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PROGRAM & ABSTRACTS

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Pradaxa® dabigatran etexilate

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PRADAXA® LE SEUL ANTICOAGULANT ORAL AVEC UN AGENT NEUTRALISANT SPÉCIFIQUE DISPONIBLE PARTOUT ET REMBOURSÉ EN BELGIQUE[13-16]

▼ Ce médicament fait l'objet d'une surveillance supplémentaire qui permettra l'identification rapide de nouvelles informations relatives à la sécurité. Les professionnels de la santé déclarent tout effet indésirable suspecté. Voir rubrique « Effets indésirables » pour les modalités de déclaration des effets indésirables.

DÉNOMINATION DU MÉDICAMENT Praxbind® 2,5 g/50 mL, solution injectable/pour perfusion **COMPOSITION QUALITATIVE ET QUANTITATIVE** Chaque mL de solution injectable/pour perfusion contient 50 mg d'idarucizumab. Chaque flacon de 50 mL contient 2,5 g d'idarucizumab. L'idarucizumab est produit par la technologie d'ADN recombinant dans des cellules ovariennes de hamsters chinois. Excipients à effet notoire: Chaque flacon de 50 mL contient 2 g de sorbitol et 25 mg de sodium. **FORME PHARMACEUTIQUE** Solution injectable/pour perfusion. Solution translucide à légèrement opalescente, incolore à légèrement jaune. **INDICATIONS THERAPEUTIQUES** Praxbind est l'agent de réversion spécifique du dabigatran et est indiqué chez les patients adultes traités par Pradaxa (dabigatran étexilate) quand une réversion rapide de ses effets anticoagulants est requise : -Pour une urgence chirurgicale ou des procédures urgentes -En cas de saignements menaçant le pronostic vital ou incontrôlés. **POSOLOGIE ET MODE D'ADMINISTRATION** Réservé à l'usage hospitalier. Posologie La dose recommandée de Praxbind est de 5 g (2 x 2,5 g/50 mL). Chez un sous groupe de patients, la réapparition de concentrations plasmatiques de dabigatran libre et l'allongement concomitant des tests de coagulation ont été constatés jusqu'à 24 heures après l'administration d'idarucizumab. L'administration d'une deuxième dose de 5 g de Praxbind peut être envisagée dans les cas suivants: -réapparition d'un saignement cliniquement pertinent en même temps qu'un allongement des temps de coagulation, ou -lorsqu'un nouveau saignement risque de menacer le pronostic vital et qu'un allongement des temps de coagulation est observé, ou -patients nécessitant une deuxième intervention chirurgicale ou des procédures urgentes alors que les temps de coagulation sont allongés. Les paramètres de coagulation pertinents sont le temps de céphaline activé (TCA), le temps de thrombine dilué (TTd) ou le temps d'écarine (ECT). La posologie quotidienne maximale n'a pas été recherchée. Reprise du traitement antithrombotique Le traitement par Pradaxa (dabigatran étexilate) peut être réintroduit 24 heures après l'administration de Praxbind si l'état du patient est cliniquement stable et si une hémostase adéquate a été obtenue. Après l'administration de Praxbind, un autre traitement antithrombotique (héparines de bas poids moléculaire, par exemple) peut être commencé à tout moment si l'état du patient est cliniquement stable et si une hémostase adéquate a été obtenue. L'absence de traitement antithrombotique expose les patients au risque thrombotique lié à leur maladie ou affection sous jacente. Patients insuffisants rénaux Aucune adaptation de la dose n'est nécessaire chez les patients insuffisants rénaux. L'atteinte de la fonction rénale n'a pas modifié l'effet de réversion de l'idarucizumab. Patients insuffisants hépatiques Aucune adaptation de la dose n'est nécessaire chez les patients insuffisants hépatiques. Patients âgés Aucune adaptation de la dose n'est nécessaire chez les patients âgés de 65 ans et plus. Population pédiatrique La sécurité et l'efficacité de Praxbind chez les enfants âgés de moins de 18 ans n'ont pas encore été établies. Aucune donnée n'est disponible. Mode d'administration Voie intraveineuse. Praxbind (2 x 2,5 g/50 mL) est administré par voie intraveineuse sous forme de deux perfusions consécutives de 5 à 10 minutes chacune, ou sous forme de bolus. Pour des instructions d'utilisation et de manipulation supplémentaires, se reporter à la rubrique 6.6 du Résumé des Caractéristiques du Produit. **CONTRE INDICATIONS** Aucune. **EFFETS INDÉSIRABLES** La sécurité d'emploi de Praxbind a été évaluée chez 224 volontaires sains ainsi que chez 123 patients dans un essai de Phase III en cours, présentant des saignements non contrôlés ou nécessitant une intervention chirurgicale ou des procédures d'urgence et qui recevaient un traitement par Pradaxa (dabigatran étexilate. Aucun effet indésirable n'a été identifié. Déclaration des effets indésirables suspectés La déclaration des effets indésirables suspectés après autorisation du médicament est importante. Elle permet une surveillance continue du rapport bénéfice/risque du médicament. Les professionnels de santé doivent déclarer tout effet indésirable suspecté via : -Belgique: Agence fédérale des médicaments et des produits de santé, Division Vigilance, EUROSTATION II, Place Victor Horta 40/40, B-1060 Bruxelles. Site internet: www.afmps.be / e-mail: adversedrugreactions@fagg-afmps.be -Luxembourg: Direction de la Santé – Division de la Pharmacie et des Médicaments. Site internet: http://www.ms.public.lu/fr/activites/pharmacie-medicament/index.html **MODE DE DELIVRANCE** Médicament soumis à prescription médicale **TITULAIRE DE L'AUTORISATION DE MISE SUR LE MARCHÉ** Boehringer Ingelheim International GmbH Binger Str. 173 55216 Ingelheim am Rhein Allemagne **NUMÉRO D'AUTORISATION DE MISE SUR LE MARCHÉ** EU/1/15/1056/001 **DATE DE PREMIÈRE AUTORISATION** 11/2015 **REPRÉSENTANT LOCAL** Boehringer Ingelheim Avenue Ariane 16 1200 Bruxelles Editeur responsable: SCS Boehringer Ingelheim Comm.V, Avenue Ariane 16, 1200 Bruxelles.

