Increased Immunogenicity of Factor IX Administered Subcutaneously vs. Intravenously Demonstrated in Hemophilia B Mice Expressing a Human MHC II Allele.

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Background: The development of neutralizing alloantibodies (inhibitors) directed against factor IX (FIX) following replacement therapy is a poorly understood and potentially devastating complication of hemophilia B that results in loss of efficacy of infused replacement clotting factor. Although inhibitors develop in only ~2.4% of individuals with severe hemophilia B, the unique combination of FIX inhibitors and their relative resistance to treatment strategies for re-inducing immunologic tolerance demand improved understanding of FIX inhibitors.

Method: 4 “Humanized” Factor IX deficient mice (FIX(-/-) [n = 10-11/group] expressing the common human MHC II DR B*1501 allele were administered FIX via one of three different routes: subcutaneous (SC), intravenous (IV via tail vein), or into the retro-ovalt venous plexus (RO) at a dose of 200 IU kg⁻¹ for a total of 9 doses on a weekly basis from weeks 0 through 8. Retro-ovalt blood collection was performed at weeks 4, 6, 8, and 12. FIX Bethesda inhibitor antibody was evaluated in all treated groups at each of the time points using a START Coagulation Analyzer. At week 12, all the treated mice are sacrificed and further evaluated for IgG1 subclass FIX binding immunoglobulins.

Result: After FIX clotting factor replacement, 90% of mice in the subcutaneous treatment group developed high titer FIX inhibitors (>5 Bethesda inhibitor units, BU) by week 4 and 100% by week 6. These inhibitors persisted through week 12 with a mean of 24.5 BU ml⁻¹ (range 8.3-45 BU ml⁻¹). In comparison, 38% of mice in the intravenous treatment group developed inhibitors by week 4 and 75% by week 12, none of which were high titer (mean of positive values 1.5 BU ml⁻¹, range 0.48-1.9 BU ml⁻¹). Inhibitors detected in 44% of the retro-ovalt treatment group at week 12 were near or below the lower level of sensitivity of the assay (mean of positive values 0.6 BU ml⁻¹, range 0.4-0.75 BU ml⁻¹). (Figure 1) Mice treated with subcutaneous FIX had a mean IgG1 level of 718 mcg ml⁻¹, while the mice treated via the intravenous or retro-ovalt route had a mean IgG1 level of 2 mcg ml⁻¹ and 0.75 mcg ml⁻¹, respectively. (Figure 2) Multiplex cytokine analysis of serum from the terminal collection (week 12 samples) is ongoing.

Conclusion: Subcutaneous administration of FIX clotting factor replacement is strongly immunogenic when compared to intravenous delivery, as evidenced by the generation of high titer inhibitors and FIX IgG1 binding immunoglobulins in mice expressing a common human MHC II haplotype. The time course of inhibitor development in these studies suggests that the study of pro-inflammatory signaling at weeks 4-6 of the subcutaneous FIX exposure will be informative.

Safety and Efficacy of FACTOR X, a New High Purity Factor X Concentrate: A Phase III Study in Patients with Hereditary Factor X Deficiency

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Background: Congenital factor X (FX) deficiency is a rare bleeding disorder affecting 1 in 500,000 to 1 in 1 million of the population. There is limited published data on the pharmacokinetics of FX with existing treatments for FX deficiency, and no specific replacement factor concentrate exists. A high-purity plasma-derived FX concentrate (FACTOR X) has been developed for treatment of FX deficiency.

Objectives: To assess the pharmacokinetics (PK), safety and efficacy of FACTOR X in the treatment of bleeding episodes in patients with hereditary severe or moderate FX deficiency (basal FXC ≤ 5 IU dl⁻¹).

Method: Subjects aged 12 years or older, who had received replacement therapy for at least one spontaneous or menorrhagic bleed in the past 12 months, received 25 IU kg⁻¹ FACTOR X for a PK assessment. Subjects received one dose of FACTOR X (doses: 25 IU kg⁻¹ for 6 months to 2 years. The PK assessment was repeated after a minimum of 6 months. Efficacy of FACTOR X was assessed by the subject for all bleeds, and by the investigator for bleeds treated at the hospital. At study end, each investigator assessed the overall efficacy of FACTOR X. Safety assessments were adverse events, development of inhibitors, viral sequence conversions and changes in laboratory parameters.

Results: Sixteen subjects were enrolled to the study; 10 were female and 6 male. The median age was 27.1 years (range 12-58 years); 5 subjects were aged under 18 years. Fourteen subjects had severe FX deficiency (FXC level ≤ 1 BU dl⁻¹) and two had moderate FX deficiency (plasma FX ≥ 4 < 8 BU dl⁻¹). The median (IQR) incremental recovery and half-life of FACTOR X in 16 patients were 2.12 (1.79-2.37) IU h⁻¹ per IU kg⁻¹ and 28.6 (25.8-33.1) h, respectively. 468 infusions of FACTOR X were administered. 187 bleeds treated with FACTOR X were included in the efficacy analysis, of which 98 (52%) were major and 88 (47%) were minor. Of 187 bleeds, 155 (82.9%) were treated with one infusion of FACTOR X, 28 (15.0%) with 2 infusions, 3 (1.6%) with 3 infusions and 1 (0.5%) with 4 infusions. The mean (±SD) number of infusions to treat a bleed was 1.2 (±0.47) and median (IQR) dose to treat a bleed was 25.0 (24.4-26.7) IU kg⁻¹. Efficacy of FACTOR X was excellent in 90.9%, good in 7.5%, and poor in 1.1% of bleeds. For the 15 subjects who completed the study, overall efficacy of FACTOR X was rated by the investigator as excellent in 12 (80%) and good in 3 subjects (20%). There were no serious adverse reactions, hypersensitivity reactions or clinically significant trends in any laboratory safety parameters.

Conclusions: FACTOR X is a highly purified factor X concentrate developed for patients with hereditary FX deficiency. FACTOR X was judged to be safe and effective in treating bleeds in subjects with severe or moderate FX deficiency at a nominal dose of 25 IU kg⁻¹. The pharmacokinetics of FACTOR X support the observed hemostatic effect of the product in patients aged 12 years and older. Presented by Dr S. Austin on behalf of the FX Investigators Group.

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An Immunodominant B Cell Epitope is Present on the C1 Domain of FVIII

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Background: Approximately 30% of individuals with severe hemophilia A develop inhibitors to infused concentrates of factor VIII. The C1 domain of factor VIII (FVIII) is a major immunological determinant in the immune response to FVIII. Prior studies have shown that the C1 domain participates in binding to activated platelets, phospholipid membranes, and with Willebrand factor (VWF) as well as FVIII uptake by dendritic cells. Though we have gained some knowledge of C1 domain function, extensive investigation into the B cell epitopes of the C1 domain potentially involved in the immunologic response to FVIII and their clinical relevance are lacking.

Objectives: The main objectives of this study are to produce a repertoire of anti-human C1 monoclonal antibodies (MAbs) in hemophilia A mice model to characterize the effect of these antibodies on FVIII function and to map the recognized B-cell epitopes within the C1 domain.

Method: E16 knockout mice were immunized with intravenous doses of B-domain deleted FVIII. Splens from the mice were harvested and NS-1 hybridomas were produced using standard Kohler-Milstein technology. Hybridoma supernatants containing antibodies to the C1 domain of factor VIII were isolated and purified for MAbs.

Results: We have produced and purified 7 anti-C1 domain MAbs designated: B153, B156, I41, I84, I88, M6143, and B136 from four splenins. Anti-C1 MAb 2A9 was
null
Pharmacokinetic and Cost Considerations for Transition to Extended Half-life (EHL) Factor VIII and IX Products

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Background: Extended half-life (EHL) factor VIII and factor IX products signal potential improvements in treatment of hemophilia. Although lumped into the same “extended-half-life” designation, these products differ markedly in pharmacokinetic profile. The potential impact on patients with Hemophilia A and Hemophilia B must be considered separately.

Objective: To investigate and compare the pharmacokinetic parameters (target peak and trough) driving prophylactic dosing regimens and the regimen-specific annual costs for standard and EHL VIII and FIX products.

Design/Methods: A comparative analysis of factor product coverage (standard and EHL) modeled on different half-lives and prophylaxis dosing regimens and corresponding cost analysis was performed.

Results: Patients on standard factor 8 replacement of 12 h should achieve adequate coverage with an EHL prophylaxis regimen of 25 IU per kilogram every 4-3 days; however, those with a standard product half-life of < 8 h (as do many pediatric patients) will not have sufficient half-life extension to increase the dosing interval, for example, from 48 h to every 72 h. AlprolixTM dosing strategy using 100 IU per kilogram every 10 days results in a 40% higher annual cost, compared to 50 IU weekly dosing, without expectation of either improved efficacy or higher trough levels. Another way of evaluating the mathematical and treatment assumptions of the models is to calculate the expected “cost per dose saved” (i.e. the annual increment in cost) divided by the annual number of doses saved. For a 70 kg patient, this is an annual cost of $209,475 per 15 doses saved ($13,965 per infusion not given). Inadequate nominal zonal size availability, particularly for pediatric patients, hinders the provider’s ability to titrate dosing.

Conclusion: The approved FC-fusion VIII and FIX products each propose two possible dosing strategies in their package insert; these strategies are not equivalent with respect to total factor dosage used, factor coverage, number of infusions, or cost. As stewards of the precious resource of expensive, innovative factor products, providers are obliged to think critically about the balance of convenience and cost and have frank discussions with patients about these elements before transitioning to an EHL product.

SEC: 2014 HTRS/Novo Nordisk Clinical Fellowship Award, 2014 PANCRS/Medical Affairs, Plainsboro, New Jersey, USA

Safety and Efficacy of recombinant factor VIIa (rFVIIa) by pediatric age-cohort: Reassessment of compassionate use and trial data supporting US Label

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Background: First approved by FDA in 1999 for treatment of patients with congenital hemophilia with inhibitors (CHwI), rFVIIa (NovoSevenTM) has been studied in compassionate/emergency use programs and randomized trials for nearly 30 years. It has been used in compassionate/emergency use programs and randomized trials for nearly 30 years.

Objective: To analyze rFVIIa safety and efficacy data in pediatric CHwI patients to support FDA directives on updating pediatric use labeling.

Methods: Trial and safety database for studies included in current US prescribing information were queried to identify pediatric patients by diagnosis and 4 age cohorts. Results: During the investigational phase of product development in CHwI, rFVIIa was studied in 172 pediatric patients (age 0-16) for 3184 bleeding episodes and 28 surgical procedures. Pediatric patients treated for bleeding episodes (compassionate use 125 bleeds, emergency use 387 bleeds, dose-finding 72 bleeds, home treatment 600 bleeds) included 16 aged 0-2 years for 151 episodes, 34 aged 2-6 years for 158 episodes, 56 aged 6 to <12 for 406 episodes and 38 aged 12-16 years for 469 episodes. rFVIIa was effective across age groups in compassionate/emergency use and home treatment studies (age >2, 95% 2 to <6, 94%; 6 to <12, 97%; 12-16, 95%). In a double-blinded, randomized comparison trial of 35 vs. 70 mcg kg-1 dosing in the treatment of 28 children with joint, muscle and mucocutaneous bleeds, rFVIIa was effective in 89% 35 mcg kg-1 group and 98% 70 mcg kg-1 group. In a dose comparison study (90 vs. 35 mcg kg-1) 22 children were treated for minor/major elective procedures. Effective intraoperative hemostasis was achieved in 21/22 (95%). At 48 h satisfactory hemostasis was maintained in 10/10 (100%) in the 90 mcg kg-1 group and 11/12 (92%) in the 35 mcg kg-1 group. By day 5, hemostasis was 10/10 (100%) in the 90 mcg kg-1 group and 10/12 (83%) in the 35 mcg kg group. In the trial comparing bolus and continuous infusion in major surgery (6 children aged 30-35, 3 per group), both regimens were 100% effective intra-operatively, the first 24 h, and at day 5, and 83% at day 10 or discontinuation of therapy. Adverse drug reactions in pediatric patients were similar to the types generally reported in clinical trials, including one thrombotic event probably related to rFVIIa in a 4-year-old with internal jugular vein thrombosis after port-a-cath placement (35 mcg kg-1 group in surgery that resolved completely).

