Table 1: Demographic features, snake type and clinical effects for patients allocated to FFP compared to those allocated to no FFP treatment.

	FFP Treatment (n=41)		No FFP (n=24)	
Sex (male)	25	61%	15	63%
Age (median, IQR); years	39 (18 to 53)		45 (29 to 59)	
Snake Type				
Brown snake ¹ <i>Venom concentration (median, IQR);ng/mL</i>	30 1.0 (0.2 to 5.4)	73%	17 1.2 (0.3 to 3.7)	71%
Tiger Snake, Rough-scaled snake, Hoplocephalus ²	11	27%	6	25%
Taipan	0		1	4%
State³				
NSW	9	22%	7	29%
QLD	12	29%	6	25%
WA	20	49%	10	42%
Time to antivenom (median, IQR); hours	4.5 (2.9 to 5.8)		3.5 (1.9 to 4.9)	

¹ Only sufficient numbers of venom concentrations for brown snake comparison.

² These snakes all contain a group D prothrombin activator (Factor Xa-like) compared to a group C prothrombin activator in brown snake venom (Factor Xa-Va-like).[3];

³ One patient was recruited from South Australia.

Table 2: Comparison of the patients given FFP who responded to treatment with the 11 patients given FFP who did not respond.

	Response to FFP Treatment (n=30)	%	No Response to FFP (n=11)	%	P value
Sex (male)	19	63%	6	55%	0.72
Age (median, IQR); years	43 (18 to 60)		38 (10 to 41)		0.49
Snake Type					
Brown snake	21	70%	9	82%	0.69
Tiger Snake, Rough-scaled snake, Hoplocephalus	8	27%	2	18%	n/a
Time to antivenom (median, IQR); hours	5 (4.1 to 6)		2.4 (1.5 to 4.4)		0.001
Time to FFP (median, IQR); hours	7.3 (6.1 to 8)		4.7 (4.2 to 6.7)		0.002
Time from antivenom to FFP (Median hrs, IQR)	2 (1.3 to 2.9)		2.7 (1.5 to 3)		0.32
Number of units of FFP -					
4	30	100%	8	73%	0.24
2 to 3	0		2	18%	n/a
0	0		1	9%	n/a

Table 3: Secondary outcomes for patients allocated to FFP compared to those allocated to no FFP treatment.

	FFP Treatment (n=41)	No FFP (n=24)	P value
Time from AV to discharge (median, range); hours	34 (14 to 230)	39 (14 to 321)	0.44
Major Haemorrhage	1	0	-
Death	1	0	-
Reactions post-antivenom			
Systemic hypersensitivity reaction	3 (7%)	4 (17%)	0.41
Severe anaphylaxis	1 (2%)	1 (4%)	1.0
Time to INR <1.3 (normal) [median; IQR]; hours	16.5 (4 to 58) n=33	31 (12 to 69) n=16	0.0051

