patients was 1.5 (range 1-6). Strict adherence to recent NICE guidelines and local policy is expected to improve the donor exposure rate in autoSCT, particularly in those not previously transfused.

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COAGULATION PROFILE AND OUTCOME OF BLOOD COMPONENT THERAPY IN SNAKE BITE VICTIMS

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Background: Bites from poisonous snakes cause substantial human morbidity and mortality in tropical and subtropical countries. Hemotoxic envenoming is quite common in many parts of India, but only limited data exist on its accurate incidence in the country, and clinical effects. Herein we present findings from an observational study on coagulation profile and outcomes of blood component therapy in patients with hemotoxic snake bites
Aims: To assess the coagulation profile and outcome of blood component therapy in snake bite victims
Methods: We undertook an observational study between January 2013 and June 2014, on patients admitted to the medical ICU of our tertiary hospital, with a history of snake bite and satisfying the inclusion criteria. The patients underwent detailed evaluation of envenoming by clinical and laboratory investigations. The patients were screened by a standard, 20-minute Whole Blood Clotting Time (WBCT) test (repeated four times at 30 min. intervals) and prolongation of at least one test result indicated hemotoxic envenoming. Routine blood counts, Bleeding Time (BT), Prothrombin Time (PT), International Normalized Ratio (INR), activated Partial Thromboplastin Time (aPTT), fibrinogen and D-dimer levels were also monitored. Samples of plasma separated from a citrated blood sample collected at the occurrence of deranged WBCT were flash frozen and assayed for coagulation factors. Blood component therapy and its outcomes were also monitored in the patients.
Results: Among 595 patients reporting with snake bites, 445 were adults. Absence of signs of envenoming suggested non-venomous bites 282 patients. One hundred and sixty three patients manifested features of envenoming, and received Anti-Snake Venom (ASV). Species identification of the biting snake was not possible in 50% of cases. Among the rest, Russel's viper and Pit viper accounted for 66 and 9 cases, respectively. Only the cases where the biting species could be identified were studied further. Delayed treatment resulted in severe local and systemic manifestations in patients. Hematological manifestations developed following bites from Russel's viper, pit viper and unidentified species as well. Acute Renal Failure (ARF) due to Disseminated Intravascular Coagulation (DIC) and capillary leak syndrome developed only in patients with bites from Russels viper, and necessitated hemodialysis. Coagulopathies occurred in most patients with bites from Russel's viper and pit viper. Patients bitten by Russel's viper manifested severe deficiency of Factor V and Factor X, while it did not occur in pit viper bites. Qualitative testing for Factor XIII revealed its deficiency specifically in cases of Russel's viper bite. Most patients with hemotoxic envenomation received transfusion of fresh frozen plasma (FFP), 24 hours after administration of ASV. Severe thrombocytopenia necessitated the administration of platelet concentrate in seven patients. Packed Red Blood Cells (PRBC) was administered in 4 patients with anemia. Blood component therapy significantly increased hospital stay of the patients. No statistically significant complications or mortality occurred after transfusion therapy.
Summary/Conclusions: Rural areas had higher incidence of snake bites. Though BT and CT were sufficient to identify hemotoxicity, costlier and time-consuming assessment of PT, aPTT, serum fibrinogen and D-dimers had more sensitivity. Hematological manifestations occurred following bites from Russel's viper and pit viper, while the former produced major bleeding diatheses and a higher incidence of DIC and ARF. The public need to be made aware of the need for early identification and appropriate treatment of snake bites

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IMPACT OF PROTHROMBIN COMPLEX CONCENTRATE (PCC) USE ON TRANSFUSION REQUIREMENTS AND BLOOD LOSS IN CARDIAC SURGERY