Conclusion: Analysis of pediatric CHwI patients within the compassionate/emergency use and clinical trials in the US labeling supports that rFVIIa is effective in the bleeding treatment and surgeries, with no apparent differences observed among age groups. Efficacy is similar to that observed in adult patients. Safety data confirm low risk of thrombosis in pediatric CHwI patients and represent substantial evidence for the use of rFVIIa in this population.

This research was conducted through financial support from Novo Nordisk.

Experience of FACTOR X, a New High Purity Factor X Concentrate in Subjects with Factor X Deficiency Undergoing Surgery

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Background: Hereditary factor X (FX) deficiency affects in 500,000 to 1 in 1 million of the population and the specific replacement product FX concentrate is not available in the US. A high-purity, high potency, plasma-derived factor X concentrate (FACTOR X) has been developed to address this unmet need.

Objective: To assess the safety and efficacy of FACTOR X in patients with hereditary severe to mild FX deficiency (factor FX:C ≤ 20 IU dL-1) undergoing surgery.

Methods: Subjects aged ≥12 years received FACTOR X to raise plasma FX activity to 70-90 IU dL-1 pre-operatively and to maintain levels ≥50 IU dL-1 until no longer at risk of bleeding due to surgery. Efficacy assessments included blood loss during surgery, requirement for blood transfusion, post-operative bleeding, and changes in hemoglobin (Hb) levels. Safety assessments included adverse events, development of inhibitors (Bethesda assay), viral seroconversions and other clinically significant changes in laboratory parameters.

Results: Five subjects with severe to mild FX deficiency underwent 7 surgical procedures (Table 1). Subjects 1 and 2 had a history of coronary artery disease and hypertension. A median (range) of 48.83 (30.88-54.41) IU kg-1 FACTOR X was administered pre-surgery, resulting in plasma FX levels ranging from 0.77 to 1.32 IU dL-1. The median (range) incremental recovery (IR) for the pre-surgical dose was 2.21 (1.67-2.34) IU dL-1 per kg IU-1. In all 7 procedures, FACTOR X was assessed by the investigator as excellent in the control of bleeding. There were no blood transfusions or post-operative bleeds for any procedure. Compared with a similar patient without a coagulation disorder undergoing the same surgery, blood loss was ‘as expected’ in 5 procedures and ‘less than expected’ in 2 procedures. There were no adverse events considered to be related to FACTOR X, no viral seroconversions, and no other clinically relevant safety findings. All inhibitor results were negative.

Conclusion: FACTOR X is a highly-purified factor X concentrate developed for patients with hereditary factor X deficiency. Data from this study show that FACTOR X was safe and effective as replacement therapy for five subjects with mild to severe FX deficiency undergoing a variety of surgical procedures on seven occasions.

Development and Validation of a Chromogenic Factor Xa Inhibitor Assay on the ACL TOP 700 for Quantifying Rivaroxaban Levels

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Background: Rivaroxaban is an oral, specific, direct inhibitor of Factor Xa (FXa) that binds to the active site of FXa, directly blocking FXa-mediated procoagulant activity. Rivaroxaban is an oral, specific, direct inhibitor of Factor Xa (FXa) that binds to the active site of FXa, directly blocking FXa-mediated procoagulant activity. Rivaroxaban inhibits free, prothrombinase-bound, and clot-associated FXa in a concentration-dependent manner, preventing thrombin generation by inhibiting FXa generated by the intrinsic and extrinsic coagulation pathways. Patients on therapeutic

Table 1. Surgical procedures and exposure to FACTOR X

| Subject | No. infusions | FX total exposure (IU kg-1) | Hb pre-dose, post-operatively & at discharge (g dL-1) |
|---------|---------------|-----------------------------|------------------------------------------------------|
| 1       | 1             | 151.9                       | 46.5, 15.1, 11.3                                     |
| 2       | 5             | 210.1                       | 15.3, 15.5, 13.2                                    |
| 3       | 1             | 209.4                       | 16.2, 12.6, 11.4                                    |
| 4       | 4             | 24.4                        | 15.0, 14.9, 14.8                                    |
| 5       | 2             | 45.0                        | 12.7, 11.1, 11.5                                    |

Rivaroxaban is an oral, specific, direct inhibitor of Factor Xa (FXa) that binds to the active site of FXa, directly blocking FXa-mediated procoagulant activity. Rivaroxaban inhibits free, prothrombinase-bound, and clot-associated FXa in a concentration-dependent manner, preventing thrombin generation by inhibiting FXa generated by the intrinsic and extrinsic coagulation pathways. Patients on therapeutic

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doses of rivaroxaban do not require routine coagulation monitoring due to its dose-dependent predictable pharmacological profile. However, in some situations (e.g., urgent surgery, severe bleeding, thrombosis despite treatment, overdose) it may be beneficial to accurately measure the level of anticoagulant present.

**Objective:** To develop and validate a chromogenic direct FXa inhibitor assay on the A. HILL, J. SHATZEL and D. ORNSTEIN

**Background:** Dabigatran is a direct thrombin inhibitor that binds to the active site of thrombin, inhibiting both free and clot-bound thrombin. Dabigatran etexilate is the prodrug that is metabolized to its active form after oral administration. Due to its dose-dependent predictable pharmacological profile, patients on therapeutic doses of dabigatran do not require routine coagulation monitoring. However, situations may arise where it is beneficial to accurately measure the level of anticoagulant present (i.e., urgent surgery, severe bleeding, thrombosis despite treatment, overdose, patients with a high risk of dabigatran accumulation or potential drug interactions).

**Objective:** To develop and validate a chromogenic direct thrombin inhibitor assay on the ACL TOP 700 for the determination of dabigatran levels in human plasma.

**Methods:** The test principle is based on the in vitro thrombin inhibition by dabigatran, in which excess thrombin is added to the dabigatran-treated plasma sample and the residual thrombin hydrolyzes the chromogenic substrate, releasing pNA that is measured photometrically at 405 nm. Concentrations are determined by measuring the inhibition of thrombin activity by dabigatran in plasma samples.

**Results:** The calibration curve consisted of 15 plasma samples using a modification of the thrombin inhibition activity (THI) method described by Shearman et al. (2015). The calibration curve spanned multiple days, 11 plasma samples were free of anticoagulants, and 4 plasma samples containing dabigatran (high, medium, low concentration) were treated with dabigatran calibration standards (anamur). For all runs, an r² value of 1.0 was observed. To evaluate accuracy, 3 plasma samples containing dabigatran (high, medium, low concentration) were compared to the mass spectrometry determined value. A dose-dependent predictable pharmacological profile was determined. However, situations may arise where it is beneficial to accurately measure the level of anticoagulant present (i.e., urgent surgery, severe bleeding, thrombosis despite treatment, overdose, patients with a high risk of dabigatran accumulation or potential drug interactions).

**Conclusions:** We have developed and validated an accurate, precise, sensitive and robust chromogenic assay on the ACL TOP 700 for the determination of dabigatran concentration in human plasma. This assay may prove useful in certain clinical circumstances (urgent surgery, severe bleeding, thrombosis despite treatment) for the assessment of anticoagulation status.

**Efficacy and Safety of a Recombinant von Willebrand Factor for Bleed Treatment in Patients with Severe von Willebrand Disease**

**Objective:** To evaluate the efficacy of rVWF in subjects with severe VWD.

**Methods:** Eligible subjects had VWD of severe type 1 or 2A (VWF:RCo <20 IU dL⁻¹), 2N (FVIII:C <20%), and 3N (FVIII:C <10%) from assigned value for samples ≤100 ng mL⁻¹ or within 15 ng mL⁻¹ of assigned values for all other samples). Assay results were minimally affected by unfractionated heparin up to 1 U mL⁻¹, but were affected by LMWH heparin at 2 U mL⁻¹. Serum resulted in >10% difference from the standard plasma, indicating assay interference. Samples thawed and refrozen up to 2 times are acceptable.

**Conclusions:** We have developed and validated an accurate, precise, sensitive and robust chromogenic assay on the ACL TOP 700 for the determination of von Willebrand concentration in human plasma. This assay may prove useful in certain clinical circumstances (urgent surgery, severe bleeding, thrombosis despite treatment) for the assessment of anticoagulation status.

**Table 1. Characteristics of bleeding episodes (N = 192)**

| Severity         | N   | %  |
|------------------|-----|----|
| Major/severe**   | 7   | 3.65|
| Minor            | 122 | 63.3|
| Moderate         | 61  | 31.8|
| Unknown          | 2   | 1   |

**Location**

| Location        | N   | %  |
|-----------------|-----|----|
| Joint           | 59  | 30.8|
| Gastrointestinal| 6   | 3.15|
| Mucosal**       | 106 | 55.3|

**Other***

| (Genital tract) | 32  | 16.75|
| (Nasopharyngeal) | 42  | 22.12|
| (Mouth/oral cavity) | 26  | 13.69|

**Other*** includes e.g., superficial, body cavity, soft tissue and muscle bleeds.
malignancy or immune dysregulation. We recently identified two patients with acquired factor V inhibitors. The first patient developed an inhibitor in association with a new intramuscular vaccine, a recognized risk factor for an inhibitor. The second patient, however, acquired a factor V inhibitor during an acute influenza A infection, representing a previously undescribed association.

Case Report: A 75 year-old female presented with hemarthrosis. Laboratory evaluation showed anemia (Hgb 9.6 g dL \textsuperscript{–1}) and prolonged clotting times (PT 47.9 s (INR 5.0), aPTT >160 s). Neither clotting time corrected in a mixing study, suggesting the presence of an inhibitor to a coagulation factor in the common pathway. Factor II and factor X activity levels were normal (>50%), but factor V activity was undetectable and a factor V inhibitor titer was 2.4 Bethesda units (B.U.). She was treated with a platelet transfusion, recombinant factor VIII and FEIBA to control bleeding and underwent a cytoresection and resection of a low-grade papillary urothelial tumor. She received a 4-week course of weekly rituximab, which resulted in eradication of the inhibitor and normalization of clotting times. She subsequently developed a recurrence of the inhibitor that was treated successfully with cyclophosphamide and prednisone.

An 84 year-old female presented with dyspnea, lightheadedness and anemia (Hgb 6.5 g dL \textsuperscript{–1}) due to gastrointestinal bleeding. A test for influenza A was positive. Clotting times were prolonged (PT 32.1 s (INR 2.9), aPTT 140) and showed only partial correction in a mixing study. Factor II and factor X activity levels were normal but factor V activity was 13%, and a factor V inhibitor titer was 1.6 B.U. She required daily red blood cell transfusions due to continued blood loss from her GI tract despite treatment with platelet transfusions and FEIBA. The patient did not wish to pursue further therapy and opted instead for hospice care at home.

Conclusions: Malignancy-acquired factor V inhibitors are recognized and may reflect antigenic cross-reactivity between tumor antigens and factor V. Viral infection-associated factor V inhibitors are unusual and have not previously been reported with influenza A. We hypothesize that the emergence of a factor V inhibitor in our patient with influenza A may reflect antigenic drift in this year’s influenza strains. Given the limited efficacy of the current influenza vaccine, we expect an increase in influenza-related events and recurrent autoimmunity and antiphospholipid syndrome.

Renal Function in Patients with Atrial Fibrillation on Target Specific Oral Anticoagulants
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Background: Atrial fibrillation (AF) is a major risk factor for embolic stroke. The target specific oral anticoagulants (TSOAs) are approved for the prevention of stroke in patients with AF. These agents are renally cleared to varying extents and patients with a glomerular filtration rate (GFR) of <40 ml min \textsuperscript{–1} are at increased risk for bleeding when treated with TSOAs.

Objectives: (i) To determine the prevalence of decreased GFR in outpatients with AF. (ii) To determine if renal function testing (RFT) using estimated GFR is routinely obtained prior to initiation of TSOAs in outpatients with AF.

Methods: Outpatients with AF were identified from the Fairview Hospital System cardiology clinics using ICD-9 codes. All patients presenting to the cardiology clinics during October, 2011 were identified to address Objective 1 in a cross-sectional manner. To address our second objective, we identified a cohort of all AF patients during October, 2011 were identified to address Objective 1 in a cross-sectional manner. To address our second objective, we identified a cohort of all AF patients during October, 2011 were identified to address Objective 1 in a cross-sectional manner.