Figure 1

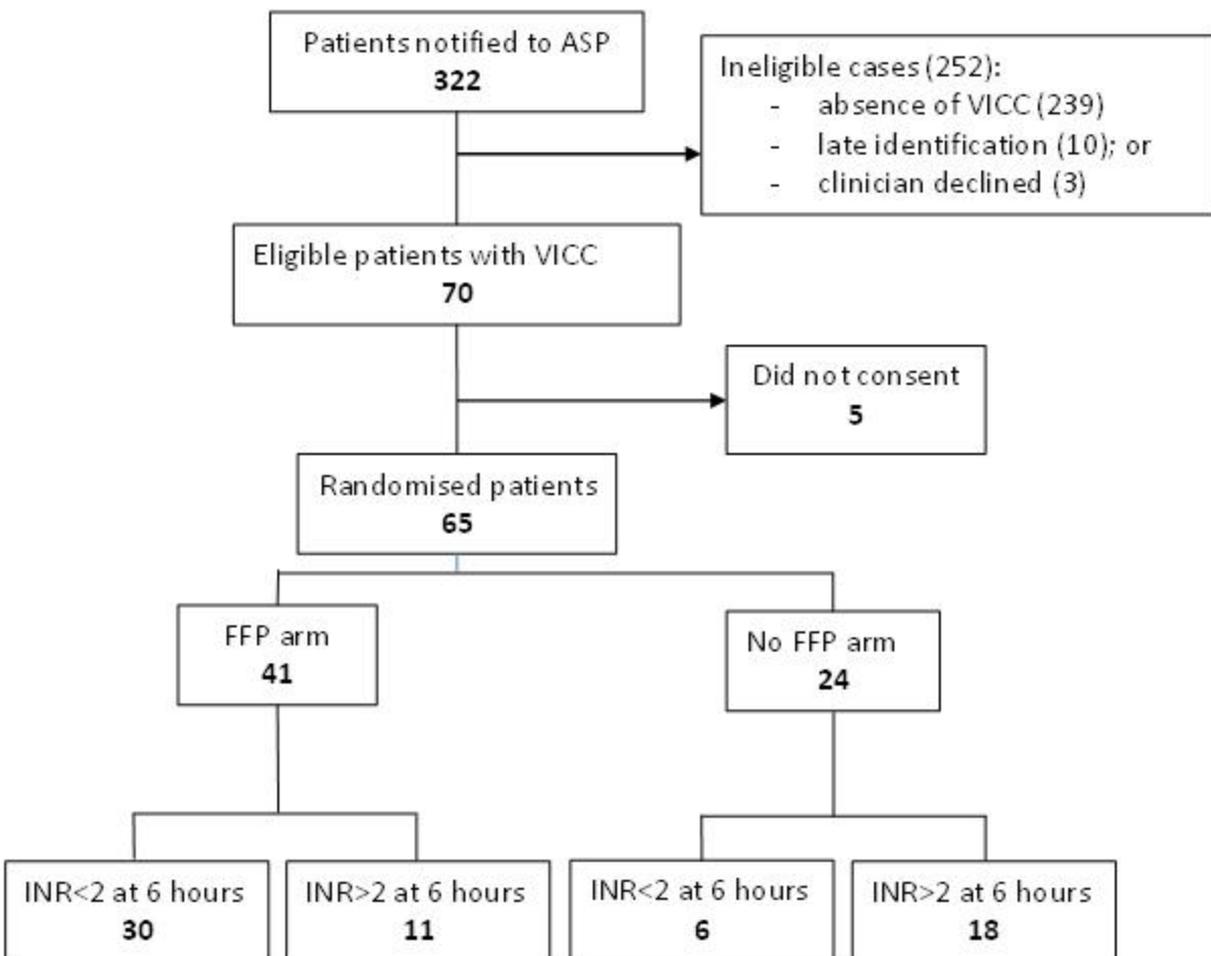
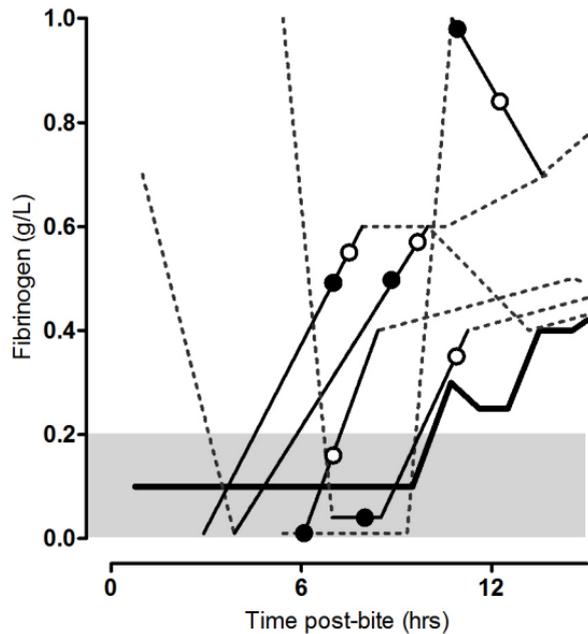