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DENOMINATION DU MEDICAMENT Pradaxa 150 mg, gélules **COMPOSITION QUALITATIVE ET QUANTITATIVE** Chaque gélule contient 150 mg de dabigatran etexilate (sous forme de mésilate). **FORME PHARMACEUTIQUE** Gélule.Gélule composée d'une coiffe opaque bleu clair et d'un corps opaque de couleur crème de taille 0, rempli de granules jaunâtres. La coiffe porte le logo imprimé de Boehringer Ingelheim, le corps est imprimé du code « R 150 ». **INDICATIONS THERAPEUTIQUES** Prévention de l'accident vasculaire cérébral (AVC) et de l'embolie systémique (ES) chez les patients adultes atteints de fibrillation atriale non valvulaire (FANV) et présentant un ou plusieurs facteur(s) de risque tels que : antécédent d'AVC ou d'accident ischémique transitoire (AIT) ; âge ≥ 75 ans ; insuffisance cardiaque (classe NYHA ≥ II) ; diabète ; hypertension artérielle. Traitement des thromboses veineuses profondes (TVP) et des embolies pulmonaires (EP), et prévention des récidives de TVP et d'EP chez l'adulte. **POSOLOGIE ET MODE D'ADMINISTRATION** Posologie *(prévention des AVC/ES liés à la FA, TVP/EP)* *Prévention de l'AVC et de l'ES chez les patients adultes présentant une FANV associée à un ou plusieurs facteurs de risque (prévention des AVC/ES liés à la FA)* La dose quotidienne recommandée de Pradaxa est de 300 mg, soit une gélule de 150 mg deux fois par jour. Le traitement doit être poursuivi au long cours. Traitement des thromboses veineuses profondes (TVP) et des embolies pulmonaires (EP), et prévention des récidives de TVP et d'EP chez l'adulte (TVP/EP) La dose quotidienne recommandée de Pradaxa est de 300 mg, soit une gélule de 150 mg deux fois par jour après un traitement par un anticoagulant par voie parentérale pendant au moins 5 jours. La durée du traitement doit être individualisée après une évaluation approfondie des bénéfices du traitement par rapport au risque de saignement. Une courte durée de traitement (au moins 3 mois) doit être basée sur les facteurs de risque transitoire (par exemple, chirurgie récente, traumatisme, immobilisation) et des durées plus longues doivent être basées sur les facteurs de risque permanents ou la présence de formes idiopathiques de TVP ou d'EP. Prévention des AVC/ES liés à la FA, TVP/EP Pour les groupes de patients suivants, la dose recommandée de Pradaxa est de 220 mg par jour, soit 1 gélule de 110 mg deux fois par jour: -patients âgés de 80 ans ou plus -patients traités de façon concomitante par du vérapamil Pour les groupes suivants, la dose quotidienne de Pradaxa de 300 mg ou 220 mg doit être choisie d'après l'évaluation individuelle du risque thromboembolique et du risque de saignement: -Patients âgés de 75 à 80 ans -Patients présentant une insuffisance rénale modérée -Patients présentant une gastrite, une oesophagite ou un reflux gastro oesophagien -Autres patients présentant un risque augmenté de saignement. Pour la TVP/EP, la recommandation relative à l'utilisation de Pradaxa à la dose de 220 mg, administrée sous forme d'une gélule de 110 mg deux fois par jour, est basée sur des analyses pharmacocinétiques et pharmacodynamiques et n'a pas été étudiée dans ce contexte clinique. Voir ci dessous. En cas d'intolérance au dabigatran, les patients doivent être prévenus de la nécessité de consulter immédiatement leur médecin traitant afin de passer à d'autres alternatives thérapeutiques adaptées pour la prévention de l'AVC et de l'ES associée à une fibrillation atriale ou pour la TVP/EP. *Sujets âgés (prévention des AVC/ES liés à la FA, TVP/EP)* La dose quotidienne chez les patients âgés de 75 à 80 ans est de 300 mg, soit une gélule de 150 mg deux fois par jour. Le médecin pourra envisager de façon individuelle une dose quotidienne de 220 mg, soit une gélule de 110 mg deux fois par jour, si le risque thromboembolique est faible et le risque hémorragique élevé. La dose quotidienne chez les patients de 80 ans ou plus doit être de 220 mg, soit une gélule de 110 mg deux fois par jour, du fait d'un risque hémorragique accru dans cette population. L'insuffisance rénale étant fréquente chez les patients âgés (>75 ans), la fonction rénale doit être évaluée en calculant la ClCr avant l'initiation d'un traitement par Pradaxa afin d'exclure les patients présentant une insuffisance rénale sévère (ClCr < 30 mL/min). Au cours du traitement, la fonction rénale doit également être évaluée au minimum une fois par an chez les patients traités par Pradaxa et, si nécessaire, plus fréquemment dans les situations cliniques pour lesquelles la fonction rénale pourrait être altérée (en cas d'hypovolémie, de déshydratation ou d'association avec certains médicaments par exemple) (voir rubrique « Contre-indications »). *Patients présentant un risque hémorragique (prévention des AVC/ES liés à la FA, TVP/EP)* Les patients présentant un risque hémorragique accru doivent faire l'objet d'une surveillance clinique étroite (recherche de signes de saignement ou d'anémie). Le médecin pourra envisager une adaptation de la dose après l'évaluation du bénéfice et du risque potentiel chez un patient donné. Un test de coagulation peut aider à identifier les patients présentant un risque hémorragique accru dû à une exposition excessive au dabigatran. En cas d'exposition excessive au dabigatran chez des patients à risque hémorragique élevé, une dose de 220 mg, soit une gélule de 110 mg deux fois par jour, est recommandée. Le traitement doit être arrêté en cas de saignement cliniquement pertinent. Une dose de 220 mg, soit une gélule de 110 mg deux fois par jour, peut être envisagée chez les patients présentant une gastrite, une oesophagite ou un reflux gastro-oesophagien du fait d'un risque élevé de saignement gastro-intestinal majeur. *Évaluation de la fonction rénale (prévention des AVC/ES liés à la FA, TVP/EP)*: Pour tous les patients: -Avant l'initiation d'un traitement par Pradaxa, la fonction rénale doit être évaluée en calculant la clairance de la créatinine (ClCr) afin d'exclure les patients présentant une insuffisance rénale sévère (ClCr < 30 mL/min) (voir rubrique « Contre-Indications »). Pradaxa est contre indiqué chez les patients présentant une insuffisance rénale sévère. -La fonction rénale doit être également évaluée lorsqu'une altération de la fonction rénale est suspectée pendant le traitement (par exemple en cas d'hypovolémie, de déshydratation et d'association avec certains médicaments). Exigences supplémentaires chez les patients présentant une insuffisance rénale légère à modérée et chez les patients agés de plus de 75 ans: -La fonction rénale doit être évaluée pendant le traitement par Pradaxa au minimum une fois par an et, si nécessaire, plus fréquemment dans les situations cliniques où une diminution ou une altération de la fonction rénale pourrait être suspectée (en cas d'hypovolémie, de déshydratation et d'association avec certains médicaments par exemple). La méthode d'évaluation de la fonction rénale (ClCr en mL/min) lors du développement clinique de Pradaxa était celle de Cockcroft-Gault. La formule est la suivante: -Si la créatininémie est exprimée en µmol/L: 1,23 x (140-age [années]) × poids [kg] (× 0,85 pour les femmes) créatininémie [µmol/L] -Si la créatininémie est exprimée en mg/dL: (140-age [années]) × poids [kg] (× 0,85 pour les femmes) 72 × créatininémie [mg/dL] Cette méthode est recommandée pour évaluer la ClCr des patients avant et pendant le traitement par Pradaxa. Populations particulières *Insuffisance rénale (prévention des AVC/ES liés à la FA, TVP/EP)* Chez les patients présentant une insuffisance rénale sévère (ClCr < 30 mL/min), le traitement par Pradaxa est contre-indiqué (voir rubrique « Contre-indications »). Aucune adaptation de la dose n'est nécessaire chez les patients présentant une insuffisance rénale légère (ClCr 50-≤ 80 mL/min). Pour les patients présentant une insuffisance rénale modérée (ClCr 30-50 mL/min), la dose recommandée de Pradaxa est également de 300 mg, soit une gélule de 150 mg deux fois par jour. Toutefois, pour les patients à risque élevé de saignement, une réduction de la dose de Pradaxa à 220 mg, soit une gélule de 110 mg deux fois par jour, devra être envisagée. Une surveillance clinique étroite est recommandée chez les patients présentant une insuffisance rénale. *Administration concomitante de Pradaxa et des inhibiteurs faibles à modérés de la P glycoprotéine (P gp) (amiodarone, quinidine ou vérapamil) (prévention des AVC/ES liés à la FA, TVP/EP)* Aucune adaptation de la dose n'est nécessaire en cas d'administration concomitante avec l'amiodarone ou la quinidine. La posologie doit être réduite à 220 mg, soit une gélule de 110 mg deux fois par jour, chez les patients traités à la fois par dabigatran etexilate et vérapamil. Dans ce cas, Pradaxa et le vérapamil doivent être pris simultanément. *Poids (prévention des AVC/ES liés à la FA, TVP/EP)* Sur la base des données cliniques et pharmacocinétiques disponibles, aucune adaptation de la dose n'est nécessaire, mais une surveillance clinique étroite est recommandée chez les patients pesant moins de 50 kg. Sexe (prévention des AVC/ES liés à la FA, TVP/EP) Sur la base des données cliniques et pharmacocinétiques disponibles, aucune adaptation de la dose n'est nécessaire. *Insuffisance hépatique (prévention des AVC/ES liés à la FA, TVP/EP)* Les patients présentant un taux d'enzymes hépatiques supérieur à deux fois la limite supérieure de la normale (LSN) ont été exclus des principales études. Aucune expérience clinique n'est disponible pour cette sous-population de patients et l'administration de Pradaxa n'est donc pas recommandée dans cette population. Une insuffisance hépatique ou une maladie hépatique susceptible d'avoir un impact sur la survie sont contre-indications (voir rubrique Contre-indications). *Changement de traitement (prévention des AVC/ES liés à la FA, TVP/EP)* Passage de Pradaxa à un anticoagulant par voie parentérale Il est recommandé d'attendre 12 heures après la dernière dose de dabigatran etexilate pour passer à un anticoagulant par voie parentérale. *Passage d'un anticoagulant par voie parentérale à Pradaxa* Il convient d'arrêter l'anticoagulant par voie parentérale et d'administrer le dabigatran etexilate 0 à 2 heures avant l'heure prévue d'administration de l'autre traitement ou au moment de l'arrêt de ce dernier dans le cas d'un traitement continu (par exemple, héparine non fractionnée (HNF) par voie intraveineuse). *Passage de Pradaxa aux antagonistes de la vitamine K (AVK)* L'initiation d'un traitement par AVK doit être adaptée en fonction de la ClCr, comme suit: -ClCr ≥ 50 mL/min, les AVK doivent être débutés 3 jours avant l'arrêt du dabigatran etexilate -ClCr ≥ 30-< 50 mL/min, les AVK doivent être débutés 2 jours avant l'arrêt du dabigatran etexilate. Pradaxa pouvant augmenter l'INR, l'INR reflétera davantage l'effet des AVK lorsque Pradaxa aura été interrompu pendant au moins deux jours. Avant ce délai, les valeurs de l'INR doivent être interprétées avec prudence. *Passage des AVK à Pradaxa.* Les AVK doivent être arrêtés. Le dabigatran etexilate peut être administré dès que l'International Normalized Ratio (INR) est < 2,0. *Cardioversion (prévention des AVC/ES liés à la FA, TVP/EP)* Les patients peuvent rester sous dabigatran etexilate lorsqu'ils sont soumis à une cardioversion. *Population pédiatrique (prévention des AVC/ES liés à la FA)* Il n'y a pas d'utilisation justifiée de Pradaxa dans la population pédiatrique dans l'indication : prévention de l'AVC et de l'embolie systémique chez les patients présentant une FANV. *Population pédiatrique (TVP/EP)* La sécurité et l'efficacité de Pradaxa chez les enfants de moins de 18 ans n'ont pas encore été établies. Les données actuellement disponibles sont présentées dans la rubrique « Effets indésirables », mais aucune recommandation sur la posologie ne peut être donnée. *Dose oubliée (prévention des AVC/ES liés à la FA, TVP/EP)* Il est toujours possible de prendre une dose de dabigatran etexilate oubliée jusqu'à 6 heures avant la dose programmée suivante. Dans les 6 heures précédant le moment d'administration de la prochaine dose, ne pas rattraper la dose oubliée. Ne pas prendre de dose double pour compenser la dose oubliée. Mode d'administration *(prévention des AVC/ES liés à la FA, TVP/EP)* Les gélules de Pradaxa peuvent être prises avec ou sans aliments. Elles doivent être avalées entières avec un verre d'eau pour faciliter la distribution dans l'estomac. Les patients doivent avoir pour instruction de ne pas ouvrir les gélules, car cela pourrait augmenter le risque de saignement. **CONTRE-INDICATIONS** -Hypersensibilité à la substance active ou à l'un des excipients mentionnés à la rubrique 6.1 (« Liste des excipients ») du RCP. -Insuffisance rénale sévère (ClCr < 30 mL/min) (voir rubrique « Posologie et mode d'administration ») -Saignement évolutif cliniquement significatif -Lésion ou maladie jugée à risque significatif de saignement majeur. Ceci s'applique à une ulcération gastrointestinale en cours ou récente, à la présence de néoplasies malignes à haut risque de saignement, à une lésion cérébrale ou rachidienne récente, à une intervention chirurgicale cérébrale, rachidienne ou ophtalmique récente, à une hémorragie intracrânienne récente, aux varices oesophagiennes connues ou suspectées, aux malformations artérioveineuses, à un anévrisme vasculaire ou à une anomalie vasculaire majeure intrarachidienne ou intracérébrale -Traitement concomitant avec tout autre anticoagulant, par exemple héparine non fractionnée (HNF), héparines de bas poids moléculaire (énoxaparine, dalteparine, etc), dérivés de l'héparine (fondaparinux, etc), anticoagulants oraux (warfarine, rivaroxaban, apixaban, etc), sauf dans les circonstances particulières de changement de traitement anticoagulant (voir rubrique « Posologie et mode d'administration ») ou en cas d'administration d'HNF aux doses nécessaires au maintien de la perméabilité d'un cathéter central veineux ou artériel -Insuffisance hépatique ou maladie du foie susceptible d'avoir un impact sur la survie -Traitement concomitant avec le kétoconazole administré par voie systémique, la ciclosporine, l'itraconazole et la dronédarone -Porteur de prothèses valvulaires cardiaques nécessitant un traitement anticoagulant **EFFETS INDESIRABLES** Résumé du profil de sécurité d'emploi Dans l'étude pivot évaluant la prévention de l'AVC et de l'ES chez les patients présentant une fibrillation atriale, 12 042 patients au total ont été traités par dabigatran etexilate. Parmi ceux ci, 6059 ont reçu 150 mg de dabigatran etexilate deux fois par jour et 5983 ont reçu des doses de 110 mg deux fois par jour. Au cours des deux études contrôlées avec comparateur actif sur le traitement de la TVP/EP, RE COVER et RE COVER II, un total de 2 553 patients ont été inclus dans l'analyse de la sécurité du dabigatran etexilate. Tous les patients ont reçu des doses de dabigatran etexilate de 150 mg deux fois par jour. Les effets indésirables des deux traitements, le dabigatran etexilate et la warfarine, ont été comptabilisés à partir de la première prise de dabigatran etexilate ou de warfarine, après que le traitement par voie parentérale ait été interrompu (période de traitement par voie orale uniquement). Cela inclut tous les effets indésirables survenus au cours du traitement par le dabigatran. Tous les effets indésirables survenus au cours du traitement par la warfarine sont également inclus, à l'exception de ceux observés au cours de la période de chevauchement entre la warfarine et le traitement par voie parentérale. Au total, 2 114 patients ont été traités dans l'étude contrôlée avec comparateur actif sur la prévention de la TVP/EP, RE MEDY, et dans celle contrôlée par un placebo sur la prévention de la TVP/EP, RE SONATE. Tous les patients ont reçu des doses de dabigatran etexilate de 150 mg deux fois par jour. Au total, 22 % des patients présentant une fibrillation atriale traités pour la prévention de l'AVC et de l'ES (traitement à long terme allant jusqu'à 3 ans), 14 % des patients traités pour une TVP/EP et 15 % des patients traités pour la prévention d'une TVP/EP ont présenté des effets indésirables. Les effets indésirables les plus fréquemment rapportés ont été des saignements, survenant au total chez 16,6 % des patients présentant une fibrillation atriale traités au long cours en prévention de l'AVC et de l'ES et chez 14,4 % des patients traités pour une TVP/EP. En outre, des saignements sont survenus chez 19,4 % des patients au cours de l'étude RE MEDY sur la prévention de la TVP/EP et chez 10,5 % des patients au cours de l'étude RE SONATE sur la prévention de la TVP/EP. Dans la mesure où les populations de patients traités dans les trois indications ne sont pas comparables et où les événements hémorragiques sont répartis dans divers systèmes classe organe (SOC), un résumé des hémorragies majeures et des hémorragies de tout type classées par indication est présenté dans les tableaux ci dessous. Bien que de fréquence faible dans les essais cliniques, des saignements majeurs ou sévères peuvent survenir et, indépendamment de la localisation, peuvent conduire à un handicap, à une menace du pronostic vital, voire même à une issue fatale. Tableau résumé des effets indésirables Le tableau ci-dessous présente les effets indésirables identifiés lors des études de prévention de l'AVC d'origine thromboembolique et de l'ES chez les patients présentant une fibrillation atriale, et de prévention de la TVP/EP. Ils sont classés par système classe organe et fréquence selon la convention suivante : très fréquent (≥ 1/10) ; fréquent (≥ 1/100 à < 1/10) ; peu fréquent (≥ 1/1000 à< 1/100) ; rare (≥ 1/10 000 à < 1/1000) ; très rare (< 1/10 000) ; fréquence indéterminée (ne peut pas être évaluée à partir des données disponibles). Tableau 4 : Effets indésirables Prévention de l'AVC et de l'ES chez les patients présentant une fibrillation atriale Traitement et prévention de la TVP/EP Système classe organe/terme préférentiel Affections hématologiques et du système lymphatique Anémie Fréquent Peu fréquent Diminution de l'hémoglobinémie Peu fréquent Fréquence indéterminée Thrombopénie Peu fréquent Rare Diminution de l'hématocrite Rare Fréquence indéterminée Affections du système immunitaire Hypersensibilité médicamenteuse Peu fréquent Peu fréquent Rash Peu fréquent Peu fréquent Prurit Peu fréquent Peu fréquent Réaction anaphylactique Rare Rare Angio-œdème Rare Rare Urticaire Rare Rare Bronchospasme Fréquence indéterminée Fréquence indéterminée Affections du système nerveux Hémorragie intracrânienne Peu fréquent Rare Affections vasculaires Hématome Peu fréquent Peu fréquent Hémorragie Peu fréquent Peu fréquent Affections respiratoires, thoraciques et médiastinales Epistaxis Fréquent Fréquent Hémoptysie Peu fréquent Peu fréquent Affections gastro-intestinales Hémorragie gastro-intestinale Fréquent Fréquent Douleurs abdominales Fréquent Peu fréquent Diarrhée Fréquent Peu fréquent Dyspepsie Fréquent Fréquent Nausée Fréquent Peu fréquent Hémorragie rectale Peu fréquent Fréquent Hémorragie hémorroïdaire Peu fréquent Peu fréquent Ulcère gastro-intestinal, incluant l'ulcère de l'oesophage Peu fréquent Peu fréquent Gastro-oesophagite Peu fréquent Peu fréquent Reflux gastro-oesophagien Peu fréquent Peu fréquent Vomissements Peu fréquent Peu fréquent Dysphagie Peu fréquent Rare Affections hépatobiliaires Anomalie de la fonction hépatique/anomalie des tests de la fonction hépatique Peu fréquent Peu fréquent Augmentation de l'alanine aminotransférase Peu fréquent Peu fréquent Augmentation de l'aspartate aminotransférase Peu fréquent Peu fréquent Augmentation des enzymes hépatiques Rare Peu fréquent Hyperbilirubinémie Rare Fréquence indéterminée Affections de la peau et du tissu sous-cutané Hémorragie cutanée Fréquent Fréquent Affections musculo-squelettiques et systémiques Hémarthrose Rare Peu fréquent Affections du rein et des voies urinaires Hémorragie du tractus uro-génital, incluant l'hématurie Fréquent Fréquent Troubles généraux et anomalies au site d'administration Hémorragie au site d'injection Rare Rare Hémorragie au site d'un cathéter Rare Rare Lésions, intoxications et complications liées aux procédures Hémorragie traumatique Rare Peu fréquent Hémorragie au site d'incision Rare Rare Saignement Prévention de l'AVC et de l'ES chez les patients adultes présentant une FANV associée à un ou plusieurs facteurs de risque (prévention des AVC/ES liés à la FA) Le tableau ci-dessous présente les événements hémorragiques classés en hémorragies majeures et hémorragies de tout type au cours de l'étude pivot qui a évalué la prévention de l'AVC d'origine thromboembolique et de l'ES chez les patients présentant une fibrillation atriale. Tableau 5 : Événements hémorragiques au cours d'une étude évaluant la prévention de l'AVC d'origine thromboembolique et de l'ES chez les patients présentant une fibrillation atriale Dabigatran etexilate 110 mg deux fois par jour Dabigatran etexilate 150 mg deux fois par jour Warfarine Sujets randomisés 6015 6076 6022 Hémorragies majeures 347 (2,92 %) 409 (3,40 %) 426 (3,61 %) Hémorragie intracrânienne 27 (0,23 %) 39 (0,32 %) 91 (0,77 %) Hémorragie GI 134 (1,13 %) 192 (1,60 %) 128 (1,09 %) Hémorragie fatale 26 (0,22 %) 30 (0,25 %) 42 (0,36 %) Hémorragies mineures 1566 (13,16 %) 1787 (14,85 %) 1931 (16,37 %) Hémorragies de tout type 1759 (14,78 %) 1997 (16,60 %) 2169 (18,39 %) L'hémorragie majeure était définie comme répondant à un ou plusieurs des critères suivants. Hémorragie associée à une réduction du taux d'hémoglobine d'au moins 20 g/L ou entraînant une transfusion d'au moins 2 unités de sang ou de culots globulaires. Hémorragie symptomatique dans une zone ou un organe critique : intracoulaire, intracrânienne, intrarachidienne ou intramusculaire associée à un syndrome des loges, une hémorragie rétropéritonéale, une hémorragie intra-articulaire ou une hémorragie péricardique. Les hémorragies majeures étaient considérées comme menaçant le pronostic vital si elles correspondaient aux critères suivants : Hémorragie fatale ; hémorragie intracrânienne symptomatique ; réduction du taux d'hémoglobine d'au moins 50 g/L ; transfusion d'au moins 4 unités de sang ou de culots globulaires ; hémorragie associée à une hypotension nécessitant le recours à des médicaments inotropes intraveineux ; hémorragie nécessitant une intervention chirurgicale. Les patients randomisés dans le groupe dabigatran etexilate 110 mg deux fois par jour ou 150 mg deux fois par jour présentaient un risque significativement inférieur d'hémorragies menaçant le pronostic vital et d'hémorragies intracrâniennes par rapport aux patients sous warfarine [p < 0,05]. Le dabigatran etexilate aux deux doses présentait également un taux global de saignement significativement plus faible. Les patients randomisés dans le groupe dabigatran etexilate 110 mg deux fois par jour présentaient un risque significativement inférieur d'hémorragie majeure par rapport aux patients sous warfarine (risque relatif 0,81 [p=0,0027]). Les patients randomisés dans le groupe dabigatran etexilate 150 mg deux fois par jour présentaient un risque significativement plus élevé d'hémorragies GI majeures par rapport aux patients sous warfarine (risque relatif 1,48 [p=0,0005]). Cet effet a été principalement observé chez les patients ≥ 75 ans. Le bénéfice clinique du dabigatran dans la prévention de l'AVC et de l'ES ainsi que la diminution du risque d'HIC par rapport à la warfarine est maintenu dans les sous-groupes, par exemple en fonction de l'insuffisance rénale, de l'âge, de l'utilisation concomitante de médicaments tels que des antiplaquettaires ou des inhibiteurs de la P-gp. Alors que certains sous-groupes de patients sont exposés à un risque majoré d'hémorragies majeures lorsqu'ils sont traités par un anticoagulant, le risque accru de saignement pour le dabigatran est dû aux hémorragies GI, généralement observées dans les 3-6 premiers mois suivants l'initiation du traitement par dabigatran etexilate. Traitement des thromboses veineuses profondes (TVP) et des embolies pulmonaires (EP) et prévention des récidives de TVP et d'EP chez l'adulte (traitement de la TVP/EP) Le tableau ci-dessous présente les événements hémorragiques survenus au cours des études pivots poolées RE COVER et RE COVER II portant sur le traitement de la thrombose veineuse profonde (TVP) et de l'embolie pulmonaire (EP). Au cours des études poolées, les critères principaux de sécurité (hémorragie majeure, hémorragie majeure ou cliniquement pertinente et hémorragie de tout type) ont été significativement inférieurs par rapport à la warfarine à un niveau alpha nominal de 5 %. Tableau 6 : Événements hémorragiques survenus au cours des études RE COVER et RE COVER II portant sur le traitement de la thrombose veineuse profonde (TVP) et de l'embolie pulmonaire (EP) Au cours des études poolées, les critères principaux de sécurité (hémorragie majeure, hémorragie majeure ou cliniquement pertinente et hémorragie de tout type) ont été significativement inférieurs par rapport à la warfarine à un niveau alpha nominal de 5 %. Tableau 6 : Événements hémorragiques survenus au cours des études RE COVER et RE COVER II portant sur le traitement de la thrombose veineuse profonde (TVP) et de l'embolie pulmonaire (EP) Dabigatran etexilate 150 mg deux fois par jour Warfarine Risque relatif vs warfarine (intervalle de confiance à 95 %) Patients inclus dans l'analyse de sécurité 2 456 2 462 Événements hémorragiques majeurs 24 (1,0 %) 40 (1,6 %) 0,60 (0,36 ; 0,99) Hémorragie intracrânienne 2 (0,1 %) 4 (0,2 %) 0,50 (0,09 ; 2,74) Hémorragie GI majeure 10 (0,4 %) 12 (0,5 %) 0,83 (0,36 ; 1,93) Hémorragie menaçant le pronostic vital 4 (0,2 %) 6 (0,2 %) 0,66 (0,19 ; 2,36) Événements hémorragiques majeurs / hémorragies cliniquement significatives 109 (4,4 %) 189 (7,7 %) 0,56 (0,45 ; 0,71) Hémorragies de tout type 354 (14,4 %) 503 (20,4 %) 0,67 (0,59 ; 0,77) Tout type d'hémorragie GI 70 (2,9 %) 55 (2,2 %) 1,27 (0,90 ; 1,82) Les événements hémorragiques des deux traitements ont été comptabilisés à partir de la première prise de dabigatran etexilate ou de warfarine, après que le traitement par voie parentérale ait été interrompu (période de traitement par voie orale uniquement). Cela inclut tous les événements hémorragiques survenus au cours du traitement par le dabigatran etexilate. Tous les événements hémorragiques qui sont survenus au cours du traitement par la warfarine sont également inclus, à l'exception de ceux observés au cours de la période de chevauchement entre la warfarine et le traitement par voie parentérale. La définition des événements hémorragiques majeurs (EHM) a suivi les recommandations de la Société internationale de thrombose et d'hémostase. Un événement hémorragique a été classé comme un EHM s'il remplissait au moins l'un des critères suivants: -Hémorragie fatale -Hémorragie symptomatique dans une région ou un organe critique, par exemple intracrânienne, intrarachidienne, intraoculaire, rétropéritonéale, intra articulaire ou péricardique, ou intramusculaire avec syndrome des loges. Pour qu'une hémorragie dans une région ou un organe critique soit classée comme un EHM, elle devait être associée à un tableau clinique symptomatique -Hémorragie provoquant une diminution de l'hémoglobine de 20 g/L (1,24 mM/L) ou plus, ou entraînant la transfusion d'au moins deux unités de sang total ou de globules rouges. Le tableau ci-dessous présente les événements hémorragiques survenus au cours de l'étude pivot RE MEDY portant sur la prévention de la thrombose veineuse profonde (TVP) et de l'embolie pulmonaire (EP). Certains événements hémorragiques (EHM/événements hémorragiques cliniquement significatifs (EHCS), hémorragies de tout type) ont été significativement inférieurs à un niveau alpha nominal de 5 % chez les patients recevant du dabigatran etexilate par rapport à ceux traités par la warfarine. Tableau 7: Événements hémorragiques survenus au cours de l'étude RE MEDY portant sur la prévention de la thrombose veineuse profonde (TVP) et de l'embolie pulmonaire (EP) Dabigatran etexilate 150 mg deux fois par jour Warfarine Risque relatif vs warfarine (intervalle de confiance à 95 %) Patients traités 1 430 1 426 Événements hémorragiques majeurs 13 (0,9 %) 25 (1,8 %) 0,54 (0,25 ; 1,16) Hémorragie intracrânienne 2 (0,1 %) 4 (0,3 %) Non calculable* Hémorragie GI majeure 4 (0,3 %) 8 (0,5 %) Non calculable* Hémorragie menaçant le pronostic vital 1 (0,1 %) 3 (0,2 %)) Non calculable* Événements hémorragiques majeurs / hémorragies cliniquement significatives 80 (5,6 %) 145 (10,2 %) 0,55 (0,41 ; 0,72) Hémorragies de tout type 278 (19,4 %) 373 (26,2 %) 0,71 (0,61 ; 0,83) Tout type d'hémorragie GI 45 (3,1 %) 32 (2,2 %) 1,39 (0,87 ; 2,20) * Le risque relatif ne peut pas être estimé car aucun événement n'est survenu dans l'un(e) ou l'autre des cohortes/traitements. La définition des EHM a suivi les recommandations de la Société internationale de thrombose et d'hémostase décrites dans les études RE COVER et RE COVER II. Le tableau ci-dessous présente les événements hémorragiques survenus au cours de l'étude pivot RE SONATE portant sur la prévention de la thrombose veineuse profonde (TVP) et de l'embolie pulmonaire (EP). Les taux des EHM/EHCS combinés et des hémorragies de tout type ont été significativement inférieurs à un niveau alpha nominal de 5 % chez les patients recevant le placebo par rapport à ceux traités par le dabigatran. Tableau 8 : Événements hémorragiques survenus au cours de l'étude RE SONATE portant sur la prévention de la thrombose veineuse profonde (TVP) et de l'embolie pulmonaire (EP) Dabigatran etexilate 150 mg deux fois par jour Placebo Risque relatif vs placebo (intervalle de confiance à 95 %) Patients traités 684 659 Événements hémorragiques majeurs (0,3 %) 0 Non calculable* Hémorragie intracrânienne 0 0 Non calculable* Hémorragie GI majeure 2 (0,3 %) 0 Non calculable* Événements hémorragiques majeurs / hémorragies cliniquement significatives 36 (5,3 %) 13 (2,0 %) 2,69 (1,43 ; 5,07) Hémorragies de tout type 72 (10,5 %) 40 (6,1 %) 1,77 (1,20 ; 2,61) Tout type d'hémorragie GI 5 (0,7 %) 2 (0,3 %) 2,38 (0,46 ; 12,27) * Le risque relatif ne peut pas être estimé car aucun événement n'est survenu dans l'un ou l'autre traitement. La définition des EHM a suivi les recommandations de la Société internationale de thrombose et d'hémostase décrites dans les études RE COVER et RE COVER II. Infarctus du myocarde Prévention de l'AVC et de l'ES chez les patients adultes présentant une FANV associée à un ou plusieurs facteurs de risque (prévention des AVC/ES liés à la FA) Dans l'étude RELY, il a été observé une augmentation du taux annuel d'infarctus du myocarde dans les groupes dabigatran par rapport à la warfarine. Le taux était de 0,64 % dans le groupe warfarine, 0,82 % dans le groupe dabigatran etexilate 110 mg deux fois par jour et 0,81 % dans le groupe dabigatran etexilate 150 mg deux fois par jour. Traitement des thromboses veineuses profondes (TVP) et des embolies pulmonaires (EP), et prévention des récidives de TVP et d'EP chez l'adulte (TVP/EP) Dans les trois études contrôlées avec comparateur actif, un taux supérieur d'IDM a été rapporté chez les patients ayant reçu du dabigatran etexilate par rapport à ceux ayant reçu de la warfarine : 0,4 % vs 0,2 % au cours des études à court terme RE COVER et RE COVER II ; 0,8 % vs 0,1 % au cours de l'étude à long terme RE MEDY. L'augmentation a été statistiquement significative dans cette étude (p = 0,022). Dans l'étude RE SONATE, qui a comparé le dabigatran etexilate à un placebo, le taux d'IDM a été de 0,1 % chez les patients ayant reçu du dabigatran etexilate et de 0,2 % chez les patients ayant reçu le placebo. Population pédiatrique (TVP/EP) Dans l'étude clinique 1160.88, au total 9 adolescents (âgés de 12 à < 18 ans) présentant un diagnostic d'ETEV primaire ont reçu une dose orale initiale de dabigatran etexilate de 1,71 (± 10 %) mg/kg de poids corporel. Sur la base des concentrations de dabigatran déterminées par le test du temps de thrombine dilué et l'évaluation clinique, la dose a été ajustée à la dose cible de 2,14 (± 10 %) mg/kg de poids corporel de dabigatran etexilate. Deux patients (22,1 %) ont présenté des effets indésirables non graves (reflux gastro œsophagien / douleur abdominale ; gêne abdominale) et un patient (11,1 %) a présenté un événement indésirable grave non relié au traitement (récidive d'ETEV de la jambe) au cours de la période post traitement au-delà de trois jours après l'arrêt du dabigatran etexilate. Déclaration des effets indésirables suspectés La déclaration des effets indésirables suspectés après autorisation du médicament est importante. Elle permet une surveillance continue du rapport bénéfice/risque du médicament. Les professionnels de santé doivent déclarer tout effet indésirable suspecté via. -Belgique: Agence fédérale des médicaments et des produits de santé Site internet: www.afmps.be / e-mail: adversedrugreactions@fagg-afmps.be -Luxembourg: Direction de la Santé – Division de la Pharmacie et des Médicaments Site internet: http://www.ms.public.lu/fr/activites/pharmacie-medicament/index.html **MODE DE DÉLIVRANCE** Médicament soumis à prescription médicale. **TITULAIRE DE L'AUTORISATION DE MISE SUR LE MARCHÉ** Boehringer Ingelheim International GmbH Binger Str. 173 D-55216 Ingelheim am Rhin Allemagne **NUMÉROS D'AUTORISATION DE MISE SUR LE MARCHÉ** EU/1/08/442/011 (60 x 1 gél.) EU/1/08/442/012 (3x (60 x1) gél.) **DATE DE MISE À JOUR DU TEXTE** 01.2016 **REPRÉSENTANT LOCAL** Boehringer Ingelheim Avenue Ariane 16 1200 Bruxelles Editeur responsable: SCS Boehringer Ingelheim Comm.V, Avenue Ariane 16, 1200 Bruxelles.