Results: 219 patients presented to the cardiology clinics during October, 2011. The mean GFR was 60.72 ml min \textsuperscript{–1}. 7.2% of the patients had a GFR <30 ml min \textsuperscript{–1}. For the remainder of the patients the GFR distribution in ml min \textsuperscript{–1} was as follows: 30–59 (N = 82, 37.4%), 60–89 (N = 96, 43.8%) and >90 (N = 25, 36.4%). From the cohort of patients initiated on TSOAs during the study period, 173/200 had complete data on the date of initiation of the medication. The mean GFR at the time of initiation of TSOAs was 70.9 ml min \textsuperscript{–1}. Of these 53.1% (N = 92) patients had RFT within 1 week of TNOA initiation, 14.6% (N = 25) had RFT within 8–30 days, 10.4% (N = 18) between 31 and 59 days and 21.9% (N = 38) had not had RFT in >60 days. 9/38 patients from the latter subgroup did not have RFT in the 60 days preceding initiation of the anticoagulant. 21 of the 21 patients (12.3%) developed either a major or minor bleeding event while on anticoagulation. We found no difference in the mean interval between RFT and initiation of anticoagulation developed either a major or minor bleeding event while on anticoagulation. We found no difference in the mean interval between RFT and initiation of anticoagulation.

Conclusions: With newly emerging data demonstrating the safety and efficacy of TSOAs in real-life cohorts, the use of this class of medications is likely to increase several-fold in the coming years. In our study, mild renal insufficiency was common in patients with AF, with most patients having GFR between 60–89 ml min \textsuperscript{–1}. Further, a sizeable number of patients (21.9%) were initiated on TSOAs without baseline testing of renal function. This highlights an important need to develop standard guidelines not only for initial testing but also for routine monitoring of renal function in this group of patients.

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BAY 81-8973: A New Third-Generation rFVIII Created Through State-of-the-Art Manufacturing, Offering Dosing Flexibility to the Hemophilia A Community
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Background: BAY 81-8973 is a new full-length recombinant factor VIII (rFVIII) produced by recombinant DNA technology. BAY 81-8973 has the same amino acid sequence as Bayer’s sucrose-formulated rFVIII (rFVIII-FS).

Objectives: To introduce a new rFVIII, its manufacturing process, and its potential for dosing flexibility for prophylaxis in hemophilia A.

Methods: Human- and animal-derived raw materials are not used in the cell culture, purification, and formulation processes of BAY 81-8973. To achieve a high level of virological safety, the manufacturing process incorporates dedicated viral clearance steps which include detergent-mediated virus inactivation and 20-nm filtration for removal of viruses (including small and nonenveloped ones). The process includes robust elimination of high molecular weight protein aggregates. Purification methods of ion exchange chromatography, monoclonal antibody immunosorbent chromatography, and other chromatographic steps are designed to purify BAY 81-8973 and remove process- and product-related impurities. In the clinical development program, BAY 81-8973 was evaluated in adolescent and adult patients with hemophilia A in 2 registration protocols. Patients received 2x/week or 3x/week prophylaxis with BAY 81-8973 in both trials (and on-demand treatment in the second trial); in the first trial physicians assigned the dosing regimen and in the second trial all patients were randomized to 1 of the 2 prophylaxis regimens or on-demand. The BAY 81-8973 dose in the first trial was 20–50 IU kg \textsuperscript{–1} for both arms; in the second trial the dose was 20–30 IU kg \textsuperscript{–1} (low dose) for the 2x/week arm and 30–40 IU kg \textsuperscript{–1} for the 3x/week arm.

Results: A total of 142 patients were evaluated in the 2 trials. The mean dose per infusion for prophylaxis was similar across the 4 arms studied. Annualized bleeding rates (ABRs) are summarized in the table. Median (quartile 1; quartile 3) annualized point bleeding rates were very low: 1.0 (0.3); 2.0 (0.6) for the first and second trials, respectively. Incidence of treatment-related adverse events was low, and no inhibitors developed in this previously-treated population.

Conclusions: BAY 81-8973, developed using a state-of-the-art process, demonstrated efficacy in prophylaxis with both a lower-dose 2x/week regimen and a standard-dose 3x/week regimen, offering the possibility for reduced frequency of infusions, flexibility of dosing, and cost savings for prophylaxis in hemophilia A. This study was funded by Bayer HealthCare AG (Leverkusen, Germany).

Children’s Hospital-Acquired Thrombosis Database (CHAT): A Multi-institutional Database for Prospective Identification of Independent Risk Factors
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Background: The incidence of venous thromboembolism (VTE) in hospitalized pediatric patients is rising, leading to increased morbidity, mortality, and healthcare costs. Early VTE risk assessment and risk-based prophylaxis is standard of care in
hospitalized adults and prophylactic anticoagulation has been shown to reduce the incidence of VTE. Previous work in this field is limited to single-institution, retrospective studies, and thus there is a paucity of high-quality, case-control research to delineate what truly constitutes a risk factor for pediatric hospital-acquired VTE (HA-VTE).

Objectives: With this initial pilot study, we aim to assess the feasibility of creating the first large-scale, multi-institutional, database of pediatric HA-VTE patients in order to identify pertinent risk factors and create a scoring system for risk stratification.

Methods: This study includes a preliminary demographic overview from 3 large pediatric hospitals (Children’s Hospital Los Angeles, Children’s Hospital Colorado and Children’s Hospital Orange County). Patients aged 0–21 years of age who were identified as having a VTE, with radiological confirmation, after 48 h of hospital admission from January 2012 to December 2014, were included. Patient data were collected by electronic health record (EHR) review, and included patient sex, age and hospital unit location at the time of HA-VTE diagnosis, as well as VTE location and nature of a central venous catheter (CVC) relationship, if any.

Results: 313 subjects were diagnosed with HA-VTEs and were included in the database. As seen in Figure 1, 137 subjects (44%) were female and 176 (56%) were male, with a median age of 4 years (IQR = 0.42–14). Almost two-thirds of the subjects were either younger than 1 year (32%) or 12 years and older (33%). Two-hundred and twenty-two (71%) subjects had catheter-related VTE, and the most common location was the upper extremity (138 cases, 43%), as seen in Figure 2. The majority of HA-VTE (190 cases, 61%) occurred in patients admitted to an intensive care unit (pediatric, neonatal or cardiac ICU).

Conclusions: This preliminary HA-VTE evaluation in 3 large pediatric hospitals reveals a trend towards HA-VTE in male patients, <1 year of age or adolescent, having a CVC, and admitted to an ICU. Further work is planned, including an extensive medical record review of the patients’ medical history, comorbid conditions, and hospital course to evaluate multiple proposed risk factors for VTE. Control subjects will also be added and incorporated into the risk model development and validation. The number of participating hospitals will also expand up to 100 pediatric centers throughout the U.S. This work will be the foundation for future clinical studies to retrospectively identify and prospectively validate HA-VTE risk factors, as well as the development of randomized clinical trials to evaluate safety and efficacy of pediatric HA-VTE prevention strategies.

Economic Burden of von Willebrand Disease (VWD) in the Hospital Setting Among a US Population

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Background: Individuals diagnosed with von Willebrand Disease (VWD), the most common bleeding disorder, have either a defect, reduced level, or a lack of von Willebrand factor leading to poor ability or inability to clot and consequent bleeding manifestations. Those with severe disease may require hospitalization to treat major bleeding episodes with factor replacement therapy to achieve
hemostatic efficacy. The cost burden associated with such hospital stays has not been well described.

Objectives: Determine total treatment cost and length of stay associated with inpatient treatment of bleeds among VWD patients prescribed factor replacement therapy in the US.

Methods: Adult VWD patients (≥18 years) were retrospectively analyzed using the US Premier Hospital database from 2000 to 2013. Demographics, length of stay (LOS), and total treatment costs were assessed for patients who met two criteria: a secondary ICD-9 diagnosis for VWD (286.4) and receiving factor replacement therapy. Results from this analysis suggest that VWD individuals treated in the inpatient and outpatient settings of care would provide a comprehensive understanding of the total cost burden in VWD.

Research was funded by Baxter Healthcare.

Table 1. Median Cost and LOS by Bleed Location in VWD

| Bleed Location          | % of VWD Inpatient Admissions | Medium LOS (days) | Total Median Inpatient Costs (2013USD) |
|-------------------------|-------------------------------|-------------------|---------------------------------------|
| Gastrointestinal (GI)   | 10.4%                         | 5                 | $37,655 (range $2,355-$591,181)       |
| Epistaxis               | 1.8%                          | 4                 | $23,129 (range $5,951-$275,505)       |
| Intercranial bleed      | 1.3%                          | 8                 | $64,327 (range $14,619-$313,614)      |
| Menorrhagia             | 3.4%                          | 2                 | $14,579 (range $4,568-$399,964)       |
| Joint bleed             | 1.1%                          | 3                 | $29,982 (range $8,597-$608,159)       |
| Muscular                | 1.0%                          | 4                 | $38,571 (range $4,487-$101,110)       |

Results: Most patients had been treated on-demand only prior to enrollment into this study. Their mean annualised bleeding rate (ABR) within 6 months prior to study entry was 47.9.

Conclusion: The data suggest that personalised prophylaxis with Human-cl rh FVIII leads to a more convenient treatment for more than half of the patients with lower factor consumption and a lower ABR while remaining safe and efficacious. This study was supported by Octapharma.

Clots in Adolescent and Young Adult Cancers (CAYAC)

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Background: Little is known about the prevalence, risk factors, and outcomes of thromboembolism (TE) in adolescents and young adults (AYA) with cancer. In a retrospective, pediatric hospital discharge database review, venous thromboembolism (VTE) occurred in 5.3% of 9,721 15-24 year-old oncology patients (O’Brien, et al., Pediatr 2011). Another study showed that TEs occurred in 16% of the pediatric and young adult patients (4-32 year olds) with sarcomas at the NIH Clinical Center (Paz-Puel et al., J Clin Oncol, 2007). The aim of this retrospective study was to determine characteristics and risk factors among AYA patients at Children’s Hospital Los Angeles (CHLA) with VTE.

Methods: At CHLA, all oncology patients with VTE are consulted on by the Coagulation Team and are captured in our Thrombosis Database. This retrospective chart review included all AYA patients (15-23 years old) with malignancy and VTE identified in the CHLA Thrombosis Database between January 1, 2000 and April 31, 2014. The following data was extracted from the patients’ medical records: demographics, medical history, time from surgery, central-venous catheter (CVC) data, VTE diagnostic data, laboratory findings, status of VTE at each subsequent imaging study, and VTE treatment and duration. Descriptive statistics are used to describe the patients and outcomes.

Results: There were 30 patients who fit the inclusion criteria with 37 total VTEs diagnosed; 7 patients had two VTEs. There were 18 males (60%) and 12 females (40%). The mean age of the patients at the diagnosis of malignancy was 15.37 ± 3.48 (standard deviation) years. The mean age at all first VTE episodes was 17.1 ± 1.98 years. Mean age at 2nd VTE (7 patients) was 17.71 ± 2.56 years. Oncologic diagnoses included 14 patients (46.7%) with Pre-B ALL at the time of VTE diagnosis, 5 (16.7%) with AML, 4 (13.3%) with Hodgkin’s Lymphoma, 4 (13.3%) with brain tumors, 2 (6.7%) with sarcoma, and 1 (3.3%) with renal cell carcinoma. Twenty-six of 37 VTEs (70.3%) were found in the upper extremity, while 11 (29.7%) were in the lower extremity. Twenty-one (56.8%) of the VTEs were CVC-related. None were surgery-related. With regards to thrombophilia evaluations, we discovered the following results: 7 patients had decreased protein S activity, 5 had decreased protein C activity, 5 had elevated FVIII activity, 4 had decreased antithrombin activity, 3 patients were heterozygous and 1 was homozygous for Factor V Leiden, and 1 patient had an elevated homocysteine level.

Conclusions: AYA cancer patients have a number of risk factors, including CVCs, type of malignancy, and abnormal thrombophilia results that predispose them to VTE. They are an understudied group of patients who require further investigation. By understanding the characteristics and risk factors that predispose them to VTE, future studies could aim to help prevent this cause of morbidity and mortality.