Figure 4

A

Responders



B

Non-responders

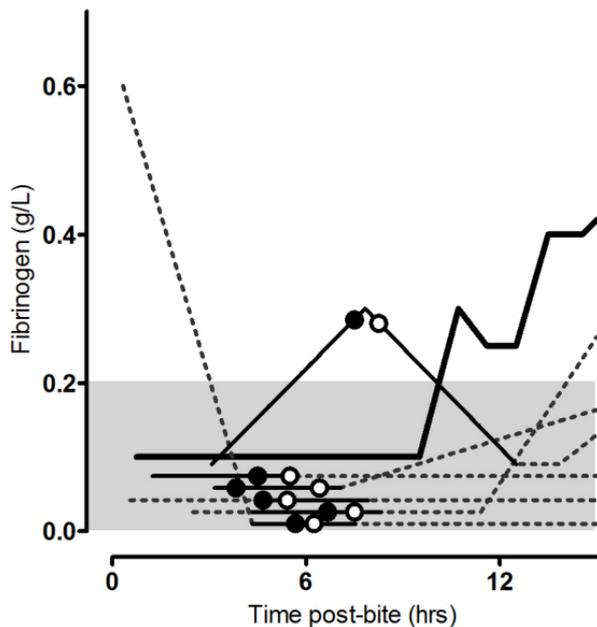
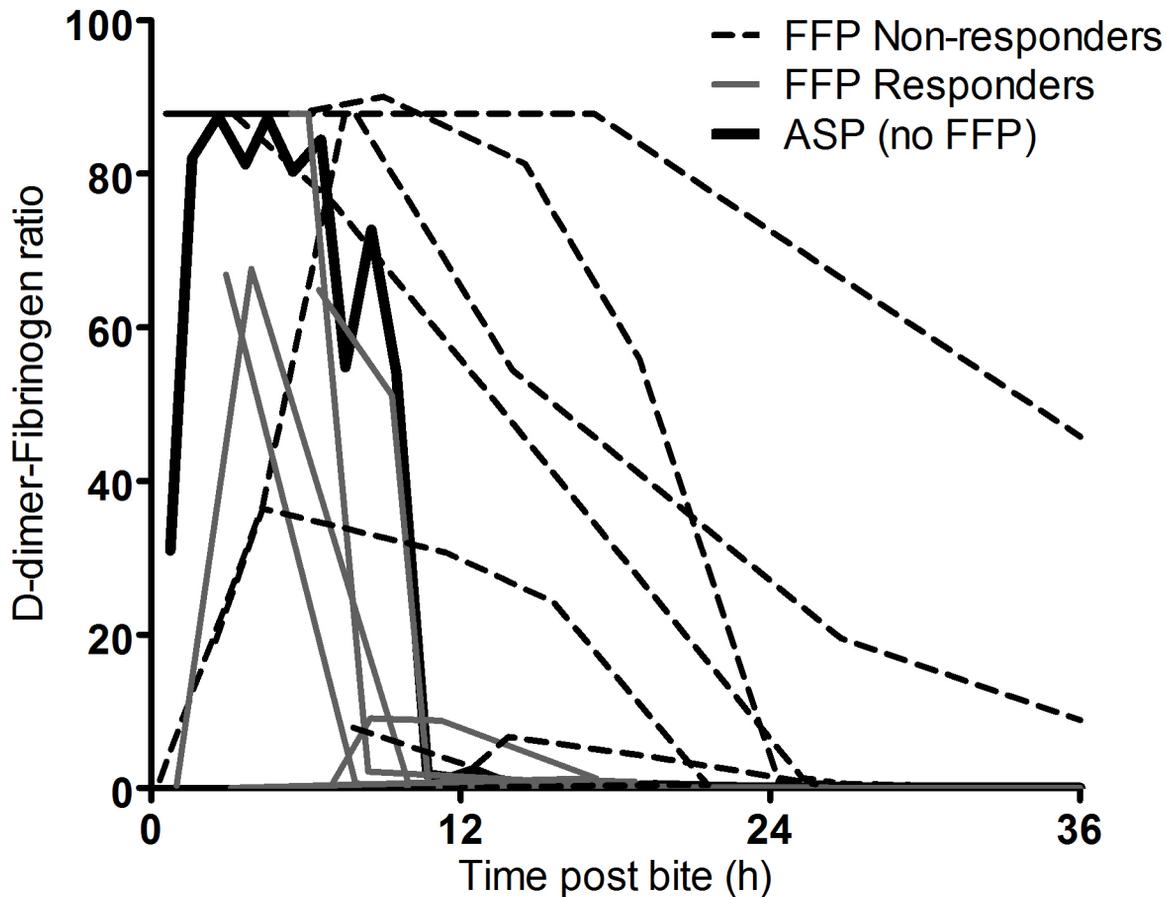
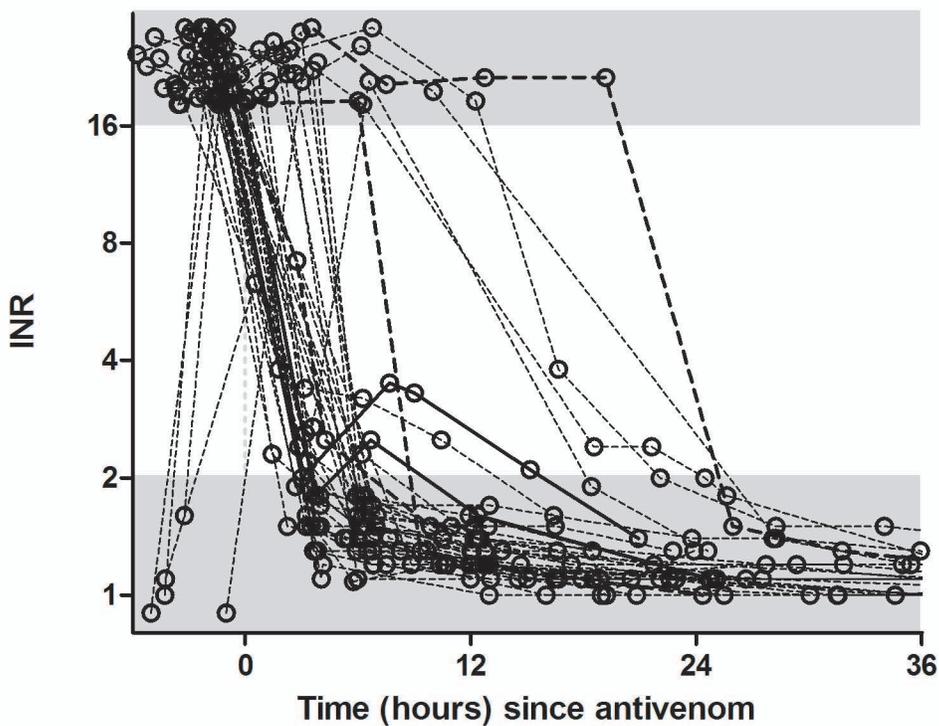


Figure 5



Supplementary Figure 1

FFP



No FFP