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TABLE OF CONTENTS

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CONGRESS MANAGEMENT

For information about sponsor options, symposia and exhibition, please contact:

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GENERAL INFORMATION

Language:
The official language of the BSTH Annual Meeting is English. There will be no simultaneous translation.

Technical Equipment:
Only Power Point projection will be available. All slides will be presented from a central server.Presenters are being requested to bring their presentation on a memory stick, ZIP drive, CD rom or DVD.

Posters:
Posters should be put on display on Thursday morning upon arrival and have to be removed on Friday in the afternoon after closure.

Accreditation:
Participants will receive a certificate of attendance and accreditation.

Liabilitiy:
Neither the organizers nor the BSTH accept liability for accidents, damages and/or losses and any kind which may be incurred by meeting participants.

WELCOME TO THE 24TH ANNUAL MEETING

Dear participants,

I would like to welcome all participants to the 2016 Annual Meeting of the Belgian Society on Thrombosis. This is the third time the Annual Meeting is held in Mechelen, which is beginning to feel like home now. Many of you will know the venue of previous years.

The scientific organization of the 2016 Annual Meeting of the BSTH has been entrusted to the colleagues of Liége, prof dr Muriel Sprynger of the department of cardiology at the CHU Liége and dr Cécile Oury of the GIGA-Cardiovascular Sciences Laboratory of Thrombosis and Haemostasis at the University of Liège. They have put together a programme about the interface between cardiology and haemostasis, with the state-of-the art lectures focused on Platelets and Thrombosis, and Flow and Thrombosis in Cardiology. In contrast, the satellite symposia are all concerned with bleeding problems, focusing on haemophilia A and B. With the von Willebrand Factor gaining importance in cardiology, all aspects of haemostasis are covered at this meeting. Our thanks go out to Muriel and Cécile for their hard work in putting this meeting together.

We cordially invite all participants to the Networking Reception after the meeting on Thursday where Mechelen will meet Liège in a relaxed atmosphere. Afterwards we have the culinary walk through Mechelen, an initiative which proved successful last year.

The satellite symposia are evidence of the continued support that we enjoy from the pharmaceutical industry. Most companies active in the area of haemostasis and thrombosis are present at this meeting in one form or another, be it with a satellite symposium, and exhibition stand or through financial support. I would like to ask all participants to show appreciation for their support and visit the exhibition and interact with company representatives.

This year will also see the first Annual CSL Behring Encouragement Award being given out on Friday. Like all new initiatives it will take a little time to build momentum but this is an important tool to encourage young people working in haemostasis and coagulation.

The BSTH is growing and becoming more and more professional, not always an easy process. I would like to thank all who are working hard to achieve this. I am also pleased that we have an ungoing rejuvenation of the board with this year another two candidates hoping to be elected to the BSTH board.

Above all I hope that we have another great Annual Meeting of the BSTH with interesting scientific topics, good interaction and a relaxed atmosphere.

Alain Gadisseur, MD, PhD
President of BSTH

PROGRAM THURSDAY 24 NOVEMBER

08h30	Registration	
09h25	Welcome	
09h30	**Educational I:**	
Chair:	S. De Meyer and C. Oury	
	Platelet function in coagulation and thrombus formation	J. Cosemans (Maastricht)
10h15	**Satellite Symposium: Taking the next step together**	
Chair:	K. Peerlinck	
	Improving outcomes in bleeding disorders	P. Mathew (Albuquerque)
	Real Life Experience with the new treatment option from Bayer in hemophilia A	N. Marquardt (Bonn)
	Management of comorbidities in patients with hemophilia A	P. Schinco (Turin)
11h15	Break	
11h45	**Oral Communications: Clinical & Laboratory**	
Chair:	A. Demulder and K. Jochmans	
	Neutrophil extracellular traps in thrombi from patients with acute ischemic stroke	E. Laridan (Kortrijk)
	Thrombus composition reflects stroke etiology	F. Denorme (Kortrijk)
	N-acetylcysteine in preclinical animal models of thrombotic thrombocytopenic purpura	C. Tersteeg (Kortrijk)
	Plasma ADAMTS13 adopts a closed conformation in healthy individuals	E. Roose (Kortrijk)
	The p.Arg498Cys mutation in the cysteine rich domain of ADAMTS13 results in a secretion deficiency	A.S. Schelpe (Kortrijk)
12h45	Reward of the Paul Capel Prize Clinical & Laboratory	
13h00	Lunch	
14h00	**BSTH General Assembly**	
14h30	**STATE OF THE ART I: Platelets and Thrombosis**	
Chair:	S. Motte and C. Orlando	
	GPVI: more than a collagen receptor and more than an antithrombotic target	P. Mangin (Strasbourg)
	P2Y12 antagonists	M. Cattaneo (Milan)
16h00	Break	
16h30	**Satellite Symposium: Extended Half-Life products: protection or convenience?**	
Chair:	K. Peerlinck and C. Lambert	
	Converting to EHL rFVIIIFc	R. Klamroth (Berlin)
	New Treatment opportunities with EHL products in hemophilia B	K.J. Pasi (London)
Chair:	K. Devreese and A. Demulder	
	Factor Assays, much ado about nothing?	S. Kitchen (Sheffield)
17h30	Closure of day program	

P8

The Satellite symposia are open for healthcare professionals only.

EVENING PROGRAM

At Thursday night 17:40 all registered participants are invited to join the welcome reception.

At 19:00 a special program with dinner is being organized. Each participant including delegates of companiers can join for a fixed price per person. Please register to participate.