Saban Scholar Award from The Saban Research Institute of Children’s Hospital Los Angeles.
### Demographics

| Total No. of Patients | 30 |
|----------------------|----|
| Age at Diagnosis of Malignancy | Mean: 15.37 years | Std Dev: 3.48 years |
| Sex | Male: 18 (60%) | Female: 12 (40%) |
| Ethnicity | Hispanic: 15 (50%) | Caucasian: 11 (36.7%) | African-American: 3 (10%) | Asian: 1 (3.3%) |
| Cancer type | ALL (Pre-B): 14 (46.7%) | AML (including APML): 4 (13.3%) | Hodgkin’s Lymphoma: 4 (13.3%) | Brain Tumor: 4 (13.3%) | Sarcoma: 2 (6.7%) | Renal Cell Carcinoma: 1 (3%) |
| Thrombophilia | Decreased protein S activity: 7 | Decreased antithrombin activity: 5 | Elevated Factor VIII Activity: 5 | Factor V Leiden Mutation: 3 Heterozygous, 1 Homozygous | Decreased Protein C activity: 3 | Elevated Homocysteine: 1 |

### VTE Diagnostic Data

| Total No. of VTEs | 37 |
|------------------|----|
| Age at 1st VTE Episode (n = 30) | 17.1 years, 1.98 years |
| Age at 2nd VTE Episode (n = 7) | 17.71 years, 2.56 years |
| BMI (n = 37) | 24.54 kg/m², 7.05 kg/m² |

| Upper Extremity VTE | 26 (70.3%) |
| Lower Extremity VTE | 11 (29.7%) |
| CVC-related | 21 (56.8%) |
| Due to PICCs | 20 (59.5%) |
| Due to Tunneled Lines | 1 (4.8%) |
| Non-CVC-related | 16 (43.2%) |
| Surgery-related | 0 (0%) |
| Symptomatic Finding | 30 (81.1%) |
| Asymptomatic Finding | 7 (18.9%) |
| Due to Tumor Compression | 2 (5.4%) |

### Conclusions

Results of a Pivotal Clinical Trial Evaluating a Full-length Pegylated Recombinant Factor VIII (PEG-rFVIII) with Extended Half-life in Hemophilia A

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Background: Turoctocog alfa is a new B-domain-truncated recombinant factor VIII (rFVIII) for routine prophylaxis and treatment of bleeds in hemophilia A. The pivotal guardianTM1, 2, and 3 trials investigated the safety and previously tried efficacy of turoctocog alfa in previously treated, severe hemophilia A patients. GuardianTM2 is an ongoing, multinational, open-label, non-controlled extension trial involving patients who were previously enrolled either in guardianTM1, guardianTM3, or a third small preceding PK trial.

Objectives: To evaluate the long-term safety of turoctocog alfa in patients of all ages using data from the ongoing guardianTM2 extension trial, and pooled data from guardianTM1, guardianTM3, or a third small preceding PK trial.

Methods: Patients with severe hemophilia A have <1% of normal FVIII levels and experience frequent bleeding, which can be controlled by prophylaxis with FVIII. A full-length FVIII (ADVATE) coherently bound with 20 kDa PEG (PEG-FVIII) demonstrates comparable preclinical, structural, and functional characteristics with ADVATE.

Methods: Prophylactic treatment with PEG-FVIII was examined in previously treated severe hemophilia A patients aged ≥12 years. Based on previous treatment, patients were assigned either to twice weekly prophylaxis (40-50 IU kg⁻¹ for ≥50 exposure days; n = 120) or episodic treatment (10-50 IU kg⁻¹ for 6 months; n = 17).

Results: PEG-FVIII extended half-life by 1.4- to 1.5-fold, increased AUC, and decreased CL compared to the ADVATE. Incremental recovery was comparable. These results confirmed the phase 1 trial. The primary endpoint was met by demonstrating a 90% reduction in mean annualized bleeding rate (ABR) for the prophylactic vs. episodic group using a negative binomial model, which was significantly greater than the predefined 50% reduction (P < 0.0001). In the prophylactic group, the median interquartile range ABR was 1.9 (5.8) and 39.6% had no bleeding, compared to 41.5 (19.4) in the episodic group. More than half (57.4%) of patients treated prophylactically had no hemorrhagic or spontaneous bleeding. ABRs were 0.0 (2.0) and 0.0 (2.2), respectively. The mean dose per prophylactic infusion was 44.4 IU kg⁻¹ at a median interval of 3.6 days. PEG-FVIII was effective for the treatment of bleeding with no major complications. PEG-FVIII was safe and well tolerated. No deaths or other SAEs considered related to PEG-FVIII. None of the patients developed inhibitors or persistent binding antibodies to FVIII, PEG-FVIII, or PEG. Seven adverse reactions were in 6 patients, including headache, diarrhea, nausea, and flushing.

Conclusion: These pivotal trial results demonstrate that PEG-FVIII is safe and efficacious for treating bleeding episodes and for the prevention of ABRs when administered prophylactically twice weekly. PEG-FVIII prophylaxis may permit the use of fewer infusions while maintaining prophylactic efficacy.

This clinical study was sponsored by Baxter Healthcare Corporation and Baxter Innovations GmbH.
Management of Adult Non-severe Hemophilia A patients with Inhibitors: A Practice-pattern Survey
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Background: The most significant treatment complication in modern hemophilia care is inhibitor development. The risk of inhibitor development in mild and moderate hemophilia A patients increases with the number of exposure days and can occur throughout their lifetime, with the median age of onset at about 30 years.

Objectives and Methods: Given the lack of evidence-based guidelines addressing the management of inhibitors in adult non-severe hemophilia A patients, we performed a web-based survey of hematologists from Hemophilia Treatment Centers (HTCs) across the United States to examine current practice patterns. An email invitation explaining the study and containing a link to the survey was sent to a list of HTC physicians (both adult and pediatric) available on the Centers for Disease Control and Prevention (CDC) website. The survey consisted of three patient case scenarios, each with multiple-choice responses representing a range of clinically reasonable management options.

Results: The survey response rate was 16.6%. Of 39 eligible responses, the majority (n = 29, 74%) of hematologists had >10 years of experience caring for adult patients with hemophilia A, and most practiced at HTCs within a university hospital (n = 32, 82%). For an asymptomatic patient with a low titer inhibitor, 77% (n = 30) would observe with awareness of further factor replacement therapy, 10% (n = 4) would start immune tolerance induction (ITI) therapy ± immunosuppressive (IS) therapy and 8% (n = 3) would start single-agent IS therapy (either rituximab or methylprednisolone). Conversely, in a bleeding patient presenting with a low-titer inhibitor, 51% (n = 20) would observe, 28% (n = 11) would start ITI therapy and 13% (n = 5) would start IS therapy. In contrast, if the symptomatic bleeding patient presented with a high-titer inhibitor of 20 BU ml−1, only 18% (n = 7) would observe, 33% (n = 13) would start ITI therapy once the inhibitor titer fell <10 BU mL−1, 10% (n = 4) would start ITI therapy immediately and 22% (n = 9) would start IS therapy alone.

Conclusions: Our survey demonstrates substantial variation in the treatment approaches adopted by experienced hematologists and highlights the need for a registry-based study to determine optimal management given the rarity of this condition.

Table 1. Safety overview

|                         | Total population | US-only population |
|-------------------------|-----------------|--------------------|
| Patients (n)            | 200             | 31                 |
| Exposure (days)         | 72,320          | 8,809              |
| Adverse events (n)      | 877             | 125                |
| **Pooled analysis: guardian™ 1, 2, and 3** |                  |                    |
| Patients (n)            | 225             | 41                 |
| Total exposure (days)   | 88,788          | 11,961             |

Management of adult patients with non-severe hemophilia A with inhibitors

Fig. 1. What is your standard of practice for inhibitor eradication in the following clinical case scenarios? (a) An asymptomatic adult non-severe hemophilia A patient with a low titer inhibitor, (b) a symptomatic bleeding patient with a low titer inhibitor (2 BU/mL); and (c) a symptomatic bleeding patient with a high titer inhibitor (20 BU/mL)? Fig 8, factor VIII; ITI, immune tolerance induction; IS, immunosuppressive * This option was only available (c).

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Retrospective Evaluation of the Utility of the 4T Score in Patients with a Suspected Diagnosis of Heparin-induced Thrombocytopenia: A Cautionary Tale

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Background: The 4T score predicts the clinical likelihood of heparin-induced thrombocytopenia (HIT). Studies have shown that patients with low probability 4T scores are unlikely to have HIT, and current guidelines suggest no additional evaluation or change in treatment for such patients. The aim of this study was to evaluate the use of the 4T score retrospectively in patients with a suspected diagnosis of HIT to determine whether it is feasible to use in our institution to exclude HIT without laboratory testing.

Methods: Review of patients for whom a heparin-induced platelet antibody (Heparin-PF4 Antibody) enzyme immunoassay (EIA) was ordered from April 2011 to Dec 2013 in a tertiary care center. 4T scores were calculated retrospectively for each patient and appropriateness of testing and management were assessed according to published algorithms. Logistic regression analysis was used to evaluate the relationship between EIA optical density (OD) and thrombosis.

Results: 344 EIA were included, most of which were ordered for patients with low probability 4T scores [N = 196; 57.0%]. Intermediate probability scores were calculated for 132 patients (38.4%) and high probability scores for 16 (4.6%). Negative EIA (OD <0.3) were documented for 297 patients. The EIA was indeterminate (OD 0.3–0.39) in 11 and positive in 36 (OD ≥0.4). The mean 4T score for patients with a positive EIA was 4.2 ± 1.8, and 3.0 ± 1.9 and 3.0 ± 1.5 for those with intermediate and negative EIA, respectively. Of the 36 patients with a positive EIA, 12 (33%) had a low probability 4T score and 24 (67%) had intermediate or high probability scores. Of the 12 patients with low probability scores and positive EIA, 5 EIA were positive at >2 OD (42%) and 3 patients developed arterial or venous thrombosis. When the entire study population was examined, OD ≥0.4 was associated with an increased the risk for thrombosis compared to OD <0.4 (OR 2.6, 95% CI = 1.1–6.0, P = 0.03).
Evaluation of Venous Thromboembolism Mechanical Prophylaxis Use in Pediatric Trauma Patients

**Background:** Pediatric venous thromboembolism (VTE) is increasing in incidence and pediatric trauma patients represent a high-risk cohort. There are no clear guidelines irrespective of the presence of absence of HIT. Our study is limited by its retrospective nature, and prospective evaluation of this topic is indicated.

**Objective:** The purpose of this study was to evaluate the use of mTP modalities in pediatric trauma patients via the Pediatric Health Information System (PHIS) database. Clinical factors associated with use of mTP and VTE incidence and use of mTP were explored during a 2-year period. In addition, we evaluated use of mTP over a ten-year span.

**Methods:** We evaluated patients aged 0–18 years from the last two years, 1/1/2012 through 12/31/2013, and yearly trends of mTP use from 1/1/2004 through 12/31/2013. We utilized International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) codes to define pediatric trauma patients. The PHIS database was queried for age, length of stay (LOS), and the following risk factors associated with VTE: intensive care unit (ICU) admission, central venous catheter (CVC), acute spinal cord injury (ASCI), and hip fracture. A logistic regression model was used to evaluate the association of mTP modalities (GCS or SCD) with the following patient characteristics: age (≥12 vs. <12 years), LOS (≥7 vs. <7 days), ICU admission, CVC presence, ASCI, and hip fracture. To evaluate the likelihood of VTE for those who had mTP modalities compared to those without, adjusted for the aforementioned patient characteristics, we used propensity score (PS) analysis to minimize selection bias.

**Results:** Among 63,114 patients, there were 60.7% boys and 29.6% were at least 12 years old. Approximately 17.8% patients had LOS for 7 or more days, 22.4% were admitted to ICU. 8.2% had CVC. 0.5% had hip fracture and 0.6% had spinal cord injury. 3.5% of patients received mTP and VTE incidence was 0.7%. Use of mTP was significantly associated with each of the variables explored. Use of mTP had a non-overlap of 95% CI compared to those without, adjusted for the aforementioned patient characteristics.