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Background: Cardiac surgery is associated with significant coagulopathy and bleeding. This has led to off-label Prothrombin Complex Conccentrate (PCC) use, despite minimal data regarding its efficacy in this setting. There have been retrospective studies investigating use of four-factor PCCs in Europe showing variable efficacy, however the only PCC available in Australia is Prothrombinex-VF, a three-factor PCC containing factors II, IX and X.
Aims: We aimed to retrospectively review the perioperative off-label use of PCCs during cardiac surgery in our institution, to evaluate if PCC use reduced postoperative bleeding or transfusion requirements, and its effect on other clinical and laboratory parameters compared to patients who did not receive PCC.
Methods: A retrospective audit was performed, with the approval of the hospital Research Ethics Committee, of all patients undergoing cardiac surgery from 1st June 2012 to 31st December 2012. Data was collected via the hospital's cardiac surgery database and medical records.
Results: 193 patients underwent cardiac surgery during the study period. 66 (34%) received PCC at a median dose of 22 IU/kg. There was a significant reduction in the INR (medain INR reduced from 1.5 pre-PCC to 1.3 post-PCC), with the change in INR greatest for patients with the highest pre-PCC INRs), but no change to APTT following PCC use. Post-operative blood loss, as measured by the 4-hour chest drain output, was comparable to international studies and did not decrease significantly with PCC (290mL *versus* 270mL, p=0.29). The PCC group also had higher transfusion requirements, including more red blood cells (median 4 units *versus* 0 units), more platelet transfusions (median 2 bags *versus* 0), more Fresh Frozen Plasma (both receiving a median of no units however the PCC group had a greater IQR of 0-2 units), and more cryoprecipitate transfusions (median 3.5 units *versus* 0) (p <0.0001). These results were, however, potentially confounded by longer median cardiopulmonary bypass time and a greater proportion of aortic surgeries in the PCC group.
Summary/Conclusions: A substantial proportion of patients undergoing cardiac surgery are treated off-label with PCCs. This is associated with a reduction in the INR, but no significant difference in the post-operative blood loss or blood product transfusion rate.

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COMBINED PLASMA EXCHANGE AND ERYTHROCYTAPHERESIS FOR TREATMENT OF MULTIORGAN FAILURE DUE TO SUSPECTED FAT EMBOLISM SYNDROME IN SICKLE CELL DISEASE

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Background: Fat embolism syndrome (FES) as a consequence of bone marrow necrosis during a vaso-occlusive crisis is a devastating complication of sickle cell disease (SCD) that affects all age groups and phenotypes with a high mortality. Clinically FES is characterised by acute respiratory failure, neurological impairment, cutaneous/mucosal petechiae, acute kidney injury, and hepatic dysfunction. Additional markers that aid diagnosis include hyponatraemia, thrombocytopenia and coagulopathy. Retrospective case reviews of FES in SCD have highlighted the role of red cell exchange transfusion in improving clinical outcome though mortality remains ~30%. These have not differentiated between manual and automated red cell exchange. Previous studies have shown reduction in inflammatory mediators after manual exchange transfusion. Such an effect would not be expected from selective removal of red cells during automated erythrocytapheresis (aRCE) increasingly the modality of choice in specialist haemoglobinopathy centres. Since inflammatory mediators are likely to play a role in its pathogenesis we reasoned that combined plasma exchange (PEX) and erythrocytapheresis could improve the outcome of FES in SCD.
Aims: To report the outcome in adult SCD patients who developed multiorgan failure and met diagnostic criteria for FES treated with combined aRCE and PEX.

Table 1. Clinical details at the onset of MOFS.

	Case 1	Case 2	Case 3
Gender	F	F	F
Age	60	47	33
Genotype	HbS-beta thal	HbS-O Arab	HbSS
WC x10^6/L	2,41	7	11
Hb g/l	66	68	65
Plt x10^6/L	64	48	50
Creatinine (umol/L)	112	122	>110
ALT (iU/L)	72	113	>40
Arterial pO2 (kPa)	7.7	8.2	6.5
Glasgow Coma Score Before PEX	11	9	8
HbS%	28	23	25
Courses of PEX	3	2	1
Outcome at 2 months	Alive	Alive	Alive
Recovery (days)	9	18	16

Methods: A retrospective analysis of 3 SCD patients aged 33-67 years with suspected FES treated at Hammersmith Hospital. Clinical details of the patients are summarized in table 1. Apheresis was performed using the Spectra Optia Apheresis System according to our standard operating procedure with a target HbS of <30% and plasma volume exchanged of 3L. Consecutive aRCE and PEX were performed initially and a decision to repeat PEX subsequently based on clinical response (median number PEX 2;range 1-3). Time to recovery was defined by reversal of neurological and respiratory impairment and normalisa-

tion of platelet count, liver and renal function and coagulation parameters. Overall survival (OS) was assessed 2 months post-treatment with a median follow up of 16 months (range 2-21).
Results: All patients exhibited features of multiorgan failure compatible with the diagnosis of FES. OS was 100% at 2 months. Rapid improvement in neurological status was seen with normalisation of Glasgow Coma Scale within 24 hours of completing PEX. No long-term neurological sequelae were observed. Median time to recovery as defined above was 16 days (range 9-18).
Summary/Conclusions: Based on these favourable preliminary results in SCD patients with multiorgan failure due to suspected FES it is recommended combined aRCE/PEX is evaluated in a larger cohort of patients to determine its efficacy in treatment of this life-threatening complication.