For the latest news and updates on the program please check on www.bsth2016.be.

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DATE & VENUE

Date: 24 November 2016
17:40 Networking reception
19:00 Start dinner walk through Mechelen

Venue:

Lamot Congres- en Erfgoedcentrum
A Van Beethovenstraat 8/10, 2800 Mechelen
T 015 29 49 00 | F 015 29 49 09
W www.lamot-mechelen.be

PROGRAM FRIDAY 25 NOVEMBER

08h55	Welcome	
09h00	**Educational II:**	
Chair:	K. Vandenbosch and A. Gadisseur	
	Why do children experience a thrombotic event?	V. Labarque (Leuven)
09h45	CSL Behring **Satellite Symposium**	
Chair:	C. Hermans	
	Transforming the treatment for Haemophilia B patients	C. Négrier (Lyon)
10h45	Break	
11h15	**Oral Communications: Basic Research**	
Chair:	K. Devreese and H. Deckmyn	
	A clinically relevant hybrid adenovirus-Sleeping Beauty transposon vector for gene therapy for von Willebrand disease	I. Portier (Kortrijk)
	The role of von Willebrand factor in a malaria-associated lung pathology model	S. Kraisin (Kortrijk)
	Unrestrained endogenous plasmin activity reverses acute thrombotic thrombocytopenic purpura	C. Tersteeg (Kortrijk)
	Clopidogrel prevents colitis-associated carcinogenesis in mice by inhibiting immunosuppressive function of immature myeloid cells	L. Servais (Liège)
	Staphylococcus aureus and Staphylococcus lugdunensis bind von Willebrand Factor to overcome shear stress and cause endocarditis	L. Liesenborghs (Leuven)
12h15	Reward of the Paul Capel Prize Basic Research	
12h25	BSTH news CSL Behring Encouragement Award	
12h45	Lunch	
13h00	Poster walk	
14h00	**STATE OF THE ART II: Flow and Thrombosis in Cardiology**	
Chair:	K. Vanhoorelbeke and M. Sprynger	
	Von Willebrand factor: a new flow marker in cardiovascular pathology	S. Susen (Lille)
	Prosthetic Valve Thrombosis: mechanism, evaluation and management	P. Lancellotti (Liège)
15h30	Closure	
15h30	Reception	

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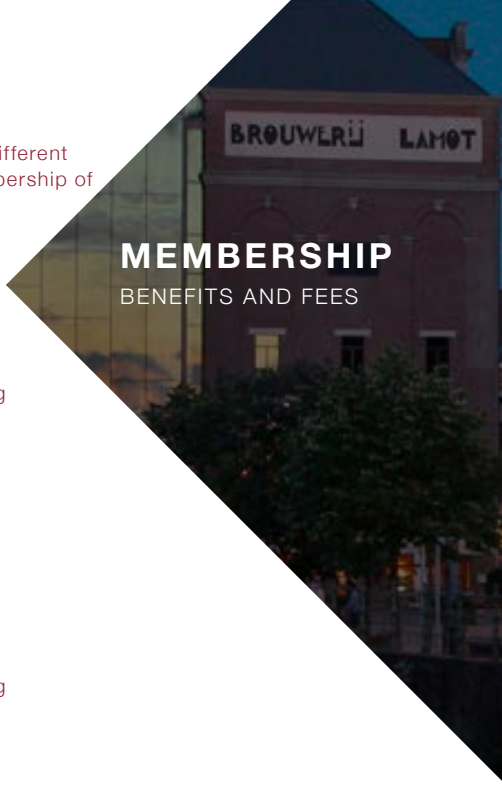

MEMBERSHIP
BENEFITS AND FEES

MEMBERSHIP BENEFITS

The BSTH council has defined the membership benefits for the different categories of membership. You will be able to register your membership of BSTH 2017 until 1 March 2017.

Standard members
- Access to member-only pages of the BSTH website
- Priority information on all BSTH activities
- Free admission to BSTH educational courses
- Reduced admission fees at BSTH Annual Scientific Meeting
- Eligibility for financial grants under auspices of the BSTH
- Eligibility for travel grants dispensed by the BSTH
- Eligibility for election to the BSTH Council
- Eligibility to participate in BSTH Council Committees
- Right to vote at the BSTH General Assembly

Associate members
- Access to member-only pages of the BSTH website
- Priority information on all BSTH activities
- Free admission to BSTH educational courses
- Reduced admission fees at BSTH Annual Scientific Meeting
- Eligibility for financial grants under auspices of the BSTH
- Eligibility for travel grants dispensed by the BSTH
- Eligibility to participate in BSTH Council Committees

Corporate members
- Acknowledgement as BSTH sponsor on the BHS website
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- Priority choice for booth space at the BSTH Annual Scientific Meeting
- Access to the BSTH address database for mailings for scientific activities (subject to approval by BSTH Council)
- Priority on proposals for satellite symposium during the BSTH Annual Scientific Meeting
- Free admission of 5 employees at BSTH Annual Meeting

ANNUAL MEETING FEES

Members	Early registration	Registration	On site registration
MD specialists, MSc specialists, PhD scientists	90 €	100 €	125 €
MD trainees, PhD students	50 €	50 €	65 €
Nurses, paramedics, technicians, data managers, students	20 €	25 €	35 €
Corporate	According to sponsorship/Exhibition booth package		

Non-members			
MD specialists, MSc specialists, PhD scientists	150 €	175 €	200 €
MD trainees, PhD students	100 €	115 €	125 €
Nurses, paramedics, technicians, data managers, students	60 €	65 €	75 €

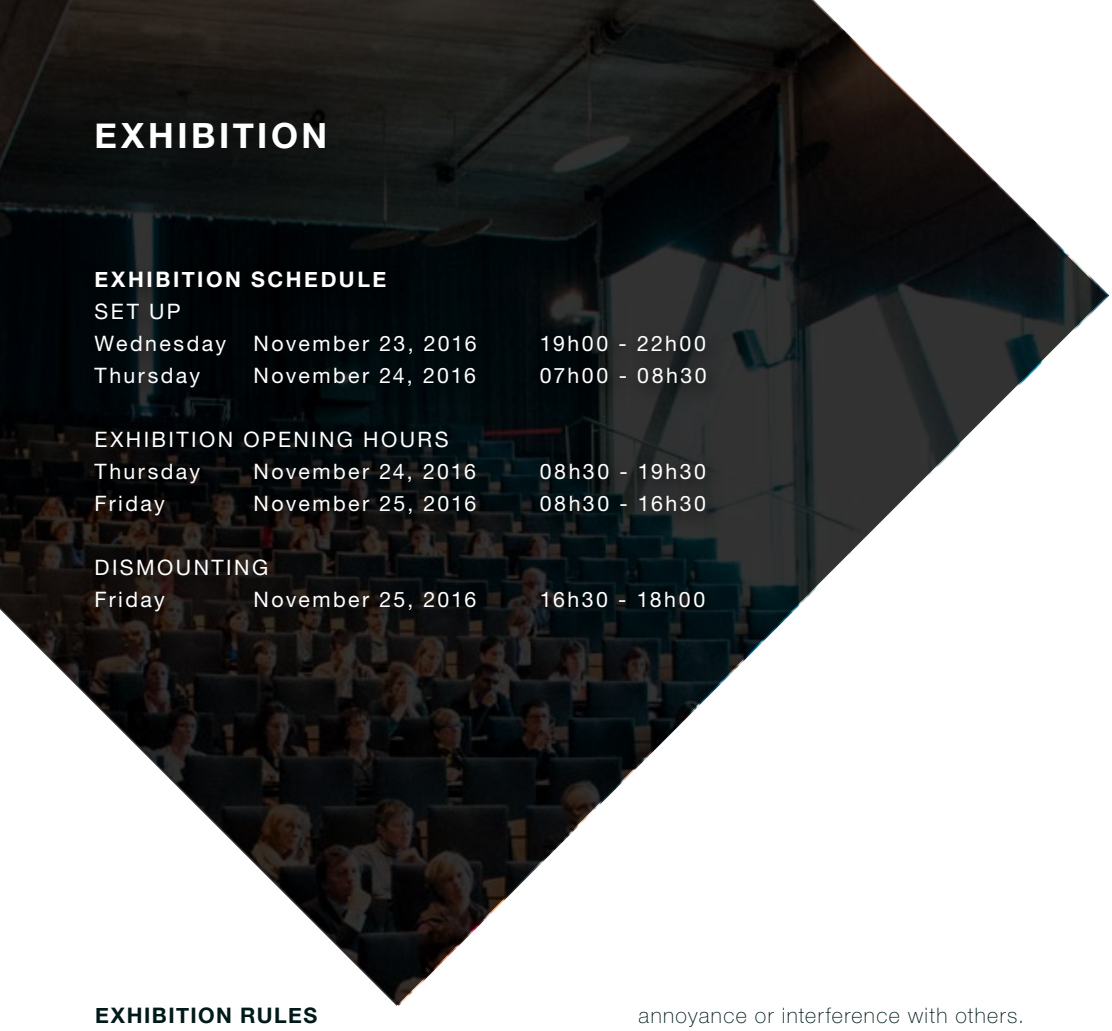
* excluding separate fee for evening programme

MEMBERSHIP FEES (ANNUALLY)

MD specialists, MSc specialists, PhD scientists	50 €
MD trainees, PhD students	35 €
Nurses, paramedics, technicians, data managers	25 €
Corporate members	5000 € (excl VAT)

At the end of the year all members are asked to renew their membership of the BSTH if they have not already done so at the occasion of the BSTH Annual Meeting.

EXHIBITION



EXHIBITION SCHEDULE

SET UP

Wednesday	November 23, 2016	19h00 - 22h00
Thursday	November 24, 2016	07h00 - 08h30

EXHIBITION OPENING HOURS

Thursday	November 24, 2016	08h30 - 19h30
Friday	November 25, 2016	08h30 - 16h30

DISMOUNTING

Friday	November 25, 2016	16h30 - 18h00

EXHIBITION RULES

At our meeting and exhibition at Lamot certain restrictions are applicable.
It is not allowed to distribute prepared food or beverages at the booth or place any food cooking equipment.

MANNING OF STANDS
Exhibitors will be required to ensure that their stands are manned during the opening hours of the exhibition and must not dismantle their stands before the published closing time.

NOISE
Exhibitors may not use audible electronic, mechanical apparatus, or open audio systems that may be heard outside the exhibitor assigned space. Con-txt, on behalf of BSTH and its organizers, reserves the right to require any exhibitor to discontinue any activity that may cause annoyance or interference with others.

COMPANY / PRODUCT PROFILE
A complete listing of all exhibitors and sponsors, including a 100-word entry will be included in the Final Program and Abstracts and is distributed to all attendees. You can submit your company / product profile by mail at bsth@con-txt.nl.

FAILURE TO OCCUPY SPACE
Exhibitors not occupying booth space by 8h30 on Thursday November 24, 2016, will forfeit their booth space without refund. The space may be resold or used by the BSTH.

SECURITY AND INSURANCE
BSTH and its organizers will not be held responsible for any accidents, loss or damage to exhibitors' goods and exhibitors are reminded that they should obtain their own insurance to cover this.

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registration
Bayer
CSL Behring
LEO Pharma
lift 1
bar and buffet
breaks and reception
entrance

STATE OF THE ART SPEAKERS

M. Cattaneo

Milan

Marco Cattaneo is Professor of Internal Medicine, Università degli Studi di Milano, Italy. MD degree at Università degli Studi di Milano; specialization in Hematology at Università di Pavia. Post-Doctoral Fellow at McMaster University, Hamilton, Ontario, Visiting Scientist at Temple University, Philadelphia and The Scripps Research Institute, La Jolla. Main research interests: pathophysiology of primary haemostasis, pharmacology of antiplatelet agents. Author of >300 original articles published in peer-reviewed scientific journals (H-index=68). In 2001 he was awarded the International Society on Thrombosis and Haemostasis (ISTH) 10th Biennial Award for Contributions to Haemostasis and Thrombosis. Editor or Associate Editor of several Scientific Journals. Chair of the Scientific Subcommittee on Platelet Physiology, ISTH Scientific and Standardization Committee and member of the ISTH Council.

P. Mangin

Strasbourg

I obtained my PhD studies in Molecular and Cellular Biology at the University of Strasbourg, France in 2003. My phD studies were focused on the signalling mechanism of the platelet receptors GPIb-IX-V and GPVI. I completed a post-doctoral internship in the Australian Centre for Blood diseases in the group of Shaun Jackson, Melbourne. There, I pursued my work on the role of adhesive platelet receptors in haemostasis and arterial thrombosis. I am currently Research Director (Directeur de Recherche Inserm) in the Inserm U949 research team and deputy scientific director of the Etablissement-Français du Sang-ALCA (Alsace, Lorraine, Champagne, Ardenne). Since 2015, I am a co-chair of the standardization subcommittee of Biorheology of the international society of thrombosis and Haemostasis.

P. Lancellotti

Liège

Patrizio Lancellotti is professor of Cardiology at University of Liège, CHU Sart Tilman, Liège Belgium. He is also director of the Cardiologist Intensive Unit at University Hospital of Liège, head of the Echo-cardiography Laboratory and the Heart Valve Clinic and director of the GIGA Cardiovascular Sciences Research Programme at the University of Liège, Belgium. Patrizio has been an active Board Member with different functions of the European Society of Cardiology, its Working Group On Valvular Heart Disease, its Committee for Practice Guidelines,the European Association of Cardiovascular Imaging. Furthermore he is member of the European association of echocardiography, the URObservationalResearch Programme committee, of several Guideline Task Forces and writing guidelines committees and Chair of the Imaging Village programme of the ESC Scientific Programme Committee 2014-2016.

S. Susen

Lille

Sophie Susen is Professor of Medicine (MD) at the department of Hematology of Lille University Hospital in Lille (France) and heads the department of Hemostasis and Transfusion. She is board-certified to practice biology, hematology and transfusion. Her Ph.D and postdoctoral fellowship was based on the role of von Willebrand factor in the context of vascular injury. Her research activity is performed at Inserm Unit 1011 (Lille Nord de France University). Her basic research activity focuses mainly in the role of Von Willebrand Factor and blood flow via different approaches. She is also involved in the French National network on VWD.

P. Mangin
(Strasbourg)

I. Platelets and Thrombosis

Glycoprotein VI: more than a collagen receptor and more than an antithrombotic target

Glycoprotein VI (GPVI) is a platelet specific receptor which is best known as the major activation receptor for collagen and increasingly accepted as a promising safe anti-thrombotic target. It was identified in 1982 as a 60-kDa glycosylated platelet membrane protein in a two-dimensional polyacrylamide gel electrophoresis. In 1987, the report of a patient lacking GPVI and whose platelets did not respond to collagen stimulation, indicated that this glycoprotein is a functional receptor for collagen. The expression of GPVI relies on its physical interaction with the FcRγ-chain, which is also key to mediate its intracellular signals.

The identification and development of GPVI-specific tools in the 90's, including the snake venom protein convulxin and CRP (collagen-related peptide), were instrumental in characterizing in great detail its signaling cascade. The cloning of GP6 in the late 90's opened a new area of investigation and notably gave insight into structural aspects of GPVI. Subsequently in the early 2000, the expression of recombinant soluble GPVI proteins, the development of blocking and immuno-depleting antibodies and the generation of genetically modified mice helped to establish that GPVI supports collagen-induced adhesion, activation and aggregation of platelets, as well as upregulation of their procoagulant activities. Beside collagen, GPVI interacts with other adhesive proteins including vitronectin and laminins, for which it promotes platelet activation. GPVI also binds EMPRINN, supporting platelet monocyte interaction and could thereby participate in inflammatory responses.