**Conclusions:** Use of mTP increased during a 2-year span and was associated with patient characteristics noted to be clinical risk factors for VTE. During a 10-year span, use of mTP increased in the latter years despite modest increase in VTE incidence. Future studies should focus on using prospective, multi-institutional study design for unbiased assessment of efficacy, safety, and cost-benefit.

**Figure 1.** Yearly incidence of mTP modalities and VTE in pediatric trauma patients from 2004–2013.

**Table 1.** Evaluation of mTP use and patient characteristics and VTE incidence

| Patient Characteristic | Association: Patient characteristic and mTP | Association: VTE and mTP |
|------------------------|-------------------------------------------|--------------------------|
| Age                    | OR (95% CI, p-value)                       | OR (95% CI, p-value)     |
| ≥12 vs <12 years       | 6.6 (7.8-9.5, <0.0001)                     | 1.3 (0.7-2.4, 0.040)     |
| Length of Stay          |                                           |                          |
| ≥7 vs <7 days          | 2.5 (2.2-2.8, <0.0001)                     | 8.9 (5.3-15, <0.0001)    |
| ICU Admission           | Yes vs No                                 | 2.8 (2.6-3.1, <0.0001)   | 0.9 (0.4-1.6, 0.636)    |
| CVC Presence            | Yes vs No                                 | 2.1 (1.8-2.4, <0.0001)   | 1.0 (0.3-3.1, 0.9526)   |
| Hip Fracture            | Yes vs No                                 | 3.3 (2.3-4.8, <0.0001)   | 1.9 (0.7-5.0, 0.2073)   |
| ASCI                   | Yes vs No                                 | 3.1 (2.3-4.2, <0.0001)   | 1.6 (0.9-3.0, 0.1264)   |

*OR: Odds Ratio, CI: Confidence Interval*
count in the aforementioned group was 490,000/mm$^3$ as compared to 226,000/mm$^3$ in subjects without thrombocytosis. Thrombocytosis was associated with significant elevations in pro-angiogenic cytokines, notably angiopoietin-2 (578.6 vs. 318.1 pg ml$^{-1}$, \(P = 0.0015\)), vascular endothelial growth factor (95.1 vs. 49.9 pg ml$^{-1}$, \(P = 0.0074\)), human growth factor (1,013 vs. 645 pg ml$^{-1}$, \(P = 0.0018\)), platelet endothelial cell adhesion molecule (5,597 vs. 3,307 pg ml$^{-1}$, \(P < 0.0001\)), platelet-derived growth factor (348.4 vs. 162.9 pg ml$^{-1}$, \(P = 0.0032\)), and interleukin-8 (243.8 vs. 98.7 pg ml$^{-1}$, \(P = 0.0002\)) as compared to subjects without thrombocytosis. Additionally, thrombocytosis was found to be associated with elevated leukocyte count, 10.7 vs. 6.5 \(\times\) 10$^9$ ml$^{-1}$ (\(P = 0.0005\)), respectively. However, TF antigen (14.5 vs. 19.4 pg ml$^{-1}$, \(P = 0.12\)) and TF MP-PCA (0.41 vs. 0.27, \(P = 0.45\)) were not significantly different. A total of 14 subjects (17.5%) experienced thromboembolic events during the study, defined as deep venous thrombosis, cerebral venous sinus thrombosis, or pulmonary embolism. 3 subjects had experienced thromboembolic events during the study, defined as deep venous thrombosis, cerebral venous sinus thrombosis, or pulmonary embolism. 3 subjects had experienced one VTE event during the investigative period, 6 of the 14 (42%) subjects who experienced VTE had thrombocytosis. There was no appreciable difference in cytokine levels between subjects who experienced VTE vs. those without VTE in this study.

Conclusions: The significance of these results again establishes a strong correlation between elevated platelet counts in malignancy and a pro-angiogenic, pro-thrombotic state, with significantly increased levels of angiopoietin-2, vascular endothelial growth factor, human growth factor and platelet-derived growth factor.

A Rare Case of Factor XIII Deficiency Along with von Willebrand Disease: Thromboelastographic Findings

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Background: Factor XIII deficiency is a rare bleeding disorder with frequency of 1 in 3-5 million people, inherited in an autosomal recessive pattern. Thromboelastography (TEG) offers new insight into the evaluation of the hemostatic disturbance in this disorder. We report the case of a patient, where an additional hemostatic abnormality was detected during TEG investigation of the FXIII deficiency.

Case Report: The patient is a 6-year-old Lebanese-American male who had significant past bleeding history that included post circumcision bleeding at birth, cranial hematomata following a fall requiring surgical evacuation at 8 months of age, and post dental surgery bleed which required plasma at 4 years of age. Family history was negative for any other individual with a significant bleeding disorder except that his sister was reported to have easy bruising, and mother had a history of two first trimester miscarriages. His investigations revealed normal PT, PTT, fibrinogen levels, but the urea clot lysis was abnormal and FXIII activity was 89% and activity to antigen ratio was 0.45. R2 values are marked and p-values displayed.

Results: TEG showed a distinct pattern of poor clot formation and exaggerated clot lysis (low angle and MA), poor strength (G) and exaggerated clot lysis (Ly30) in FXIII deficiency with VWD type 2A, when compared to those with FXIII deficiency alone or VWD type 2A alone. The clot lysis parameter (Ly30) improved after initiating plasma based FXIII replacement. TEG with tissue plasminogen activator showed rapid lysis prior to FXIII replacement and improvement with FXIII therapy.

Conclusions: Severe FXIII deficiency (plasma factor <1%) characteristically presents with spontaneous intracranial hemorrhage and umbilical cord bleeding during infancy, severe bleeding episodes, poor wound healing and spontaneous abortions. Both FXIII deficiency and VWD type 2 are rare bleeding disorders, and their co-existence is even rarer. Literature search revealed 2 other pediatric cases with this combination. TEG can help not only with the diagnosis of FXIII deficiency; it can also be employed to monitor hemostatic correction with factor replacement. TEG also offers a unique method of evaluating the clot formation capacity in those with a combination of rare bleeding disorders and thereby allows us to assess the risk of bleeding and efficacy of the treatment.

The Use of a Novel Microfluidic Assay to Evaluate Von Willebrand Disease and Mucocutaneous Bleeding

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder. Conventional assays cannot accurately detect all abnormalities in von Willebrand factor (VWF)-platelet-collagen interactions or predict a bleeding phenotype in patients with VWD.

Objective: We hypothesized that a microfluidic assay (MFA) could complement current laboratory assays and better correlate with clinical bleeding in patients with VWD or mucocutaneous bleeding (MCB), defined as clinical bleeding but normal VWF levels/platelet aggregation.

Methods: Patients: Patients with VWD or suspected to have VWD are enrolled in an IRB-approved study. Bleeding scores (BS) are obtained via the Vincenza bleeding assessment tool. Standard Assays: Samples are assayed for platelet count, platelet aggregation (collagen/ristocetin/ADP), VWF antigen (VWF:Ag), VWF activity (VWF:RCo), and PFA100 closure time (ADP and EPI). MFA: Collagen (500 µg mL$^{-1}$) is patterned in a MFA and whole blood is perfused at three shear rates, low (150 s$^{-1}$), medium (750 s$^{-1}$), and high (1500 s$^{-1}$) for five minutes. Images of platelet adhesion/aggregation are collected every 3 s at set points. Data Processing: Total platelet surface area is determined and graphed over time. Two outputs are evaluated, (i) lag time: time to reach 5% surface coverage and (ii) slope: slope during the linear portion of platelet adhesion/aggregation.

Results: Patients: 22 samples were obtained from 19 patients with VWD/MCB. 12/22 (55%) are from VWD type 1 patients, 2/22 (9%) are from VWD type 2 patients, and 8/22 (36%) are from patients with MCB. Standard Assay Correlation: Compared to the PFA100-ADP (R$^2 = 0.21$, \(P = 0.02\)) and PFA100-EPI (R$^2 = 0.25$, \(P = 0.04\)), the low shear lag time (R$^2 = 0.30$, \(P = 0.02\)), mid shear slope (R$^2 = 0.34$, \(P = 0.02\)) and high shear slope (R$^2 = 0.41$, \(P = 0.06\)) correlated better with VWF:Ag (Figure 1). For VWF:RCo, the high shear slope correlated better (R$^2 = 0.38$, \(P = 0.009\)) than the PFA100-EPI (R$^2 = 0.31$, \(P = 0.01\)) but not as well as the PFA100-ADP (R$^2 = 0.50$, \(P = 0.001\)).

Fig. 1. Correlation of Multiple MFA Parameters with VWF:Ag Levels: Comparison of clinical laboratory values with MFA parameters in all patients. Compared to the PFA100-ADP (Figure 1B) and the PFA100-EPI (1C), the low shear lag time (1D), mid shear slope (1E), and high shear slope (1F) all correlated better to VWF:Ag levels. VWF:RCo has the highest correlation with VWF:Ag (1A). R$^2$ values are marked and p-values displayed.

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**Differences in Risk Factors and Outcomes in Pediatric Pulmonary Embolism and Non-pulmonary Embolism Thrombosis: A Single Center Study**

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**Background:** The incidence of pediatric venous thrombosis (VTE), which includes both deep venous thrombosis (DVT) and pulmonary embolism (PE), has increased. In adults, significant differences exist for risk factors and outcomes between patients with PE and non-PE DVT. Such data are not known for children.

**Aims:** To evaluate differences in risk factors and outcomes between PE and non-PE DVT in a single center pediatric VTE cohort.

**Methods:** With institutional IRB approval, medical records of patients from 2001 to 2014, with confirmed cases of thrombosis from this Children’s Hospital were reviewed and analyzed with use of IBM SPSS software.

**Results:** There were 372 patients with thrombosis, 311 non-PE (83.6%) and 61 PE (16.4%) patients. Of the PE patients, there were 35 (57.4%) females, 26 (42.6%) males; 35 (57.4%) African American (AA); 20 (32.8%) Caucasian (C), 6 others. In this group, subgroups, such as those with MCF and normal VWF levels, may trend to a better predictor of bleeding than currently available tests.

**Conclusion:** PE patients were higher in PE compared to non-PE patients. Women, hemophilia A carrier status should be excluded. Accurate diagnosis is important for optimal perioperative hemostatic management.

**Von Willebrand Disease Type 1/Type 2N Compound Heterozygotes: Diagnostic and Management Challenges**

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**Background:** Co-existence of types 1 and 2N VWD is rare. In type 1 patients (pts) the FVIII:C is typically concordant or somewhat higher than the VWF:Ag, while homozygous 2N pts have disproportionally low FVIII:C. Identification of compound heterozygous pts is important for providing optimal hemostatic support: Type 1 pts respond to DDAVP, while 2N pts require FVIIa concentrate.

**Objectives:** To describe the presentation, diagnosis and outcomes of compound heterozygous types 1/2N VWD patients.

**Methods:** Retrospective chart review of VWD pts followed by the Mayo Comprehensive Hemophilia Center, Rochester MN.

**Results:** Case 1: 44 yr female with spontaneous hematomas, menorrhagia and post-surgical bleeding had VWF:Ag 47% (55–200%), VWF:RCo 50% (55–200%), FVIII:C 16% (55–200%), FVIII:C/VWF:Ag ratio 0.34. VWF FVIII binding 7% (normal >20%). No mutations were found in the F8 gene but she was heterozygous for the VWF Arg854Gln mutation.

Case 2: 25 yr female with bruising, epistaxis, menorrhagia and postsurgical bleeding had VWF:Ag 48%, VWF:RCo 53%, FVIII:C 25%, FVIII:C/VWF:Ag ratio 0.32. After receiving DDAVP for dental extraction she developed a jaw hematoma. She was identified as heterozygous for the Arg854Gln mutation and since has had uncomplicated dental extractions with Humate-P replacement.

Case 3: 19 yr female with easy bruising and menorrhagia. A younger brother was diagnosed with type 3 VWD. Testing showed VWF:Ag 14%, VWF:RCo 15%, FVIII:C 50%. FVIII:C/VWF:Ag ratio 0.71. She was heterozygous for Arg854Gln mutation. She had uncomplicated spinal surgery with Humate-P replacement.

Case 4: 23 yr male had a prolonged APTT before total hip arthroplasty. He had hemarthrosis in his late teens but no additional bleeding. VWF:Ag 35%, VWF:RCo 32%, FVIII:C ranged 1–12%, with FVIII:C/VWF:Ag ratio 0.34. He was heterozygous for Arg914Gln. He had a poor response to DDAVP for oral surgery and received Humate-P for adenosinectomies without complications.