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REDUCTION OF ABO GLYCOSYLTRANSFERASE CAUSED BY SYNONYMOUS VARIATION IN B309 ALLELE

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Background: Non-synonymous single-nucleotide polymorphism in the *ABO* gene can affect the enzyme characteristics and protein quantity producing many kinds of ABO blood type subgroups. B3 phenotype including 11 variant alleles reveals characteristic mixed field agglutination. Synonymous variations, formerly called 'silent' mutation, can result in aberrant protein expression and protein folding. Interestingly, *B309* variant (*255C>T* on exon 6 in the background of *B101*) was recently reported to have synonymous SNP (sSNP). We found B309 alleles recurrently in Korean B3 phenotype individuals.
Aims: B309 allele's effect on B antigenic expression was investigated in patients and confirmed by the experimental model.
Methods: Individuals with mixed field agglutination with anti-B were tested by direct sequencing. *B309* allele were isolated and confirmed by TA cloning. cDNAs from exon 5 to exon 7 were amplified and sequenced in B309 individuals to exclude out the possibility of splice variant. *B101* and *B309* coding sequence with *255C>T* were cloned, inserted to the vectors with fluorescent tags and transduced into the HeLa cells that have O/O genotype. The protein expression and antigenic changes by sSNP were evaluated with fluorescent protein expression and flowcytometric analysis.
Results: We found two unrelated Korean individuals and three family members in one pedigree who have *B309* alleles and all of them revealed B3 phenotypes. Exon 6 of ABO transferese gene was not spliced out in their cDNAs. Protein expression and antigenic expression were reduced in B309 variant (the median MFI: 36.85) compared to B101 (MFI: 50.03).

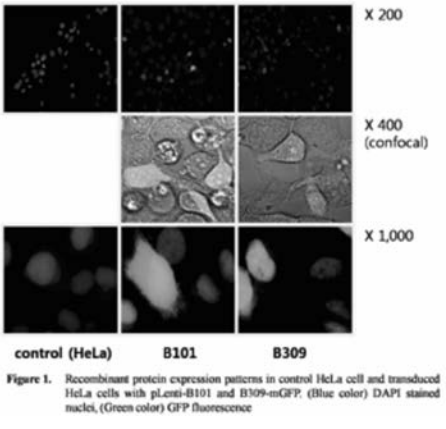


Figure 1. Recombinant protein expression patterns in control HeLa cell and transduced HeLa cells with pLenti-B101 and B309-mGFP. (Blue color) DAPI stained nuclei, (Green color) GFP fluorescence

Figure 1.

Summary/Conclusions: Individuals with *B309* allele revealed B3 phenotypes and their *255C>T* synonymous polymorphism didn't contribute to the alternative splicing. *255C>T* polymorphism was experimentally proven to cause the reduced expression of ABO transferase and the subsequent weakening of B antigens. This phenomenon implies that sSNP could also affect the expression and function of the protein, and finally alter the immunohematologic reactions.

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EFFECTS OF IMPLEMENTATION OF A MASSIVE TRANSFUSION PROTOCOL IN A TERTIARY REFERRAL HOSPITAL