The list of GPVI ligands is increasing with notably diesel exhaust particules or globular adiponectin for which the relevance of the interaction still needs to be identified. More recently we have reported that GPVI supports platelet adhesion and activation to polymerized fibrin, thereby further contributing to thrombus formation. Animal experiments revealed that the blockade, absence or immuno-depletion of GPVI did not impact the mouse tail bleeding time suggesting that GPVI does not play a key role in haemostasis. This result is in agreement with inherited or acquired GPVI deficiencies in patients which are not associated with major bleedings. On the other hand, laser, chemical or mechanical injuries in some mouse vascular beds highlighted a role for GPVI in experimental thrombosis. Moreover, it was reported that GPVI blockade also reduced infarct size in a stroke model, without resulting in intracranial hemorrhage.

Together, these results highlighted the seductive hypothesis that targeting GPVI could provide an anti-thrombotic protection with potentially no bleeding risk. The development of pharmacological agents notably a soluble dimeric form of GPVI, named Revacept, led to perform a phase I clinical trial which confirmed that impairing GPVI interaction with its ligands does not prolong the bleeding time in healthy subjects. Beside its role in arterial thrombosis, GPVI is proposed to participate in other vascular pathologies including atherosclerosis and arterial remodeling. In addition, GPVI appears to play an important role in some experimental inflammatory processes including dermatitis or rheumatoid arthritis. Finally, GPVI has been proposed to participate in tumor metastasis, through a mechanism that still remains to be identified.

ABSTRACTS STATE OF THE ART LECTURES

M. Cattaneo
(Milan)

$P2Y_{12}$ antagonists

$P2Y_{12}$ is a platelet receptor for adenosine diphosphate (ADP), which is the target of efficacious antithrombotic drugs. Ticlopidine and clopidogrel belong to the thienopyridine family of $P2Y_{12}$ antagonists: they are pro-drugs that need to be converted in vivo by the hepatic cytochrome P-450 enzymatic pathway to active metabolites, which covalently bind to $P2Y_{12}$, thereby irreversibily inhibiting the receptor. Due to its toxicity (neutropenia, thrombotic thrombocytopenic purpura), ticlopidine has been almost completely replaced by clopidogrel in the clinical practice. Despite its proven antithrombotic efficacy, clopidogrel has some drawbacks, the main one being that it induces a widely variable degree of inhibition of platelet function, which is attributable to several factors, including inter-individual differences in the extent of metabolism of the pro-drug.

About 1/3 of treated patients do not display inhibition of $P2Y_{12}$-dependent platelet function, and, as a consequence, are not adequately protected from major adverse cardiac events. Several authors proposed to tailor clopidogrel treatment based on results of platelet function tests: patients with poor response to clopidogrel should be treated with higher doses of the drug or with alternative treatments. However, the theoretical efficacy of this strategy has not been confirmed by the results of three large randomized clinical trials. Prasugrel is a third-generation thienopyridine, with more rapid and consistent inhibitory effects on platelet aggregation than clopidogrel, resulting in greater clinical benefit in patients with acute coronary syndromes (ACS) undergoing coronary revascularization by percutaneous coronary inteervention (PCI).

Ticagrelor is a cyclopentyl-triazolo-pyrimidines that directly inhibits $P2Y_{12}$: after oral administration, it rapidly and reversibly inhibits $P2Y_{12}$. Ticagrelor has an additional mechanism of action, mediated by the inhibition of cellular re-uptake of adenosine, likely contributing to its antithrombotic activity, which proved superior to that of clopidogrel in patients with ACS. The incidence of unexplained dyspnea was significantly higher in ticagrelor-treated patients, compared to clopidogrel-treated patients: this is likely due to the reversible mechanism of action of the drug. Ventricular pauses were also more frequent in ticagrelor-treated ACS patients. Despite the higher incidence of major bleeding compared to clopidogrel, the net clinical benefit of both prasugrel and ticagrelor was superior to that of clopidogrel. Ticagrelor has been tested also in stable patients with previous AMI, stroke or peripheral artery disease. Cangrelor is a direct $P2Y_{12}$ antagonist that is administered by intravenous infusion and has a half-life of 3-6 minutes. Two trials, which compared cangrelor to clopidogrel in patients requiring PCI, were prematurely terminated due to insufficient evidence of superiority of cangrelor. In a more recent study, cangrelor significantly reduced the rate of ischemic events, with no significant increase in severe bleeding, in patients undergoing either urgent or elective PCI. Finally, the BRIDGE trial showed that cangrelor could be useful as bridging therapy in patients on treatment with oral $P2Y_{12}$ antagonists who need to undergo surgery. Based on the results of experimental studies in animals and of a small, proof-of-concept randomized placebo-controlled trial with prasugrel in humans, it can be surmised that $P2Y_{12}$ antagonists might be useful also to treat patients with allergic bronchial asthma.

S. Susen
(Lille)

II. Flow and Thrombosis in Cardiology

Von Willebrand Factor: A New Flow Marker in Cardiovascular Pathology

During the last 15 years, the treatment of cardiovascular diseases has undergone revolutionary steps with the emerging of novel therapeutic options based on cardio-device implantation designed to repair or assist the diseased heart. Thus Transcatheter Aortic Valve Implantation (TAVI), a minimally-invasive procedure was developed as an alternative treatment to address aortic stenosis, in patients contraindicated to conventional surgery[2], while left ventricular assist device (LVAD) address support to patients with end-stage heart failure and ineligible for heart transplant or, at short-term, for high-risk percutaneous coronary intervention or for patients suffering from refractory cardiogenic shock. Although these innovative therapeutic approaches have been very attractive, their use is limited to a small fraction of the potential patient population, those at very high risk for other therapeutic options.

Current LVAD, known as continuous flow (CF)-LVAD, deviate significantly from the native heart function, providing a non-physiological fixed and continuous blood flow and increased shear stress. Those impairments induce an acquired Von Willebrand syndrome (aVWs) by cleaving Von Willebrand Factor (VWF) multimers. This aVWs is present in almost all patients implanted with LVAD and is identified as the main cause for the excess of bleeding observed in patients treated with these devices. Worse outcomes have been reported in CF-LVAD carriers with early surgical bleeding and recurrent gastro-intestinal bleeding.

Interestingly, bleeding secondary to aVWs is not unique to LVAD implantation, but is also observed in several other heart conditions, including severe aortic stenosis, also known as Heyde's syndrome. Other reported conditions are mitral regurgitation and hypertrophic cardiomyopathy. All these conditions have in common a marked disturbance of the regular cardiac blood flow, including increased shear stress and turbulence. These conditions provoke unfolding of the globular VWF conformation. Once unfolded, VWF becomes sensitive to proteolysis by the VWF-cleaving protease ADAMTS-13.

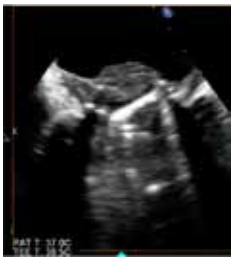
Alternatively, a narrow pulse pressure has been postulated as another major contributor to the Heyde's syndrome observed in both patients with aortic stenosis and those with CF-LVAD. Indeed, low residual arterial pulsatility in LVAD patients was associated with an increased risk of non-surgical bleeding in a retrospective single-center study. We have previously shown that this marker could act as an integrator between shear cleavage of VWF and vascular release of new VWF in response to a normal vascular pulse. We further shown that evaluation of VWF proteolysis assessment trough multimeric profile analysis and PFA-100 analysis could help to monitor TAVI procedures and predict their outcome.

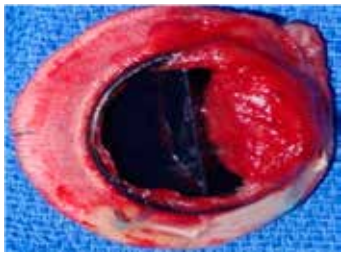
ABSTRACTS STATE OF THE ART LECTURES

Prosthetic Valvular Thrombosis

P. Lancellotti
(Liège)

University of Liège Hospital, GIGA Cardiovascular Sciences, Departments of Cardiology, Heart Valve Clinic, CHU Sart Tilman, Liège, Belgium





Valvular heart disease (VHD) is a major leading cause of cardiovascular mortality. Prevalence of VHD increases with age, reaching about 13% in patients above 75 years old. Heart valve replacement is the only therapy for the majority of patients with severe disease. Heart valve prostheses, either mechanical or biological, are currently the most widely implanted cardiovascular devices. Despite anticoagulation and/or antiplatelet treatment, thrombosis represents a frequent complication of valvular replacement (6% per patient-year), resulting in prosthesis dysfunction. The risk for prosthetic valve thrombosis depends on the prosthetic valve type and location (mitral, aortic, tricuspid, pulmonary) When it occurs, patients will need to be re-operated or will die in 50 to 60% of the cases. Thrombosis can initially develop without clinical symptoms, with an average incidence ranging from 6 up to 40%. In recent years, transcatheter aortic valve implantation has become a valuable and steadily increasing alternative to surgical valve replacement, which particularly benefits to very old patients with numerous comorbidities, who were previously considered inoperable.

The risk for prosthetic valve thrombosis depends on the prosthetic valve type and location (mitral, aortic, tricuspid, pulmonary). Clinical imaging, such as echocardiography and cardiac computed tomography are indispensible for diagnosis of prosthesis thrombosis. In practice, 2D/3D transthoracic echocardiography and transoesophageal echocardiography are used for the assessment of valve integrity, morphology, leaflet motion, and function. Cardiac computed tomography basically permits visualization of calcification, tissue degeneration, pannus formation and thrombosis in mechanical valves. Nuclear imaging currently has a very limited application other than in the setting of suspected infective endocarditis. The management of prosthetic thrombosis is high risk whatever the option taken. Surgery is high risk because it is most often performed under emergency conditions and is a re-intervention. On the other hand, fibrinolysis carries risks of bleeding, systemic embolism and recurrent thrombosis, which are higher than after surgery. Urgent or emergency valve replacement is recommended for obstructive thrombosis in critically ill patients without contra-indication to surgery.

Management of non-obstructive prosthetic thrombosis depends mainly on the occurrence of a thromboembolic event and the size of the thrombus. Surgery should be considered for large (> 10 mm) non-obstructive prosthetic thrombus complicated by embolism or which persists despite optimal anticoagulation. Fibrinolysis may be considered if surgery is at high risk but carries a risk of bleeding and thromboembolism. Subclinical thrombosis of bioprosthesis is also treated with anticoagulation using VKA and/or UFH as first-line treatment. Prevention of further thromboembolic events involves treatment of risk factors, optimization of anticoagulation control, and addition of low-dose aspirin (≤ 100 mg daily) after careful analysis of the risk-benefit ratio.

EDUCATIONAL SPEAKERS

J. Cosemans
Maastricht

Judith Cosemans is Associate Professor, CARIM School for Cardiovascular Research, Biochemistry Department, Maastricht University, The Netherlands. Dr. Cosemans became fascinated to unravel the role of platelets in arterial thrombosis during her PhD research, which focused on the dynamic regulation of thrombus stability. After finishing her PhD in 2009, she executed a multiple award winning postdoc project to develop flow chamber technology as compatible technique for experimental models of arterial thrombosis. Dr. Cosemans subsequently obtained two personal grants from the Dutch Heart Foundation (2011, 2015) and a Vidi grant from The Netherlands Organisation for Scientific Research (2016). These grants facilitate her current research line, which lies on the interface of platelet signalling and vascular biology. Her team aims to characterise the vascular-directed effects of arterial thrombi and to identify novel ways to prevent recurrent thrombotic events.

V. Labarque

Leuven

Veerle Labarque is Adjunct Clinical Head at the department of Pediatric Hemato-Oncology and Hematopoietic Stem Cell Transplantation at the University Hospitals Leuven. She is also Part-time Assistant Professor at the Catholic University of Leuven.
In 2010 she received the certification in Pediatric Hemato-Oncology and Hematopoietic Stem Cell Transplantation, Catholic University of Leuven and in 2011 Veerle got a clinical fellowship in Hemostasis and Thrombosis in the Hospital for Sick Children in Toronto (Canada).
In 2013 she won the first prize in clinical research category at the Hematology Research Day Toronto; with her Abstract: “Prospective Comparison of the Pediatric Bleeding Questionnaire (PBQ) and the International Society on Thrombosis and Haemostasis Bleeding Assessment Tool (ISTH-BAT) in children referred to a tertiary-care pediatric centre”.

J. Cosemans
(Maastricht)

ABSTRACTS EDUCATIONAL LECTURES

I. Platelet function in coagulation and thrombus formation

Judith Cosemans, CARIM School for Cardiovascular Diseases, Biochemistry Department, Maastricht University, The Netherlands

In the last decades it has become clear that platelet activation and blood coagulation are highly reciprocal processes, both contributing to hemostasis and thrombosis in an interactive way. Exposed collagen, von Willebrand factor and other components of the subendothelial matrix initiate platelet adhesion and activation, whereas exposed tissue factor simultaneously triggers the coagulation cascade. Activated platelets support and steer the coagulation process by at least four mechanisms, being: (i) exposure of the procoagulant phospholipid phosphatidylserine at their outer surface; (ii) release of coagulation factors like factor V and factor XIII, as well as anticoagulant factors like tissue factor pathway inhibitor; (iii) provision of a scaffold for the formation of fibrin fibers; and (iv) stimulation of fibrin clot retraction. In this educational lecture the coagulant roles of platelets are addressed in more detail.

II. Why do children experience a thrombotic event?

V. Labarque
(Leuven)

Thrombosis in children is very rare. The incidence of venous thrombosis is reported to be between 0.7-1.4/100,000 children per year or 53/100,000 hospital admissions per year; arterial ischemic stroke affects 3-13/100,000 children per year. There has, however, been an increase of thrombosis in children over the past decades, which is probably due to increased awareness, progress in radiologic imaging and increased survival of children with previously incurable diseases. Moreover, the incidence follows a bimodal pattern with a peak occurring in infants and adolescence. Due to developmental hemostasis, neonates have physiologically low levels of natural anticoagulants and plasminogen, but normal to relatively high levels of factor VIII. In adolescence, additional risk factors as anticonception, obesity and smoking may play a role.

Nevertheless, thrombosis in children remains much less frequent than in adults. One important reason is the presence of a diseased vessel wall in adults. In addition, it has been shown that thrombin generation in children is lower than in adults and the nanostructure of the thrombus is different, resulting in a clot which is less resistant to fibrinolysis in children than adults.

Idiopathic venous thrombosis is very rare in children and 95% of the venous thrombotic events are associated with predisposing risk factors of which the presence of a central venous catheter is the most prevalent (>85% of venous thrombotic events). Other risk factors can be divided into 3 groups according to the Virchow's triad: those resulting in stasis of the blood flow (e.g. congenital heart disease, anatomical abnormalities, malignancy,…), endothelial injury (e.g. malignancy), or hypercoagulability (e.g. dehydration, infection, inflammation, acquired or inherited thrombophilia,…). The role of inherited thrombophilia in the occurrence of a first venous thrombotic event, recurrence or persistence of thrombosis in childhood remains however controversial and will be discussed during the presentation, as well as the implications of who should (not) be tested for the presence of an inherited risk factor.

Similarly to venous thromboses, arterial stroke mostly occurs in children who have a pre-existing medical condition (eg. congenital heart disease, sickle-cell disease), but the cause of arterial thrombosis in previously healthy children remains uncertain and is probably involving an interplay of genetic predisposition, environmental effects and other risk factors (eg. non-atherosclerotic arteriopathies). Although inherited thrombophilia has been associated with arterial thrombosis in children, a large proportion of missing heritability remains to be accounted for.