Case 5: 65 yr male was seen prior to kidney transplantation for glomerulonephritis. He had a history of easy bruising and bleeding after oral surgery and was diagnosed with VWD after a transfusion reaction. Positive VWF:Ag 41%, VWF:RCo 29%, FVIII:C 31%, FVIII:C/VWF:Ag ratio 0.75, VWF:FVIII binding 12%, heterozygous for Arg854Gln. He had an uncomplicated renal transplant with Humate-P replacement.

**Conclusion:** In our series the mean VWF:C/FVIII:C was 0.34 (range 0.34 to 0.75). A low FVIII:C/VWF:Ag ratio (<0.7) should prompt performance of VWF:FVIII binding assay, with reflexive genetic confirmation if clinically indicated, to evaluate for type 2N VWD. In women, hemophilia A carrier status should be excluded. Accurate diagnosis is important for optimal perioperative hemostatic management.

**Pharmacokinetic (PK) Comparison of Two Fibrinogen Concentrates for the Treatment of Congenital Fibrinogen Deficiency**

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**Background:** Patients with congenital afibrinogenemia and hypofibrinogenemia can experience severe bleeding episodes at birth or early childhood. Bleeding may occur after a minor trauma or a small surgical intervention, into the skin, mucosa, muscles, gastrointestinal tract, or the brain. Therapeutic substitution with human fibrinogen concentrate can correct the haemostatic defect and arrest the bleeding in patients with these fibrinogen deficiencies. Octafibrin is a plasma derived, highly purified, lyophilized, fibrinogen concentrate, and includes two dedicated virus inactivation/removal steps. In a clinical study, the PK profile of this new concentrate is compared to a commercially available product (Haemocomplettan® P/RiaSTAP®). The study includes a crossover design where both products are given as a single dose in a randomized fashion separated by an observation period and washout of any fibrinogen product prior to infusion. Patients are confirmed afibrinogenemic with baseline fibrinogen activity plasma level of <0.20. All fibrinogen and MCF testing has been performed in a central lab using validated methods.

**Methods:** This ongoing study is a prospective, randomized, open-label, multinational, crossover comparison of Octafibrin to an existing marketed product, including comparison of a surrogate efficacy endpoint Maximum Clot Firmness (MCF) measured by ROTEM®. The study includes a crossover design where both products are given as a single dose in a randomized fashion separated by an observation period and washout of any fibrinogen product prior to infusion. Patients are confirmed afibrinogenemic with baseline fibrinogen activity plasma level of <0.20. All fibrinogen and MCF testing has been performed in a central lab using validated methods.

**Results:** Nine adult and adolescent patients completed the study the end of May 2014 and were included in a planned interim analysis. As of January 2015, 23 patients have completed the study and enrollment is closed. There have been no reports of adverse events (AE) related to the infusion of this novel concentrate. Comparable PK profiles between the products were seen in the interim analysis but in key parameters, Normalized Area Under the Curve (AUCnorm) and clearance (Octafibrin 0.522, Haemocomplettan® P/RiaSTAP® 0.571 h·mg/mL/mg kg·1, P-value 0.014) and washout of any fibrinogen product prior to infusion. Patients are confirmed afibrinogenemic with baseline fibrinogen activity plasma level of <0.20. All fibrinogen and MCF testing has been performed in a central lab using validated methods.

**Conclusion:** In conclusion, the interim data from this study showed in general a comparable PK profile for Octafibrin and Haemocomplettan® P/RiaSTAP® in patients with congenital fibrinogen deficiency. Octafibrin showed a significantly higher AUCnorm and lower clearance than the comparator. The haemostatic efficacy of...
Octafibrin, as measured by change in MCF as a surrogate parameter, was similar to that of the licensed comparator used in this study, and there was no related AE or SAE for Octafibrin after single-dose administration. This study was supported by Octapharma.

Assessment of Quality of Anticoagulation Therapy for Hospitalized Children: A Clinical Audit of Warfarin Therapy at Lurie Children’s Hospital

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Background: Anticoagulation medications such as warfarin are ranked among the top 50 medications causing patient harm from medication errors. Preventing harm due to anticoagulation medications has remained a National Agenda since 2008 [National Patient Safety Goal 03.05.01]. In efforts to ensure the safety of in-patients receiving anticoagulation therapy, Lurie Children’s Hospital (LCH) developed hospital-wide anticoagulation policy and established a hematologist led anticoagulation service (ACS) in 2010. The function of ACS is to monitor and round on every in-patient receiving anticoagulation therapy, to coordinate outpatient monitoring and to educate patients and their families about anticoagulation medications. This study was designed to examine the process of implementation of the anticoagulation program and barriers of success.

Objectives: 1. To assess “conformance quality” i.e. the extent to which anticoagulation policies are correctly and consistently applied; 2. To study safety of warfarin therapy; 3. To assess existence of quality gaps while receiving anticoagulation therapy and develop policies to improve overall conformance quality.

Table 1. Conformance quality measures and planned intervention

| Quality Indicator | Expectation | Observed value & percentages (N = 45 hospital admissions) | Planned Intervention |
|------------------|-------------|----------------------------------------------------------|----------------------|
| Anticoagulation consult prior therapy initiation | 100% | 10(22%) | Healthcare provider education & CPOE |
| Baseline INR | 100% | 34(76%) | CPOE |
| Loading dose given | 100%* | 0(0%) | SOP Revision CPOE |
| Bridging protocol followed for patients requiring elective or emergent surgery | 100% | 13**(54%) | CPOE |
| Discharge delay due to achievement of therapeutic INR | 0% | 5(12%) | Provider education |
| Documentation of patient education by anticoagulation service*** | 100% | g****(67%) | Discharge checklist for trainees and bedside nurse |
| Post-discharge follow up scheduled | 100% | 26(65%) | Discharge checklist for trainees and bedside nurse; Improved communication across the disciplines |
| TTR | NA | 28.47% | Provider education |
| Documentation of anticoagulation rounds | 100% | 35(78%) | Discharge checklist for trainees and bedside nurse |

Abbreviations: CPOE, Computerized Physician Order Entry; SOP, Standard Operating Procedure; TTR, Time in Therapeutic Range

*All new patients without an impending risk of bleeding
** Only 24 patients required surgical bridging
*** For all new anticoagulation patients
**** Only 12 patients were new to anticoagulation
Table 1. Ratios of Octafibrin Relative to Haemocomplettan® PRTiS® for AUC and AUCnorm (PK Population, $N = 9$)

| Parameter ratio | Mean | 90% CI of mean ratio | p-value |
|-----------------|------|----------------------|---------|
| AUC             | 139.11 | 79.25, 246.13       | 0.0173  |
| AUCnorm         | 139.96 | 80.26, 245.94       | 0.0144  |

Methods: A retrospective audit of clinical records of all inpatients receiving warfarin therapy in 2013. The exclusion criteria were: hospitalization <48 h and administration of <2 doses of warfarin. The conformance quality indicators were: indication of anticoagulation, medication administration record, dosing and monitoring of INR values, Time spent in therapeutic range (TTR), ACS documentation, bridging strategies and care-communications. Safety outcomes were evaluated by bleeding events and mortality directly related to warfarin therapy.

Results: A total of 85 admissions required warfarin therapy. Forty admissions were excluded: admissions <48 h, n = 15; warfarin dosed <2 times, n = 22; warfarin does not verified by both the Medical Administration Record and the pharmacy database, n = 3. Electronic medical records from the 45 qualifying admissions were eligible. Table 1 shows the conformance quality outcomes. It is noteworthy that 93.3% of children were on medications known to interact with warfarin (grade C or more). Despite this, ACS was consulted in 22% of children before initiating warfarin therapy while 7% of admissions had no documented ACS encounters. In regards to anticoagulation safety, bleeding events occurred in 18% of admissions and none were consulted by ACS prior to management. Recurrent thrombosis occurred in 11% (5/45) of admissions and only one was consulted prior to management.

Conclusion: The results of the audit suggested that despite implementation of ACS, the quality and safety of anticoagulation was suboptimal at LCH. Future efforts will focus on implementing anticoagulation education to healthcare providers, enforcing a safety checklist for trainees and bedside nurses, computerized clinical care pathways and provider order entry, and improving communications across the disciplines.

The Adjudication Process and Results of Assessment of Refractoriness of the Observational, Multinational Glanzmann’s Thrombasthenia Registry (GTR)

M. RECHT, 1 M. RAJPURKAR, 2 M. CHITLUR, 2 D. COOPER, 3 R. D’OIRON 4 and R. ZOTZ 5 ET AL.

Objective: The Adjudication Process and Results of Assessment of Refractoriness of the Observational, Multinational Glanzmann’s Thrombasthenia Registry (GTR) will focus on implementing anticoagulation education to healthcare providers, enforcing a safety checklist for trainees and bedside nurses, computerized clinical care pathways and provider order entry, and improving communications across the disciplines.

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Inactivation of Emerging Viruses by Pasteurization in Plasma-Derived Coagulation Factors and Wound Healing Therapies

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Emerging zoonotic viruses such as Chikungunya virus (CHIKV), Middle East Respiratory Syndrome coronavirus (MERS-CoV), West Nile virus (WNV), Dengue virus (DENV), hantavirus, and avian influenza viruses have raised concern about their potential presence in blood/plasma. To date, only DENV and WNV have been reported as being transmitted by blood components, whereas the remaining viruses present a theoretical concern. Unlike for blood-components, there have been no confirmed cases of transmission of these emerging viruses by plasma derivatives manufactured according to current state-of-the-art processes. However, there is an ongoing concern for the safety of the starting material for plasma-derived medicinal products (PDMPs) regarding
Incidence and Outcomes of Venous Thromboembolism after Surgical Repair for Congenital Heart Disease: A Retrospective PHIS Analysis

M. SILVEY, M. HALL, E. BILYNSKY and S. CARPENTER

1 Children’s Mercy Hospital, Kansas City, Missouri, USA; and 2 Children’s Hospitals

Background: Congenital heart disease (CHD) affects ~1% of all live births, has a wide range of severity, and may need several surgical procedures for complete repair. Venous thromboembolism (VTE) is increasing in frequency in hospitalized pediatric patients, with the highest rates in patients <28 days old and teenagers (Raffini et al., Pediatrics, 2009). We sought to evaluate the incidence, morbidities, and mortality rate of CHD pts who undergo surgical repair.

Objective: To determine morbidity and mortality rate of CHD patients who undergo surgical repair and develop VTE.

Methods: This retrospective study reviewed patients within the Pediatric Health Information System (PHIS) database diagnosed from 2004 to 2012 with CHD who underwent surgical repair. Demographics including diagnosis, surgical procedure, payer, length of hospital and ICU stay, mortality, and associated illness were included in the analysis. Inclusion criteria were discharge diagnosis of CHD, cardiac surgical procedure codes, and age <18 years. VTE rates were calculated by dividing the number of VTE by the total number of procedures. Demographic characteristics between those with VTE vs. without VTE were compared using Wilcoxon-rank sum or chi-squared analysis as appropriate. VTE rates were compared separately for each surgical procedure. Mortality was modeled using proportional hazards and adjusted for important clinical and demographic factors.

Results: Within the study period, 91,069 CHD pts had corrective surgery and 2655 (2.9%) of these pts developed thrombosis. Rate of VTE increased by 253% over the study period (P < 0.001) from 1.7% in 2004 to 4.4% in 2012. The procedures with the highest VTE rate were systemic to pulmonary shunt placement (34.3%) and septostomy procedure (26.1%). Of those who developed VTE, children younger than 28 days had the highest prevalence at 61% (P < 0.001). Those with VTE had longer lengths of stay with a median of 27 hospital days and 10 ICU days vs. 6 and 2 days for patients who did not have VTE (P < 0.001). Adjusted cost was higher for VTE pts at $126,257 vs. $40,773 (P < 0.001). VTE was also associated with higher rates of bacteremia and endocarditis [8.3% vs. 0.7% and 3.4% vs. 0.2%, P < 0.001]. Mortality was significantly higher in those with VTE [12.3% vs. 0.8%, P < 0.001]. The adjusted hazard ratio for mortality in patients with CHD and VTE was 5.5 (P < 0.001).