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Background: Following the study in 2007 of Borgman et al regarding early plasma product support in trauma patients receiving massive transfusion, the implementation of massive transfusion protocols (MTPs) has become the norm.Aims of utilising a MTP include pre-emptive transfusion with plasma products platelets in an effort to be proactive and overcome the effects of transfusing mostly large amounts of packed red cells on coagulation, in patients with critical bleeding.
Aims: The effect of implementing a MTP in a tertiary referral hospital, for both trauma and non-trauma–related critical bleeding was examined. Parameters include the number of MTP 'episodes' or 'activations', the proportion of 'true' critical bleeding episodes, the ratio of blood products transfused (Red Blood Cells:Fresh Frozen Plasma:Cryoprecipitate:Platelets) and blood product wastage.The appropriateness of platelet transfusion and 28-day mortality were of interest.
Methods: Data regarding the issuing of blood products which was collected by Blood Bank staff at the time of the MTP, as well as computer records regarding laboratory parameters and blood product use was assessed retrospectively.The first dataset period, denoted Period 1(P1), was prior to the implementation of the MTP and the second period (P2) post-implementation.
Results: During P1 (16.5 months), the total number of massive transfusion 'episodes' was 53 and during P2 (7.5 months) there were 59 MTP 'activations'.During P1 the average number of Red cells (RBC), Fresh Frozen Plasma (FFP), Cryoprecipitate(Cryo) and Platelets (Plt) transfused per patient were:15.1, 13.3, 13.6 and 1.5 respectively.4 RBCs or less were transfused in only 3 episodes during P1.During P2 (7.5 months), the total number of activations included 57, and the corresponding average numbers of RBC, FFP, Cryo and Plt per patient were 7.9, 6.9, 7.4 and 0.5.4 RBCs or less were transfused in 27 episodes during P2.The proportion of deaths for the 53 patients during P1 was 28% and during P2 the proportion of deaths was 31.6%.The RBC:FFP ratio in P1 was 1.13:1 and in P2 it was 1.18:1. RBC:Cryo ratio was 1.11:1 for P1 and 1.09:1 for P2. RBC:Plt ratios for P1 and P2 were 10.06:1 and 16.2:1.
Summary/Conclusions: The 2 periods contain similar numbers of patients, however the time periods during which the 'critical bleeding' episodes were recorded differ markedly (16.5/12pre *vs* 7.5/12post).This is mainly due to a large number of MTP activations in which 4 or less RBC units were transfused in the post-implementation period.The high proportion of patients who did not have truly 'massive bleeding' but for whom the MTP was activated (47%), is similar to that previously reported.Correspondingly, the number of blood products transfused per patient was much higher in the pre-MTP protocol period. A cardinal reason for the wastage of blood products is that in patients who are severely injured, the MTP is activated after they have undergone an initial assessment and plasma products are thawed, however they succumb to their injuries before receiving these.Patient mortality did not differ in the periods before and after the MTP implementation (28.3% v 31.6%).The ratio of RBC to plasma products is similar in the pre- and post-MTP implementation periods.Fewer platelets were transfused per patient in the post-MTP period than pre-implementation (0.5 v 1.5);this is most likely because the 1st MTP 'shipment' of blood products containing 4 RBCs, 4 FFPs and 4 Cryo, would not normally include platelets.In summary, implementation of a formal MTP protocol has resulted in more 'activations' than had been the case before, an increased number of 'false' activations, increased wastage of blood product, no significant changes in mortality or ratio of plasma product to RBCs transfused.Possible ways to mitigate plasma product wastage could include alteration of MTP activation criteria, extending the lifespan of thawed products and the use of lyophilised fibrinogen.

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LOW PREVALENCE OF ALLOIMMUNIZATION AFTER RED BLOOD CELL TRANSFUSION IN 182 CHILDREN WITH SICKLE CELL DISEASE

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Background: A high prevalence (around 30%) of red blood cell alloimmunization has been reported in patients with sickle cell disease (SCD), but few studies have focused on children.
Aims: Our objectives were to study prevalence and risk factors of red blood cell alloimmunization in a population of children affected with SCD.
Methods: We analyzed the medical and transfusion files of 245 children with SCD, hospitalized in our center in 2014, and identified 182 patients aged 1 to 18 years who received at least one red cell concentrate in their lifetime. The main clinical and immuno-haematological characteristics of alloimmunized and non-alloimmunized patients were compared.
Results: The 182 patients received 1 to 339 red blood cell units with a median of 10 (IQR: 4-46.7) units per patient. Six patients out of the 33 in whom a red cell genotyping was performed (18.2%) had a partial antigen in the Rh blood group system. Five patients (2.7%) had a S-s- rare blood type (either $U+^{var}$ or U-). Excluding autoantibodies and the naturally-occurring antibodies identified before any transfusion, the prevalence of alloimmunization was 13.7% (95% CI [8.6-18.6]). After excluding the probable irregular natural antibodies (anti-M, anti-Le^a, anti-Le^b), the prevalence dropped to 7.4% (95% CI [3.5-11.3]) and the alloimmunization rate was 0.17/100 units received. These antibodies were