SATELLITE SYMPOSIUM SPEAKERS

P. Mathew

Albuquerque

Dr. Mathew is Professor of Clinical Pediatrics in the Division of Pediatric Hematology/Oncology and past Director of the Hemophilia and Pediatric Hemostasis Program at the University of New Mexico Health Sciences Center in Albuquerque, New Mexico. In May 2012, Dr. Mathew joined Bayer Healthcare as the Vice-president of Global Medical Affairs, Hematology, located in New Jersey, where his main focus is in the science of Hemophilia and hematological disorders. He is a Fellow of the American Academy of Pediatrics and a member of the American Society of Hematology, the American Society of Pediatric Hematology/Oncology, the National Hemophilia Foundation, and the World Federation of Hemophilia, among others, Dr. Mathew has published many original articles, textbook chapters, and abstracts and has delivered many presentations at national and international medical conferences and symposia, while serving as chair, co-chair or as a moderator for some of these symposia. He is a past co-chair of the Pediatric hemostasis subcommittee of the Scientific and Standardization committee (SSC) of the International Society of Thrombosis and Hemostasis (ISTH), and a past member of the medical and scientific committee (MASAC) of the National hemophilia foundation, USA.

Dr. Mathew is a recipient of many awards including the Faculty Achievement Award, the Dean's Award of Distinction, the American Medical Association—Education and Research Foundation's Florence A. Carter Fellowship Award, the American Pediatric Association/Society for Pediatric Research Clinical Research Award, and the Cleveland Clinic Foundation's Bruce Hubbard Stewart Memorial Award for Humanistic Medicine, among others. Dr. Mathew is also a recipient of the 2010-2011 year Fulbright Scholarship Award from the J.W. Fulbright Foreign Scholarship Board (a US Presidentially appointed Board). He has mentored many medical students, pediatric residents and fellows through his career.

N. Marquardt

Bonn

Institute for Experimental Hematology and Transfusion Medicine, University Clinic, Bonn, Germany. Natascha Marquardt is Senior Physician at the Institute for Experimental Hematology and Transfusion Medicine, University Clinic, Bonn, Germany. She joined the faculty in 2003, and has subsequently attained specializations in both transfusion medicine and hemostaseology (in 2007 and 2008 respectively). Dr Marquardt has been an investigator in multiple clinical trials in hemophilia. She has a special interest in comorbidities in elderly hemophilia patients. Dr Marquardt also has a special interest in ultrasonography of hemophilic joints, and acts as a trainer in the HEAD US European Ultrasound Preceptorship Program.

P. Schinco

Turin

Dr. Piercarla Schinco is the Director of the Thrombosis & Hemostasis Unit at the University Hospital “Molinette” in Turin, Italy. She has also been Lecturer in Haemostasis and Laboratory Medicine at the Turin University Medical School since 1998 and Contract Professor at the Turin University Medical School since 2010. She is also a member of the European Association for Haemophilia and Allied Disorders and the Italian Association of Haemophilia Centre Directors. Her main scientific interest is concentrated on haemophilia and its innovative treatments. She is Author or co-Author of more than 70 scientific publications mainly in the haemophilia field and has been acting as Principal Investigator in several national and international collaborative trials.

P. Mathew
(Albuquerque)

Taking the next step together

Improving outcomes in bleeding disorders

Real Life Experience with the new treatment option from Bayer in hemophilia A

N. Marquardt
(Bonn)

Management of comorbidities in patients with hemophilia A

P. Schinco
(Turin)

sobi
Pioneer in Rare Diseases

SATELLITE SYMPOSIUM SPEAKERS

K.J. Pasi
London

John Pasi has been Professor of Haemostasis and Thrombosis at The Royal London Hospital, Barts and the London School of Medicine and Dentistry, since 2003. He was previously Professor of Haematology at the University of Leicester and Consultant Haematologist at the Royal Free Hospital and School of Medicine in London. He undertook his PhD in immunology along with initial haematology training in paediatrics. His clinical practice spans both adults and children and covers all aspects of haemostasis and thrombosis. His research interests cover many aspects of inherited and acquired bleeding and clotting disorders, particularly new bioengineered therapies for haemophilia, novel therapies for haemophilia including gene therapy, the link between cancer and thrombosis and the coagulopathy of major trauma. He is closely involved in the design and development of clinical trials for new therapies and evolving phase 1-4 programs. In addition, he has a major interest in the development of robust and harmonised outcome measure for haemophilia.
He has also been involved in the development of national policy and guideline formulation across a broad range of haemostatic and thrombotic disorders, and the development of novel models for haematology training initiatives such as residential academies and preceptorships for both haemostasis and thrombosis. John Pasi is a Fellow of the Royal College of Physicians, the Royal College of Pathologists, and the Royal College of Paediatrics and Child Health. He currently chairs the London Haemophilia Clinical Advisory Group and Commissioning Forum, is a member of NHS England Clinical Reference Group.

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R. Klamroth

Berlin

Robert Klamroth (Professor, MD, PhD) is Assistant Director of the Department of Internal Medicine: Angiology, Haematology and Pulmonology at Vivantes Hospital Friedrichshain, Berlin, Germany, and Director of the Haemophilia Centre/ Coagulation Clinic at the same institution. His main scientific interest is the interaction of the coagulation system with vascular disease. Since 1997, Professor Klamroth hç been involved in and helped to design more than 30 phase of haemophilia and thrombosis. He is an author of 34 papers published in the last 8 years in journals such as Vasa, Blood, Haemophilia and the British Journal of Haematology, and has presented at numerous national and international meetings.

S. Kitchen

Sheffield

Dr. Steve Kitchen is currently the Scientific Director of the UK National External Quality Assessment Scheme (NEQAS) for Blood Coagulation. He is also Head Clinical Scientist at the sheffield Haemostasis and Thrombosis Centre, with over 25 years experience in the field of laboratory testing in haemostasis.

In addition to these roles, Steve Kitchen is Director of the World Federation of Haemophilia EQA programme. Throughout his career he has had a long-term interest in standardisation and is active within the Science and Standardisation Committee of the International Society on Thrombosis and Haemostasis.

He is co-author of 'Diagnosis of Haemophilia and Other Bleeding Disorders: A Laboratory Manual', published in 2010, and editor of 'Quality in Laboratory Haemostasis and Thrombosis, 2nd Edition', published in 2013. As an expert in the field of haematological disorders, he has contributed to a vast variety of publications with a particular focus on the treatment of haemophilia A. Furthermore, he has a keen research in blood coagluation testing and screening.

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R. Klamroth
(Berlin)

Extended Half-Life products: protection or convenience?

Converting to EHL rFVIIIFc

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The goals of haemophilia treatment are to treat and avoid bleeds. The treatment of choice to avoid bleeds and joint disease in patients with severe haemophilia A is prophylaxis with the missing clotting factor VIII. Prophylactic replacement therapy is recommended in all patients with severe haemophilia A independent of age. Elevating factor VIII above 1% results in a reduction in spontaneous bleeding and is able to preserve joint health. Standard factor VIII has to be infused intravenously 3 times daily or every other day to achieve this goal.
rFVIIIFc is an extended half-life factor VIII for prophylaxis and treatment of bleeding. The extension of half-life is 1, 5-fold on average (1) but with an interindividual variability. rFVIIIFc offers the possibility to optimize prophylactic replacement therapy in patients with haemophilia A. Concerning long-term efficacy and safety there are the A-long (1) and Aspire (2) study programs in adults and children.

In Germany we have now first experience with the licensed product. Main driver for patients to switch to rFVIIIFc seems to be the possibility of fewer injections but higher protection with higher trough levels staying on the same injection frequency is a possibility too. The presentation will discuss the experience of the first patients switched to rFVIIIFc.

1. ClinTrials.gov NCT01181128; Mahlangu et al Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. Blood 2014, 123: 317-325; ClinTrials.gov 01458106, Young et al Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A. Journal of Thrombosis and Haemostasis 2015, 13: 1-11
2. ClinTrials.gov NCT1454739; Nolan et al Long-term safety and efficacy of recombinant factor VIII Fc fusion protein (rFVIIIFc) in subjects with haemophilia A. Haemophilia 2015 1-9; Pasi EAHAD 2016 Poster P070; Nolan EAHAD 2016 Poster P072

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New Treatment opportunities with EHL products in haemophilia B

K.J. Pasi
(Berlin)

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The new generation of extended half-life clotting factor concentrates offer a major therapeutic advance for patients with haemophilia B. rFIXFc, a fusion protein of rFIX expressed in HEK293 cells linked to the IgG1 Fc domain, prolongs *in vivo* survival of FIX via natural recycling through the neonatal Fc receptor (FcRn).

Using this approach in the phase 3 study B-LONG (1) rFIXFc was superior to rFIX across all clearance-related pharmacokinetic parameters with >82 hour terminal half-life for rFIXFc. Similarly, it has been shown to be safe and effective in both prevention and management of bleeding in the phase 3 and an open label extension study B-YOND (2), with patients treated now in excess of 5 years. The extension study provides a valuable insight into real world use of rFIXFc in both adults and children.

Within the extension study B-YOND patients could enroll in 1 of 4 treatment groups: weekly prophylaxis – a variable dose of 20-100 IU/kg at a fixed interval of every 7 d, individualized prophylaxis - a fixed dose 100 IU/kg at variable interval of 8-16 days, modified prophylaxis who could not achieve optimal prophylaxis with individual or weekly regimens, or episodic treatment for those >12 years. Patients could change treatment groups at any point in B-YOND.

116 subjects enrolled in B-YOND. From the start of the parent studies to the B-YOND interim data cut, the median duration of rFIXFc treatment was ~40 months and ~22 months among adult/adolescent and paediatric subjects, respectively; 73% of adult/adolescent patients and ~40% of paediatric subjects had ≥100 cumulative rFIXFc exposure days.

Overall, we see that no inhibitors have been observed, the AE profile is typical of the haemophilia B population in a clinical trial and generally similar to what is expected in the overall population of individuals with haemophilia B, with no reports of serious allergic reactions / anaphylaxis or no thromboembolic events.

Importantly from a clinical perspective median annualized bleeding rates (ABRs) were low (2.3-2.4) in all prophylaxis regimens, whether weekly, individualised or modified prophylaxis. >80% of individuals did not change their treatment interval and ~75% saw no change in prophylactic factor concentrate consumption over the course of follow up. One or two infusions were sufficient to control >95% of all bleeding episodes. These data in long term confirm the safety of rFIXFc, an absence of inhibitors, and maintenance of low ABRs with prophylactic dosing every 1–2 weeks. Examples of real world use of rFIXFc for clinical management will also be presented.

1. ClinTrials.gov NCT01027364, Powel et al Phase 3 Study of recombinant Factor IX FC Fusion Protein in Hemophilia B, New Eng J Med 2013, 369;24: 2313-2323
2. ClinTrials.gov NCT01425723; Mahlangu EAHAD 2016 Poster P044; Bennet NHF Poster CRCT01

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S. Kitchen
(Sheffield)

ABSTRACTS SATELLITE SYMPOSIUM SOBI

Factor assays for monitoring rFVIIIFc and rFIXFc: Much ado about nothing?

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The advent of modified Factor VIII (FVIII) and Factor IX (FIX) molecules with extended half-lives (EHL) compared to native FVIII and FIX represent a major advance in the field of haemophilia care with the potential to reduce the frequency of prophylactic injections and/or to increase the trough level prior to subsequent injections. Monitoring treatment through laboratory assays will be an important part of ensuring patient safety including any tailoring of prophylaxis. Some marked differences between results of different assays have been described for some EHL FVIII and EHL FIX products. (1). The laboratory should use an assay which recovers the same result as the assay used by the pharmaceutical company to assign potency so that the units reported by the laboratory agree with those used during clinical trials to establish which doses are safe and effective.

The BDD deleted rFVIII-Fc fusion protein from Biogen/SOBI (recently approved in some countries), was the subject of a field study amongst 30 laboratories using a range of different assays including One stage assays with ellagic acid, silica, kaolin and polyphenols as activators, and FVIII deficient plasmas with normal and reduced levels of VWF. The average recovery by one stage assay as a % of label was 95%, 106% and 116% at 0.8, 0.2 and 0.05 IU/ml respectively. There was on average a 20% higher activity using chromogenic FVIII assays (mean of results with 5 kits in 11 labs) as compared to one-stage clot based assay (2). UK NEQAS carried out a proficiency testing exercise in May 2016 which included a samples collected form a patient after infusion of rFVIIIFc and a spiked sample containing close to 0.5 IU/ml and in both samples chromogenic FVIII results were around 30% higher than one stage (various reagents). Taking all the studies together the results available to date therefore indicate that no special assay conditions are needed and both one stage or chromogenic assays as routinely used are suitable for monitoring rFVIIIFc.

A study assessing factor IX activity in patients receiving rFIXFc indicated that activity is dependent on the APTT reagent used for assay (3). One-stage assays using ellagic acid tended to overestimate factor activity, whereas one-stage assays using silica tended to underestimate factor activity, although both were within 15% of the labelled activity for a sample containing 0.8 IU/ml FIX. (3) and these differences should not have a significant clinical impact (e.g. dose adjustment) so the methods used would be suitable for monitoring patients receiving rFIXFc infusions. It should be noted that one-stage assays with CK Prest, Diagnostica Stago (using kaolin activators) underestimated the factor IX activity by approximately 50%. Use of this reagent in one-stage FIX assays is therefore not optimal for monitoring rFIXFc, and alternative methods should be employed for monitoring this product. A chromogenic FIX assay (Biophen) recovered 101%, 94% and 84% of nominal rFIXFc activity at 0.8, 0.2 and 0.05 IU/ml respectively indicating that chromogenic FIX assay would also be suitable for monitoring this product.

1. Dodt J, Hubbard AR, Wicks SJ, Gray E, Neugebauer B, Charton E, et al. Potency determination of factor VIII and factor IX for new product labelling and post infusion testing: challenges for caregivers and regulators. Haemophilia 2015; 21: 543-9.
2. Sommer JM, Moore N, McGuffie-Valentine B, Bardan S, Buyue Y, Kamphaus GD, Konkle BA, Pierce GF. Comparative field study evaluating the activity of recombinant factor VIII Fc fusion protein in plasma samples at clinical haemostasis laboratories. Haemophilia 2014; 20:294-300.
3. Sommer JM, Buyue Y, Bardan S, Peters RT, Jiang H, Kamphaus GD, Gray E, Pierce GF. Comparative field study: impact of laboratory assay variability on the assessment of recombinant factor IX Fc fusion protein (rFIXFc) activity Thromb Haemost. 2014; 112:932-40.

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SATELLITE SYMPOSIUM SPEAKER

C. Négrier
Lyon

Claude Négrier is Professor of Medicine at the Claude Bernard University, Lyon, France and serves as Chairman of the haematology division at Edouard Herriot University Hospital and Louis Pradel Cardiology hospital where he leads the Hemophilia and Thrombosis Comprehensive Care Center. He has been the coordinator of the French National Reference Center for Hemophilia since 2007. He received his medical degree from the University of Limoges, France, and was awarded a PhD in molecular biology at the University of Lyon. He is also an Adjunct Professor in the Department of Medicine, Division of Hematology at the University of North Carolina, Chapel Hill, North Carolina. Dr. Négrier is a member of a number of professional societies including the International Society on Thrombosis and Haemostasis (ISTH), the American Society of Hematology (ASH) and the World Federation of Hemophilia (WFH). He has authored or co-authored more than 200 publications and book chapters. Dr. Négrier's clinical and research interests include evaluation of therapeutic modalities in patients with bleeding disorders, such as haemophilia, particularly those who have developed an inhibitor. He is involved in preclinical research on molecular and cellular investigation of factor VIII and IX deficiencies, phenotypic evaluation of the coagulation system, and pathophysiology of inherited platelet disorders. In addition, he has a special interest in current and future preclinical and clinical recombinant technology, including gene therapy approaches for haemophilia.