Conclusions: VTE incidence in CHD pts who undergo surgical repair are increasing. VTE results in increased length of hospital stay, number of ICU days, and increased cost.
Men with 1 or more risk factors for VTE also have a significantly higher rate of bacteremia. Mortality is significantly higher with an increased hazard ratio. More research is needed to help understand the development of VTE. More understanding of these mechanisms, prevention of VTE may help mitigate morbidity and mortality.

Familial Thrombophilia, Facilitating Development of Osteonecrosis

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Background: Idiopathic osteonecrosis (ON) (not secondary to high dose long term corticosteroids, alcoholism, fracture-dislocation, etc) can be caused by oseous venous occlusion leading to increased oseous venous pressure, reduced arterial influx, and ischemic necrosis. Although not widely recognized, familial thrombophilia is commonly pathoetologic for the development of ON. In this study, we examined the interaction between testosterone treatment TT and previously undiagnosed thrombophilia facilitating the development of ON in 12 men and 4 women who developed idiopathic ON 6 months (median) after starting TT.

Results: ON was “idiopathic” in the cases, in the patients who had not taken high dose-long term corticosteroids, were not alcoholic, and had no hip dislocation or pinning. In 10 of the 16 cases, ON developed within 6 months after starting TT, and in all 16 cases, after a median of 6 months. Five of the 16 cases (31%) were found to be heterozygous for the Factor V Leiden mutation vs. 2/109 healthy controls (P = 0.0003). Four of the 16 cases (25%) had high (>150%) Factor VIII vs. 7/103 (7%) healthy controls (P = 0.04), 3 of 16 (19%) had high (>150%) Factor XI vs. 3/101 (3%) healthy controls (P = 0.03). Of the 16 ON patients, 14 (88%) had ≥1 abnormal precipitant (of the 8 measured) vs. 4/710 (43%) of 110 healthy controls (P = 0.0009). Of the 5 cases where serum extrudate (E2) was measured while on TT, E2 was high (≥42.6 pg ml⁻¹) in 4. High E2 while on TT is thrombophic, and interacts with the underlying familial or acquired thrombophilia.

Conclusions: When TT is given to patients with previously undiagnosed familial thrombophilia, ON commonly occurs, particularly in the presence of Factor V Leiden heterozygosity. Elevation of E2 derived from the aromatization of testosterone interacts with thrombophilia to promote development of ON. Screening for common heterozygosity. Elevation of E2 derived from the aromatization of testosterone treatment TT and previously undiagnosed familial thrombophilia facilitates the development of ON in 12 men and 4 women who developed idiopathic ON 6 months (median) after starting TT.

Supported by the Lipoprotein Research Fund, Jewish Hospital of Cincinnati.

Management of Major Surgeries in von Willebrand Disease Patients Using a High-purity Human VWF/FVIII Concentrate with a Physiological 1:1 Ratio

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Background: Major surgical procedures in patients with VWD (VWD) can be associated with a life-threatening risk of excessive bleeding, thus prothrombinic treatment with exogenous von Willebrand factor (VWF) and coagulation factor VIII (FVIII) may be required to manage these surgeries.

Methods: To calculate the individual recommended pre- and post surgical dosing, an instant recovery system (IRS) was developed in all patients at the beginning of the study. The recommended dose was to achieve a VWF:FVIII peak level of 100% and to maintain trough levels of approximately 50% until wound healing. The final dosing used during surgery was at the discretion of the treating physician. VWF activity and antigen as well as FVIII:C levels were collected throughout the study. Haemostatic efficacy was assessed intra- and post-operatively by surgeons and haematologists independently, using objective 4-point ordinal efficacy scales. Additionally, these assessments were adjudicated by an independent data monitoring committee (IDMC). Safety and immunogenicity were monitored throughout the study.

Results: Twenty-eight patients underwent 21 major and 9 minor surgeries, of which 7 (23.3%) were in VWD type 1; 2 (6.7%) in VWD type 2 (1 type 2A, 1 type 2B), and 21 (70.0%) in VWD type 3. Of the 21 major surgeries, 17 were in VWD type 3 patients, 1 in type 2 and 3 in type 1. The mean pre-operative loading dose (VWF:FVIII) for major surgeries was 55.5 IU kg⁻¹ with a mean maintenance dose of 30 IU kg⁻¹. Mean FVIII:C and VWF:FVIII peak plasma levels of each patient undergoing major surgery ranged from 120% to 145%, and 66% to 98%, respectively, during maintenance infusions (d1-d7 post surgery) with no accumulation of FVIII:C activity.

Conclusions: The efficacy of 20 of the 21 major surgeries (95.2%) managed with a VWF:FVIII concentrate with a 1:1 activity ratio was rated excellent or good. The study was terminated early because of clear and robust demonstration of efficacy, as prescribed in the protocol. None of the patients experienced excessive intra- or post-operative bleeding that was uncontrolled or required an alternate FVIII:FVIII concentrate. There was no accumulation of FVIII:C activity after repeat dosing. No thrombotic events were reported. No neutralizing inhibitors against FVIII or VWF, or study drug-related serious adverse events (SAEs) were observed.

This study was supported by Octapharma.

The FVIII Plasma Activity of rVIII-SingleChain Can Be Measured in Both the One-stage and Chromogenic Substrate Assays

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1CSL Behring, King of Prussia, Pennsylvania, USA; 2CSL Behring GmbH, Marburg, Germany

Objectives: rVIII-SingleChain is a B-domain truncated, single-chain recombinant factor VIII molecule being developed by CSL Behring (CSLB). Potency determinations and clinical monitoring in the hospital setting, CSLB initiated an international, multicenter, randomized and blinded field study. The field study was designed to

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Determine the inter-laboratory variability of measurements of both rVIII-SingleChain and Advate\textsuperscript{\textregistered} across assay methods. Methods: Plasma samples were spiked at 0.04, 0.3, 0.6 and 1.0 IU mL\textsuperscript{-1} for both rVIII-SingleChain and Advate\textsuperscript{\textregistered}, blinded, and distributed to local laboratories to be assayed by the OS and CS assays. Laboratories were instructed to follow their routine laboratory practices and use their own routine in-house VIII standard, VIII-deficient plasma and assay reagents. A standard Human Plasma standard was included in the sample kit, to allow for recalculation of results against a common standard as well as a product specific standard. Laboratories were instructed to run the samples on three separate days with no more than 15 days time between the first and third run. Results: Preliminary results suggest intra and inter laboratory variability in OS assays were similar for rVIII-SingleChain and Advate\textsuperscript{\textregistered}. For rVIII-SingleChain, the OS assay values underestimated target values at all spiked concentrations. In contrast, the CS assay values were closer to the target value for rVIII-SingleChain. Both the predictable underestimation and comparable variance of rVIII-SingleChain supports an approach to provide clinical guidance on interpretation of PIVILC measurements when the OS assay is used for monitoring.

Diagnosis of Rapid Immunoassays for Heparin-induced Thrombocytopenia: A Systematic Review and Meta-analysis

L. SUN, P. GIMOTTY and A. CUKER

Background: Diagnosis of heparin-induced thrombocytopenia (HIT), a prothrombotic complication of heparin, is based on both clinical and laboratory criteria. Widely used platelet factor 4/heparin ELISAs are highly sensitive but have limited specificity. In addition, many laboratories batch samples and only perform the ELISA once or twice a week, leaving clinicians to make critical initial management decisions without the benefit of laboratory results. Recently, there has been a proliferation of rapid immunoassays (RIs) for HIT, which are designed for single sample testing and provide results in 30 min or less. These assays hold the potential to improve clinical decision making at the point of care, but their performance characteristics remain incompletely characterized.

Objective: We conducted a systematic review and meta-analysis to evaluate the diagnostic accuracy of RIs for HIT.

Methods: We searched PubMed, Google Scholar, and IS Web of Science using the keywords [heparin-induced thrombocytopenia AND (rapid immunoassay OR lateral flow OR particle gel OR particle immunofiltration OR latex OR chemiluminescence)]. We included studies in which samples from patients with suspected HIT were tested with a RI and a functional gold standard test (e.g. serotonin release assay) against which the performance of the RI could be measured. Quality of eligible studies was assessed using QUADAS-2. Estimates of sensitivity and specificity for each RI were calculated by pooling true and false positives and negatives across all eligible studies and applying the binomial-normal model for meta-analysis of proportions.

Results: Nineteen studies met eligibility criteria. Study characteristics are summarized in Table 1. Collectively, eligible studies enrolled 4875 subjects from 11 countries. Six different RIs were evaluated. Figure 1 depicts the sensitivity and specificity of the 4 RIs that were assessed in more than one study. The sensitivity and specificity of the latex agglutination assay [1.00 (0.80–1.00); 0.76 (0.66–0.84)] and particle immunofiltration assay [1.00 (0.03–1.00); 0.68 (0.57–0.77)] were derived from single studies. All assays exhibited high sensitivity ranging from 0.96 to 1.0. There was wider variability in specificity (0.68–0.94).

Conclusions: The high sensitivity of rapid immunoassays suggests that they may serve as reliable “rule-out” tests for patients suspected of HIT. In patients who test negative, the rapid turnaround time may allow for avoidance of unnecessary treatment for HIT. However, their limited specificity indicates that a positive rapid immunoassay result cannot function as stand-alone laboratory evidence of HIT, but requires confirmation with a functional assay. Comparison between studies is hampered by differences in study population and reference standard. Quality assessment revealed several recurring methodologic limitations including non-consecutive enrollment and absence of clinical criteria in the reference standard. This work was funded by HL 112903 (to AC) from the National Institutes of Health.

Table 1. Characteristics of eligible studies.

| Study | Reference | Setting | Population | Median age (mean±SD) | No. (%) | Reference Standard |
|-------|-----------|---------|------------|---------------------|--------|-------------------|
| Parikh, J., Thromb Haemost 2013 | Advate\textsuperscript{\textregistered} | India (116) | Medical ICU and Surgical ICU (116) | 64 (6) | 84 (80–90) | SRA (radioactivity ≥ 2-fold over control) |
| Nellen, 2010 | Surgery and Medical (112) | Switzerland (112) | Medical ICU and Surgical ICU (112) | 60 | 79 | SRA (radioactivity ≥ 2-fold over control) |
| Bakchoul, L., Thromb Haemost 2008 | Surgery and medical (65) | Switzerland (65) | Medical ICU and Surgical ICU (65) | 63 | 82 | SRA (radioactivity ≥ 2-fold over control) |
| Althaus, Br J Haematol 2010 | Surgical (109) | Germany (109) | Medical ICU and Surgical ICU (109) | 73 | 47 | SRA (radioactivity ≥ 2-fold over control) |

Table 1. Continued.

| Study | Reference | Setting | Population | Median age (mean±SD) | No. (%) | Reference Standard |
|-------|-----------|---------|------------|---------------------|--------|-------------------|
| Prakhya, Thromb Res 2009 | Surgery and Medical (102) | Australia (102) | Medical ICU and Surgical ICU (102) | 65 | 82 | SRA (radioactivity ≥ 2-fold over control) |
| Meyer, Berlin 2005 | Surgical and Medical (48) | Germany (48) | Medical ICU and Surgical ICU (48) | 68 | 77 | SRA (radioactivity ≥ 2-fold over control) |
| Nellen, J. Thromb Haemost 2013 | Surgery and Medical (49) | Germany (49) | Medical ICU and Surgical ICU (49) | 63 | 74 | SRA (radioactivity ≥ 2-fold over control) |
| Legnani, Blood Coagul Fibrinolysis 2013 | Surgical and Medical (48) | Italy (48) | Medical ICU and Surgical ICU (48) | 64 | 83 | SRA (radioactivity ≥ 2-fold over control) |

Table 2. Diagnostic accuracy of rapid immunoassays for HIT.

| Study | Reference | Setting | Population | Median age (mean±SD) | No. (%) | Reference Standard |
|-------|-----------|---------|------------|---------------------|--------|-------------------|
| Legnani, Thromb Haemost 2014 | Medical ICU and Surgical ICU (112) | Italy (112) | Medical ICU and Surgical ICU (112) | 64 | 83 | SRA (radioactivity ≥ 2-fold over control) |
| Nellen, 2010 | Surgery and Medical (102) | Switzerland (102) | Medical ICU and Surgical ICU (102) | 63 | 74 | SRA (radioactivity ≥ 2-fold over control) |
| Lenzoni, Br J Haematol 2014 | Medical ICU and Surgical ICU (112) | France (112) | Medical ICU and Surgical ICU (112) | 64 | 83 | SRA (radioactivity ≥ 2-fold over control) |

Abbreviations: RI, not reported; RIAT, platelet aggregation test; HFA, heparin-induced platelet activation assay; SRA, serotonin release assay; ELISA, enzyme-linked immunosorbent assay.