Transforming the treatment for Haemophilia B patients

C. Négrier
(Lyon)

Hemophilia B is a congenital X-linked bleeding disorder caused by a deficit of coagulation FIX. Current prophylaxis therapy for hemophilia B requires frequent intravenous injections of factor IX in order to maintain FIX trough levels sufficient top recent bleeding. A recombinant fusion protein linking recombinant coagulation factor IX with recombinant albumin (rIX-FP) has been developed. rIX-FP has an improved pharmacokinetic (PK) profile with a greater than 5-fold half-life compared to standard recombinant and plasma-derived FIX and 7-fold increase in both area under the curve and clearance and high incremental recovery (Santagostino 2012).

The PROLONG-9FP study program investigated the efficacy, safety and pharmacokinetics of rIX-FP in patients with severe hemophilia B (FIX ≤ 2%) treated prophylactically, on demand or perioperatively. Here, the data on prophylaxis and on prevention of bleeding during and after surgery from two phase III-studies will be presented (Santagostino 2016, Kenet 2016, Négrier 2016).

The improved PK profile of rIX-FP enables FIX activity trough levels to be maintained above recommended thresholds even at 14-days post-injection, with FIX consumption that is lower than on prophylaxis with standard FIX products. The impact of this improvement in trough levels is a median annualized spontaneous bleeding rate of 0 across all prophylaxis regiments (7-,10-, 14-days) (Santagostino 2016). In addition to the impact on efficacy, a favorable safety profile for rIX-FP has been demonstrated across the two phase III studies with safety date from more than 7000 rX-FP injections in 90 patients, with 75% of patients having ≥ 50 exposure days. Importantly no inhibitors were detected against FIX.

Santagostino E et al. Safety and pharmacokinetics of a novel recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in hemophilia B patients. Blood 2012;120:2405-2411

Santagostino E et al. Long-acting recombinant coagulation factor IX albumin fusion protein (rIX-FP) in hemophilia B: results of a phase 3 trial. Blood 2016; 127(14):1761-1769

Kenet G et al. Long-acting recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in children. Thromb. Haemost 2016;116(4): 659-668

Négrier C et al. Efficacy and safety of ling-acting recombinant fusion protein linking factor IX with albumin in haemophilia B patients undergoing surgery. Haemophilia 2016;22(4):e259-66V

ABSTRACTS

A-122
Neutrophil extracellular traps in thrombi from patients with acute ischemic stroke

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Background / Introduction: Ischemic stroke is caused by blood thrombi that occlude blood vessels in the brain, leading to focal impairment of the downstream blood flow and irreversible damage of the associated brain tissue. For unknown reasons, use of t-PA leads to dissolution of occluding thrombi in some cases, but not in others. Although the thrombus itself is the primary target for acute ischemic stroke intervention, little is known about the exact composition of thrombi that cause ischemic stroke. In particular, neutrophil extracellular traps (NETs) have been reported in various settings of thrombosis but their presence in ischemic stroke thrombi remains unknown.

Aims: To investigate the presence of neutrophil extracellular traps in thrombi from ischemic stroke patients.

Methods / Materails: Sixty-nine thrombi from acute ischemic stroke patients were collected following thrombectomy. Thrombi were fixed, embedded and sectioned. Besides standard H&E staining, NETs were visualized via immunohistochemistry using antibodies against citrullinated histone H3 (H3Cit), granulocyte marker CD66b and neutrophil-specific marker, neutrophil elastase. Extracellular DNA was visualized via DAPI. Per thrombus, H3Cit and neutrophil amount was quantified in regard to stroke etiology. Extracellular DNA was targeted by performing ex vivo lysis with DNase1 and t-PA on six patient thrombi.

Results: Neutrophils were detected extensively throughout all thrombi with an average of 7042 ± 4075 per mm^2. Citrullinated histone H3 (H3Cit), a hallmark of NETs, was observed in almost all thrombi. H3Cit-positive areas ranged from 0.03% to 13.45% among thrombi. Co-localization of H3Cit with CD66b and extracellular DNA confirmed the presence of citrullinated histones on extracellular DNA released by neutrophils. Strands of extracellular DNA were also observed in neutrophil-rich areas on H&E staining. Different stages of NETosis could be detected, including neutrophils that contain only intracellular decondensated chromatin and neutrophils that have formed extracellular networks of DNA fibers. The majority of collected thrombi were of cardioembolic origin and showed a significantly higher amount of H3Cit compared to other stroke origins ($p<0.05$). The addition of DNase1 to tPA treatment accelerated ex vivo lysis of patient stroke thrombi after two hours ($p<0.05$).

Summary / Conclusions: We report neutrophils to play a role in thrombo-embolic occlusions in stroke. We demonstrate the presence of NETs in human ischemic stroke thrombi and provide initial evidence to further investigate DNase1-based therapeutic strategies, which could improve thrombus dissolution in acute ischemic stroke.

A-124
Thrombus composition reflects stroke etiology

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Background / Introduction: Notwithstanding recent advances in stroke prevention and therapy, ischemic stroke is still a leading cause of death and permanent disability in the world. Current ischemic stroke therapy consists of achieving fast recanalization, either via pharmacological thrombolysis or mechanical thrombectomy of the culprit thrombus. Unfortunately, little information is available regarding the composition of these thrombi. This knowledge is however indispensible to improve stroke treatment and reduce ischemic stroke risk.

Aims: To better understand stroke thrombus composition and correlate histological findings with stroke etiology.

Methods / Materials: Seventy-five stroke patient thrombi were included in this study. Of these patients, clinical data were collected and stroke etiology was determined according to the TOAST criteria. Thrombus specimens were stained with H&E, Martius Scarlet Blue (MSB) or antibodies against von Willebrand factor (VWF). Red blood cell (RBC), fibrin and VWF content were quantitatively analyzed. Thrombi were histopathologically classified as fresh, lytic or organized. Fresh thrombi were defined by the presence of layered patterns of fibrin and intact blood cells. Lytic thrombi were identified by colliquative necrosis and karyorrhexis of granulocytes, while organized thrombi were characterized by presence of organized fibrin and connective tissue.

Results: Stroke etiology was classified as cardioembolic (44 patients, 58.7%), large artery atherosclerosis (8 patients, 10.7%) and other known (7 patients, 9.3%) or unknown (16 patients, 21.3%) etiology. Other known causes included 4 patients with a carotid artery dissection and 3 with a paradoxical embolism. Thrombi contained on average 44.2% ± 24.2% RBCs, 36.8% ± 19.5% fibrin and 22.5% ± 11.8% VWF. Thrombi were classified as fresh in 44% (n=33), lytic in 30.7% (n=23) and organized in 25.3% (n=19). Fresh thrombi contained on average 15.1% ± 4.6% VWF, which was significantly lower compared with lytic thrombi (23.9% ± 9.4%; p=0.0005) and organized thrombi (32.8% ± 14.0%; p=0.0001). This puts forward a role for VWF in thrombus organization and possibly also stabilization, which could become a useful target in future stroke therapy as we recently demonstrated a thrombolytic effect of ADAMTS13 on VWF-rich thrombi.

Since thrombus composition is most likely determined by its etiology, we next compared the composition of clots from different stroke subtypes. Cardioembolic thrombi contained significantly less RBCs compared with emboli originating from an atherosclerotic plaque or thrombi from other known etiology (37.6% ± 23.3% vs. 61.8% ± 12.2% or 63.3% ± 20.8%, respectively; p=0.04). Accordingly, cardioembolic thrombi contained more fibrin (42.7% ± 21.6%) compared with atherosclerotic emboli (26.5% ± 13.8%; p=0.08) and thrombi from other known etiology (20.6% ± 10.4%; p=0.03). Strikingly, thrombi originating from the heart contained almost twice as much VWF (24.6% ± 10.5%) compared with emboli from an atherosclerotic lesion (14.5% ± 4.7%; p=0.01) or thrombi from other known etiology (15% ± 6%; p=0.04).

Summary / Conclusions: Stroke thrombi were found to display a heterogeneous morphology with thrombi originating from the heart being less rich in RBCs, but more rich in fibrin and VWF. If validated in larger cohorts, thrombus histology could assist in the process of etiology determination. Furthermore, older thrombi contained higher amounts of VWF, implying a role for VWF in thrombus organization and stabilization. This puts forward VWF as a promising target to improve thrombolytic therapy.

A-108
N-acetylcysteine in preclinical animal models of thrombotic thrombocytopenic purpura

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Background / Introduction: Thrombotic thrombocytopenic purpura (TTP) is a microangiopathic disorder diagnosed by thrombocytopenia and hemolytic anemia, and associated with a deficiency in von Willebrand factor (VWF) cleaving protease ADAMTS13. Current treatment is based on fresh frozen plasma infusion for congenital TTP, or plasma exchange, often in combination with immunosuppressive agents, for acquired TTP. These treatment methods are however not always effective and therefore new treatment options are highly necessary. N-acetylcysteine (NAC), an FDA-approved anti-mucolytic agent, could be a possible new treatment strategy for TTP as it was demonstrated to reduce

disulfide bonds in VWF, thereby decreasing VWF multimer size and hence its prothrombotic potential. So far, a limited number of patients suffering from TTP and non-responsive to plasma exchange therapy has been treated with NAC. The results of these case reports are however variable and always examine the effect of NAC treatment as an adjuvant to standard plasma exchange.

Aims: In the current study, we investigated whether NAC, without concurrent plasma exchange and immunosuppressive therapy, is effective in preventing and resolving TTP signs using well-established murine and baboon models for TTP.

Methods / Materials: Adamts13-/- mice were triggered with 2000 U/kg rVWF and received 800 mg/kg NAC 15 minutes before (prophylactic administration) or one and 12 hours after (treatment) injection of rVWF. Baboons (Papio ursinus) were injected intravenously every 48 hours with 600 µg/kg inhibitory anti-ADAMTS13 antibody 3H9. TTP symptoms developed within 72 hours, at which time treatment was initiated by intravenous injections of 400 mg/kg NAC every 12 hours for five days.

Results: In mice, prophylactic administration of NAC was effective in preventing severe TTP signs. After 24 hours, 492 ± 181 x103 platelets/ µL were measured compared to 226 ± 113 x103 platelets/ µL in mice without NAC administration (both n=12). This was supported by in vitro data, demonstrating the VWF-multimer reducing properties of NAC in solution. Nonetheless, in both mice and baboons, administration of NAC was not effective in resolving existing TTP signs: thrombocytopenia, hemolytic anemia and organ damage could not be reversed, as thrombus resolution could not be achieved. In mice receiving NAC, severe thrombocytopenia persisted as the platelet count after 24 hours was 267 ± 123 x103 platelets/ µL compared to 245 ± 149 x103 platelets/ µL in non-treated mice (both n=10). Also baboons did not recover from TTP after receiving NAC for 5 days as their platelet count remained low (38 ± 11 x103 platelets/ µL (n=4), compared to 39 ± 16 x103 platelets/ µL in control baboons (n=4)). In both models, a reduction in VWF multimers was observed, demonstrating that NAC was efficient in reducing disulfide bonds in circulating VWF multimers.

Summary / Conclusions: In conclusion, prophylactic administration of NAC, without concurrent plasma exchange, is effective in preventing severe TTP signs in mice but NAC is ineffective in resolving acute TTP signs in mice and baboons.

A-131
Plasma ADAMTS13 adopts a closed conformation in healthy individuals

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Background / Introduction: The metalloprotease ADAMTS13 regulates the size of the hemostatic protein von Willebrand factor (VWF). ADAMTS13 activity is regulated by shear-induced conformational changes in VWF. Recently it was demonstrated that recombinant ADAMTS13 can also be conformationally activated and this by disrupting the interactions between its head and tail domains through addition of its substrate VWF, activating anti-ADAMTS13 antibodies or by lowering the pH. We showed that by activating ADAMTS13 cryptic epitopes in the metalloprotease domain become exposed, which would coincide with the transition from a closed to a more open conformation. Whether plasma ADAMTS13 adopts a closed conformation where the tail domains shield the head domains remains to be determined.

Aims: To identify novel anti-ADAMTS13 antibodies that recognize cryptic epitopes in closed recombinant ADAMTS13 and to identify the overall conformation of plasma ADAMTS13 in healthy donors.

Methods / Materials: In house developed anti-ADAMTS13 antibodies recognizing cryptic epitopes in the ADAMTS13 head and spacer domains were selected using ELISA. Anti-ADAMTS13 antibodies were coated and their capacity to capture recombinant ADAMTS13 in the presence (more open ADAMTS13) or absence (closed ADAMTS13) of our activating anti-ADAMTS13 monoclonal antibody (17G2 having an epitope in the ADAMTS13 CUB2 tail domain) was analysed. To study the conformation of plasma ADAMTS13 in healthy donors, anti-ADAMTS13 antibodies recognizing a cryptic epitope in recombinant ADAMTS13 were coated, plasma ADAMTS13 from 24 healthy donors was added in the presence and absence of our activating anti-ADAMTS13 antibody 17G2 and bound ADAMTS13 was detected using biotinylated anti-ADAMTS13 antibodies and HRP labelled streptavidin.

Results: Five in house developed anti-ADAMTS13 antibodies (1C4, 3B8, 3H9, 5C11 and 6A6) were selected to identify whether they are directed against a surface or cryptic epitope in ADAMTS13. The selected antibodies were coated and

closed recombinant ADAMTS13 was added. While the antibodies 3B8, 3H9 and 5C11 could readily capture closed recombinant ADAMTS13, this was not the case for the antibodies 1C4 and 6A6. Moreover, when ADAMTS13 was opened up by the antibody 17G2, the epitopes of 1C4 and 6A6 became exposed, which indicates that the epitopes of 1C4 and 6A6 were initially cryptic. Finally, the plasma of 24 healthy donors was tested to determine the overall conformation of ADAMTS13 in plasma. Therefore, the antibodies 1C4 and 6A6 were coated and plasma was added in the presence or absence of the activating antibody 17G2. Plasma ADAMTS13 alone was not captured by the antibodies 1C4 and 6A6, while plasma ADAMTS13 binding could be detected when an activating antibody was added indicating that plasma ADAMTS13 adopts a closed conformation.

Summary / Conclusions: We proved that the physiological conformation of ADAMTS13 in healthy people is mainly closed and can be conformationally activated by the addition of an activating antibody.