1 All four chemiluminescent assays included both the pspspecific and lgspecific chemiluminescent assays.
Figure 1. Forest plot of sensitivity and specificity of rapid immunoassays for heparin-induced thrombocytopenia.
Design: A single center prospective clinical study was conducted. A total of 23 pediatric patients with ALL getting a single dose of PEG-A during induction cycle were recruited for the study. Baseline TEG was done prior to administration of PEG-A. Follow-up TEG was performed 4 days (± 2 days) and 18 days (± 2 days) after the administration of PEG-A. PT, PTT, fibrinogen and antithrombin were also measured.

Results: The demographic data of the study population is described in Figure 1 Table 1. There was a statistically significant decrease in TEG parameters for Clot Kinetics (Angle), Clot strength (MA) and Fibrinolysis (LY30) following PEG-A. Data from this series suggests that the subjects displaying abnormal TEG parameters appear to be at an increased risk of hemorrhage. However there was also a significant decrease in antithrombin following PEG-A suggesting increased risk of thrombosis but no significant change in clotting time (R) on TEG. The results from the statistical analysis have been summarized in Figure 1 Table 2.

Conclusion: Pediatric ALL has a 90% survival rate. Aggressive treatment with chemotherapy has significant short and long term side effects. As more children are cured, the focus is shifting towards reducing toxicities associated with treatment. This study was designed to identify evidence of coagulation abnormalities using TEG before evidence of bleeding or thrombosis occur. The data supports further evaluation using TEG as a means of guiding therapy for hemostatic abnormalities. TEG is a better global assay of coagulation, which may be more appropriate in this setting, where traditional coagulation testing is inadequate. Clinical prospective studies are necessary to understand the extent of hemostatic changes, standardize assays and design treatment algorithms to validate the use of TEG.

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Table 2. Results of TEG and coagulation studies

| Variable                  | Mean ± Standard Deviation | P Value | Proportion | 95% Confidence Interval | Proportion | 95% Confidence Interval |
|---------------------------|---------------------------|---------|------------|-------------------------|------------|-------------------------|
| TEG Parameters            |                           |         |            |                         |            |                         |
| R: Clotting Time          | 5 to 10 min               | 6.1 ± 1.4 | 0.14       | 0.12 - 0.72             | 0.67       | 0.36 - 0.57             |
| A: Clot Kinetics          | 50 to 70 degree           | 43.8 ± 18.9 | 0.01*      | 0.06 - 0.13             | 0.75       | 0.50 - 0.94             |
| K: Clot Kinetics          | 1 to 3 min                | 5.6 ± 6.6  | 0.1        | 0.04* - 0.09           | 0.75       | 0.50 - 0.94             |
| MA: Clot Strength         | 41.1 ± 14.8               | 38.6 ± 12.8 | 0.01*      | 0.06 - 0.13             | 0.75       | 0.50 - 0.94             |
| Ly30: Clot Stability      | 0 to 9%                   | 0.6 ± 0.9  | 0.04*      | 0.02* - 0.05           | 0.75       | 0.50 - 0.94             |
| Coagulation parameters    |                           |         |            |                         |            |                         |
| PT                        | 15.4 ± 15                  | 56.2 ± 17.1 | 0.01*      | 0.06 - 0.13             | 0.75       | 0.50 - 0.94             |
| PTT                       | 20.8 ± 6.7                | 15.3 ± 9.4  | 0.01*      | 0.06 - 0.13             | 0.75       | 0.50 - 0.94             |
| Fibrinogen                | 129 ± 10                  | 122 ± 9.1  | 0.01*      | 0.06 - 0.13             | 0.75       | 0.50 - 0.94             |

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Fig. 1–Table 1. Demographic data of the 23 study patients.

Fig. 1–Table 2. Results of TEG and coagulation studies.
When to Scan: Computed Head Tomography and Detection of Intracranial Hemorrhage in Children with Hemophilia after Head Trauma

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Background: Hemophilia is a rare X-linked bleeding disorder. Decreased production of Factor VIII or Factor IX impairs hemostasis and places patients at risk of serious bleeding after minor trauma. In the pediatric population, head trauma can result in catastrophic intracranial hemorrhage (ICH) with significant morbidity and mortality. Children with hemophilia presenting after head trauma or with symptoms concerning for increased intracranial pressure (ICP) often undergo a head computed tomography (CT) to rule out ICH. Patients with multiple accidental head injuries over time undergo repeat CT scans and are exposed to significant cumulative doses of radiation, further adding to the morbidity of their underlying disease. There are currently no consensus guidelines to help stratify the need for head imaging based on clinical presentation in this particular patient population.

Clinical questions: (i) What is the incidence of CT scans and ICH in a pediatric hemophilia cohort? (ii) What are the symptoms and signs most frequently associated with CT-demonstrated ICH?

Methods: We reviewed all central nervous system (CNS) events in a cohort of pediatric hemophilia patients followed at our institution over a period of 19 years.

Results: Among seventy-six patients in our cohort, forty-six patients were evaluated with one or more CNS events. A total of 99 CT scans were obtained and eight intracranial hemorrhages were identified, corresponding to a prevalence of 8%. Emesis was the most common complaint at time of presentation, occurring in 62.5% patients with ICH. Twenty-two patients received one or more CT scan, with the total number of scan per patient over the study period ranging from 1 to 17.

Conclusions: We observed a wide range of age and mechanism of injury at time of ICH presentation in our pediatric hemophilia cohort. Our ICH prevalence rate of 8% is in keeping with other published studies. Emesis was reported most often and may be an important indicator in creating imaging guidelines in these high-risk patients.

Target Joint Spontaneous Annualized Bleed Rate Reduction: Results from a Pivotal Trial of Once Weekly BeneFIX (nonacog alfa) in Hemophilia B

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Background: A recent pivotal study of 1/wk BeneFIX prophylaxis with 100 IU kg⁻¹ dose demonstrated a low median spontaneous annualized bleed rate (sABR) of 1.0. Because spontaneous bleeding in target joints can be difficult to control, prevention of such bleeds is a key goal of a prophylaxis regimen.

Objectives: This report examines the reduction of sABR in target joints among patients who experienced any spontaneous bleeding during the prophylaxis period of the study.

Methods: Moderately severe to severe hemophilia B patients (FIX:C ≤ 52%) aged 12-65 and with ≥100 exposure days, who sustained ≥2 bleeding episodes (6 of which must have been joint bleeds) in the prior year were screened (N = 25) in a multicenter, open-label, sequential, 2-period study: 6 months on-demand BeneFIX therapy (dose at investigator’s discretion) followed by 12 months BeneFIX prophylaxis at 100 IU kg⁻¹, 1/wk. The primary endpoint was ABR, regardless of etiology. Data for this post hoc analysis were extracted from the study report.

Results: Of the 25 patients, 12 (48%) did not bleed during the prophylaxis period, and 13 (52%) experienced at least one spontaneous bleeding episode during prophylaxis. The table depicts the reduction in sABR (in those 15 patients who had a spontaneous bleed during prophylaxis) between the On-Demand and Prophylaxis periods for any spontaneous bleeds (left side) and target joint spontaneous bleeds (right side). All 13 patients experienced a reduction in both any spontaneous bleeds and target joint spontaneous bleeds. The target joint sABR during the prophylaxis period was 0.0 for 38.5% of the 13 patients (5/13). Of these 13 patients, 92.3% (12/13) experienced target joint sABR reductions of 66.3% or more.

Conclusion: The 1/wk BeneFIX 100 IU/kg prophylaxis dose regimen lowered the ABR for any spontaneous bleeds and for target joint spontaneous bleeds compared to on-demand dosing in the 13 patients with any spontaneous bleeding during prophylaxis although no statistical testing was done. Over one-third of the 13 patients (38.5%) experienced no target joint spontaneous bleeding at all during the prophylaxis period. Of those patients who did experience a target joint spontaneous bleed in the prophylaxis period, 92.3% had an ABR reduction of 66.3% or more.

This trial was supported by Pfizer, Inc.

Alpha-Granule Deficient Platelets Have Defective Procoagulant Activity and Form Fewer Coated Platelets

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Background: Patients with Gray Platelet Syndrome (GPS) are known to have moderate bleeding symptoms, but the molecular mechanisms of bleeding are incompletely understood. Our lab and others have recently identified the gene responsible for GPS, NBEAL2 (neurobeachin-like-2) (Kahr WH et al. Blood 2013). This trial was supported by Pfizer, Inc.

Objectives: Our objective is to further characterize platelets from Nbeal2 KO mice by evaluating their ability to form coated platelets, a subpopulation of highly activated platelets that retain alpha-granule contents on their surface in response to dual agonist stimulation (Dale GL, J Thromb Haemost 2005), and by measuring their procoagulant activity.

Methods: First, we evaluated the ability of Nbeal2 KO vs. wildtype (WT) platelets to form coated platelets in response to stimulation with thrombin and convulxin. Flow

| Patient | Any spontaneous bleeds | | Target joint spontaneous bleeds | |
| --- | --- | --- | --- | --- |
| A | 35.4 | 7.2 | 97.7 | |
| B | 27.9 | 4.9 | 82.6 | |
| C | 10.4 | 2.9 | 71.8 | |
| D | 48.3 | 12.5 | 74.1 | |
| E | 46.2 | 1.0 | 97.8 | |
| F | 44.0 | 13.8 | 68.5 | |
| G | 27.2 | 9.2 | 66.1 | |
| H | 13.4 | 1.9 | 85.9 | |
| I | 18.3 | 1.0 | 94.6 | |
| J | 29.8 | 2.0 | 93.4 | |
| K | 54.2 | 6.9 | 87.2 | |
| L | 22.4 | 1.0 | 95.7 | |
| M | 14.3 | 1.0 | 93.2 | |

Fig. 1. On Demand versus Prophylaxis Periods for All Spontaneous Bleeds and Target Joint Spontaneous Bleeds.

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cytometry was used to evaluate platelet surface fibrinogen (anti-fibrinogen antibody), platelet fibrinogen receptor GP IIb/IIIa exposure (Joa/A antibody), and phosphatidylserine exposure (annexin V). Coated platelets are characterized by increased surface fibrinogen retention, decreased Joa/A binding and increased phosphatidylserine exposure. We next evaluated Nbeal2 KO vs. WT platelet procoagulant activity, measuring the ability of activated washed platelets to generate thrombin in the presence of prothrombinase complex (Xa/Va) composed either of exogenous Xa + platelet-released Va (labeled Xa in figure 1) or exogenous Xa + exogenous Va in excess (labeled Xa/Va in Figure 1). Platelets were activated by either thrombin or thrombin and collagen (labeled Thr/Col in Figure 1). Thrombin production was measured using a chromogenic assay. Baseline thrombin signal from unstimulated platelets and thrombin agonist was subtracted from the activated platelet signal.

Results: Flow cytometry data indicates that Nbeal2 KO platelets form fewer coated platelets in response to thrombin and convulxin than WT platelets, but they are equally activated by thrombin or convulxin alone. When activated with thrombin and collagen, platelets from Nbeal2 KO mice convert less prothrombin to thrombin compared to WT, if prothrombinase complex Va comes exclusively from platelets (Figure 1). This effect is not seen if excess exogenous factor Va is available to form the prothrombinase complex, suggesting an important role for platelet-derived factor Va in thrombin generation on the platelet surface.

Conclusion: Defective factor Va-dependent procoagulant activity and coated platelet formation as measured in platelets from Nbeal2 KO mice may contribute to the bleeding diathesis in GPS.

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![Graph showing procoagulant experiment results](image)

**Fig. 1.** Procoagulant Experiment Results. Error bars represent standard deviation, n = 3-5, (*, p<0.012).