A-136
The p.Arg498Cys mutation in the cysteine rich domain of ADAMTS13 results in a secretion deficiency

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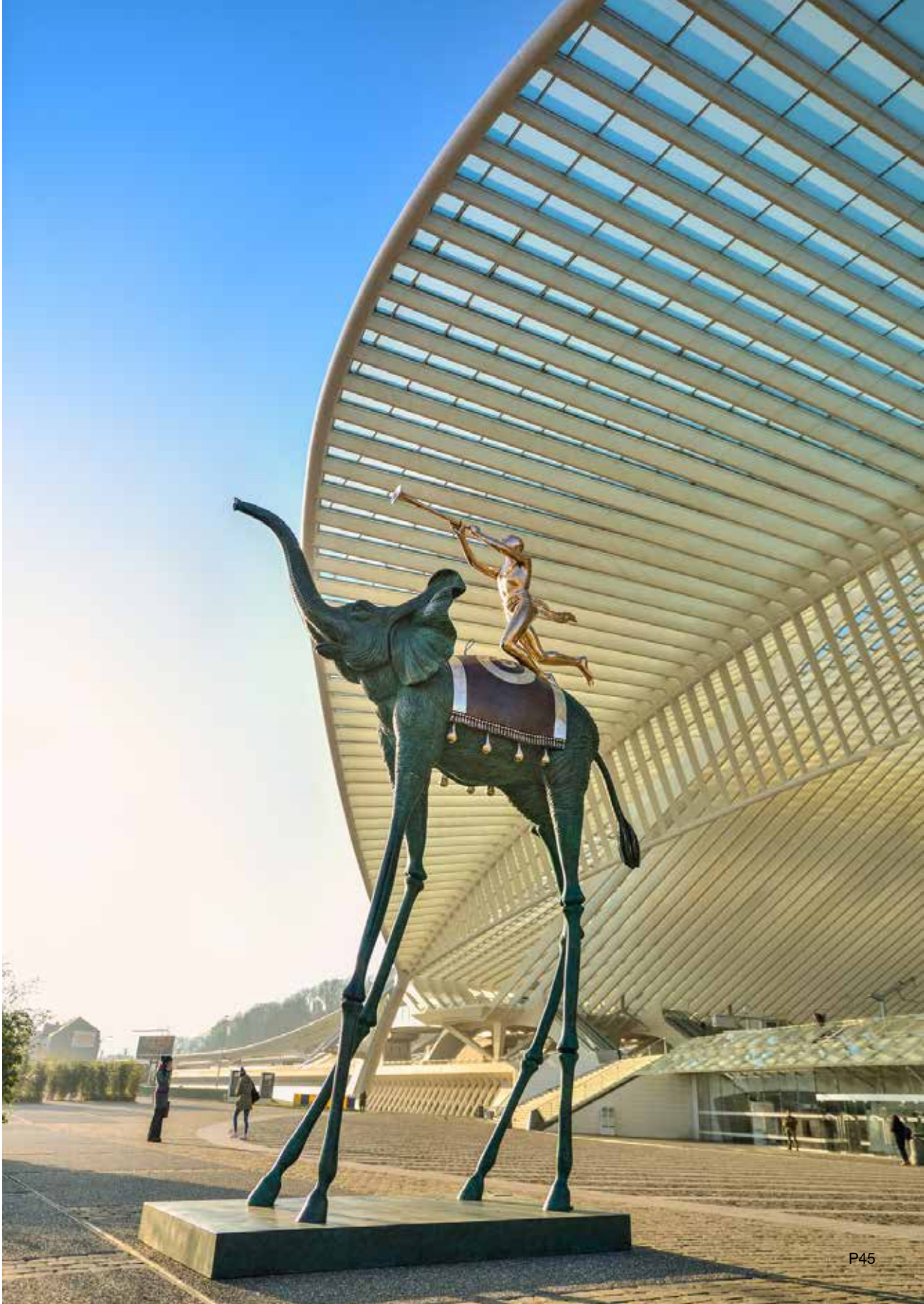
Background / Introduction: Congenital thrombotic thrombocytopenic purpura (TTP) is characterized by mutations in the ADAMTS13 gene. Defects in ADAMTS13 substrate binding, enzyme activity and/or secretion have been reported. Over 140 mutations have been identified, however the contribution of each mutation to the phenotype is not established.

Aims: We aimed at determining ADAMTS13 antigen (Ag) and activity in a congenital TTP patient, at identifying the mutation(s) in his ADAMTS13 gene and at studying the effect of the mutation(s) on ADAMTS13 Ag and activity in vitro.

Methods / Materials: ADAMTS13 Ag and activity, the presence of anti-ADAMTS13 autoantibodies, von Willebrand factor (VWF) Ag and VWF multimer distribution were determined in patient's plasma samples isolated during acute phase, remission phase before and after the initiation of prophylactic plasma infusion (PI). Genomic DNA was isolated from the propositus and all 29 ADAMTS13 exons and intron-exon boundaries were sequenced. Patient mutations were introduced in the wild type (WT) ADAMTS13 pcDNA6.1 expression vector via site-directed mutagenesis. CHO cells were transfected with WT and mutant ADAMTS13 expression plasmids and ADAMTS13 Ag present in the expression medium was determined. Cotransfections were performed with a GFP expressing plasmid to determine transfection efficiency.

Results: The patient had no detectable ADAMTS13 Ag during the acute phase and very low Ag during remission phase with a significant increase after the first prophylactic plasma infusion (pre-PI 7.37±3.51% vs. post-PI 23.40±8.95%, $p<0.05$), although ADAMTS13 activity deficiency (<10%) remained. No inhibitory anti-ADAMTS13 antibodies were detected. Total VWF Ag was increased (205.4±3.98% vs NHP 103.8±2.2%, $p<0.05$) in the acute TTP episode but not in remission. As expected, a significant decrease in high molecular weight (HMW) VWF multimers was observed during acute phase (20.55±1.23% vs. NHP 31.45±0.48%, $p<0.05$) whereas HMW VWF multimers were significantly increased during remission (pre-PI 41.18±6.68%, post-PI 39.05±8.84% vs. NHP 31.45±0.48%, $p< 0.05$) with no significant reduction after the first prophylactic plasma infusion treatment. We identified 1 novel heterozygous insertion c.775_776insCGGCGCC in the metalloprotease domain (p.G259PfsX391) resulting in a premature stop codon in the thrombospondin type 1 repeat and detected the previously defined c.1492C >T mutation (p.R498C) localized in the cysteine-rich domain of the ADAMTS13 gene. Whereas construction of the c.775_776insCGGCGCC mutant is currently ongoing, the c.1492C>T mutation was successfully introduced in the WT ADAMTS13 pcDNA6.1 expression vector via site-directed mutagenesis. Transient transfection of WT and p.R498C expression plasmids into CHO cells revealed that the p.R498C mutation resulted in a secretion defect as no mutant ADAMTS13 protein was detected in the expression medium. Transfection efficiency was similar for both WT and mutant expression plasmids (32.43±9.44% and 33.42±8.82% respectively) and resulted in 0.06±0.01 nM expression of WT ADAMTS13.

Summary / Conclusions: The patient is compound heterozygous for the c.1492C>T (p.R498C) and the c.775_776insCGGCGCC insertion (p.G259PfsX391). In vitro expression of mutant p.R498C ADAMTS13 revealed a severe secretion deficiency. ADAMTS13 Ag is absent in patient's plasma, it is likely that the c.775_776insCGGCGCC insertion which results in a premature stop codon in the ADAMTS13 gene will also lead to a secretion deficiency. In conclusion, both mutations explain the absence of ADAMTS13 Ag in the patient's plasma.



A-107
A clinically relevant hybrid adenovirus-Sleeping Beauty transposon vector for gene therapy for von Willebrand disease

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Background / Introduction: Severe von Willebrand disease (type 3 VWD) is characterized by complete absence of von Willebrand factor (VWF). Current treatment options are limited to administration of exogenous VWF/FVIII plasma concentrates, which only provide a short-term solution. Gene therapy would meet the demand for a long-term treatment and would significantly improve the patient's quality of life. Using a non-viral Sleeping Beauty (SB) transposon strategy, we previously achieved long-term and stable VWF expression in VWF-deficient mice. However, the used hydrodynamic tail vein delivery method limits translation towards more clinically relevant models. Adenoviral vectors are the most commonly used platform in clinical trials for gene therapy. High-capacity adenoviral vectors (HC-AdV), the latest generation of adenoviral vectors, are devoid of all coding viral genes, allowing transgenes up to 36 kb. Combining HC-AdV with the SB transposon technology improves the long-term therapeutic potential because of the transposon-mediated integrative features.

Aims: To develop a clinically applicable gene therapy platform for VWD based on high-capacity adenoviral vectors that deliver the SB transposon system to hepatocytes.

Methods / Materials: Construction of the HC-AdV containing VWF was based on recombineering technology. The liver-specific mVWF-transgene cassette, flanked by Flp recognition target sites and SB inverted repeats, was cloned into a bacterial artificial chromosome carrying the HC-AdV genome via homologous recombination using galactokinase as a counter selection marker, followed by established HC-AdV production. After intravenous injection of the vectors in VWF-deficient mice, blood was taken and plasma VWF antigen level, VWF multimer pattern, and FVIII activity were analyzed at regular time intervals up to 1 year. Correction of the bleeding phenotype was evaluated using a tail-clip bleeding assay and saphenous vein bleeding model.

Results: After construction and production, functionality of this hybrid adenovirus-SB transposon vector was assessed in VWF-deficient mice. Intravenous dual delivery of the constructed VWF HC-AdV in combination with HC-AdV carrying both the Flp recombinase and SB100X transposase transgenes resulted in very high and stable mVWF levels, up to 2072±383% one year after gene transfer (n=3). Moreover, the supraphysiological mVWF levels were able to restore FVIII activity to physiological levels (102±33% 1 year after gene transfer, n=3). Twelve weeks after gene transfer tail-clip bleeding was corrected in 1 out of 3 mice. While the saphenous vein bleeding model for smaller injuries resulted only in a small, but not significant reduction of the average bleeding time (109±16 s; n=3) 1 year after gene transfer in comparison to 199±63 s (n=4) in untreated VWF-deficient mice. The reduced fraction of high molecular weight multimers observed in hepatocyte produced VWF might account for the partial corrected bleeding phenotype, despite the sustained supraphysiological mVWF plasma levels.

Summary / Conclusions: Delivery of the mVWF transgene cassette by the hybrid adenovirus-SB transposon vector was very efficient, resulting in very high mVWF transgene expression for up to 1 year after gene transfer. However, no full correction of the bleeding diathesis could be observed, due to the limited hemostatic capacity of hepatocyte produced VWF. Nevertheless, the powerful hybrid adenovirus-SB transposon vector system shows great promise towards a clinically applicable gene therapy platform for VWD e.g. for targeting endothelial cells.

A-105
The role of von Willebrand factor in a malaria-associated lung pathology model

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Background / Introduction: Malaria is a global health burden, affecting more than 3 billion people, resulting in 200 million clinical cases and around 400,000 deaths each year. The pathophysiological mechanisms of severe malaria are complex and involve factors that remain poorly understood. Recent clinical studies have demonstrated that severe malaria infection is associated with acute endothelial cell activation, accumulation of highly reactive ultra large (UL-) von Willebrand factor (VWF) multimers, and a significant reduction in ADAMTS13 activity. Whether this prothrombotic state constitutes an epiphenomenon, or whether this plays an active role in the pathophysiology of malaria remains to be determined.

Aims: To investigate the role of VWF in malaria using a murine model of malaria-associated lung pathology.

Methods / Materials: Wild-type (WT) and VWF knock out (Vwf-/-) mice on C57BL/6J background were inoculated with 104 Plasmodium berghei (P.b.) NK65-infected erythrocytes via intraperitoneal injection. After infection, blood samples were taken to measure platelet counts and to assess levels and activities of VWF and ADAMTS13. Daily Giemsa-stained blood smears were prepared to measure parasitemia. Lung pathology was assessed by measuring protein levels in broncho-alveolar lavage (BAL) fluid, which is indicative for edema and alveolar leakage.

Results: Plasma VWF levels in infected WT mice significantly increased 3 days after infection (2 fold increase; $p<0.0001$), but normalized afterwards. During the course of infection, VWF multimer patterns remained normal until the end stage (day 8/9) at which a marked decrease in high molecular weight VWF multimers was observed ($p<0.0001$). This was accompanied by a significant reduction of the ADAMTS13 activity/antigen ratio ($p<0.0001$). Interestingly, severe thrombocytopenia was observed in both mouse strains starting from day 5 after infection, indicating a VWF-independent mechanism causing thrombocytopenia following P.b. NK65 infection. Overall mouse survival times were slightly but significantly shortened in Vwf-/- mice compared to WT mice (9 versus 10 days; $p=0.03$). Vwf-/- mice also showed an increased appearance of infected red blood cells, with a marked increase in mature schizont stages starting at day 7 after infection. Alveolar leakage in lungs at day 8 post-infection was significantly lower in Vwf-/- mice (Vwf-/-: 1.8±0.4 mg/mL versus WT: 3.6±0.5 mg/mL; $p=0.02$).

Summary / Conclusions: Our data demonstrate that early P.b. NK65-mediated murine malaria infection is associated with elevated levels of plasma VWF, which is indicative for endothelial cell activation and in accordance with human malaria infection. Our findings also show that VWF does not contribute to malaria-associated thrombocytopenia. Furthermore, VWF might influence the development of parasitemia and lung pathology, potentially by interfering with the sequestration of infected red blood cells.

A-109
Unrestrained endogenous plasmin activity reverses acute thrombotic thrombocytopenic purpura

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Background / Introduction: Thrombotic thrombocytopenic purpura (TTP) is an acute, rare and potentially life-threatening pathology, where patients present with occlusive VWF-rich microthrombi caused by a severe ADAMTS13 deficiency. We previously demonstrated that plasmin can cleave von Willebrand factor (VWF) multimers. Interestingly, we

also showed that endogenous plasmin is generated in patients during acute TTP episodes, however it is currently not known if plasmin-mediated digestion of VWF influences acute TTP episode outcome.

Aims: Elucidate the role of endogenous plasmin activity in acute TTP episodes.

Methods / Materials: Plasmin-α2-antiplasmin (PAP)-complex and active plasminogen activator inhibitor (PAI-1) levels were determined in TTP patient- and mouse plasma using ELISA. ADAMTS13 and PAI-1 activity were blocked using monoclonal antibodies in either α2-antiplasmin-/- or wild type mice. TTP was triggered using 2000 U/kg recombinant (r) VWF. VWF multimer analysis was performed on plasma obtained from mice to demonstrate VWF proteolysis.

Results: We here measured elevated PAP-complex levels in patients suffering from acute TTP (6.54 (0.15 – 64.91) mg/mL (median (range)); n=44) compared to TTP patients in remission (0.64 (0.00 – 6.01) mg/mL; n=40). However, no correlation was observed between PAP-complex levels and clinical symptoms (e.g. neurological disorders, impaired renal failure), indicating that plasmin had no effect on disease outcome. Increased active PAI-1 levels were also observed during acute TTP (48.8 (0.0 – 379.2) ng/mL) compared to TTP patients in remission (19.4 (0.0 – 139.3) ng/mL), suggesting that excessive plasminogen activation is controlled and limited by PAI-1. We next hypothesized that allowing unrestrained plasmin activity would allow reversal of acute TTP signs. First, mice deficient in ADAMTS13 were triggered with rVWF to develop TTP signs. Similar to acute TTP patients, mice presented with 6-fold elevated PAP-complex and 19-fold elevated PAI-1 levels. Next, unrestrained plasmin activity was allowed by using α2-antiplasmin-/- mice with PAI-1 inhibitor. These mice first presented with TTP signs after 6 hours. However, unrestrained plasmin activity resulted in a reversal of TTP signs, demonstrated by a restoration of the platelet count after 24 hours (681 ± 232 x103 platelets/ µL (mean ± SD) in mice with unrestrained plasmin activity; 146±36 x103 platelets/ µL in control mice (α2-antiplasmin+/+ mice with isotype control antibody); n=12). This restoration could be attributed to an increased proteolysis of VWF in the presence of unrestrained plasmin activity, demonstrated by VWF multimer analysis.

Summary / Conclusions: Plasmin generation occurs during acute TTP episodes alongside secretion of active PAI-1, without influencing patient outcome. Only when endogenous plasmin was unrestrained in mice, acute TTP signs could be reversed by an increased proteolysis of VWF. These data explain why plasmin generated during acute TTP is not sufficient to influence TTP outcome.

A-111
Clopidogrel prevents colitis-associated carcinogenesis in mice by inhibiting immunosuppressive function of immature myeloid cells

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Background / Introduction: The role of antiplatelet agents in tumor growth and in cancer prognosis is currently under investigation in several clinical trials. A FDA review indicates that long-term use of clopidogrel does not change the risk of death in cancer patients. Clopidogrel inhibits tumor growth and metastasis in various preclinical mouse models of cancer. In patients, it has been shown that clopidogrel therapy lowers the levels of inflammatory markers. Therefore, we hypothesized that clopidogrel could affect cancer development under chronic inflammatory conditions.

Aims: To investigate the effect of clopidogrel on colitis-induced carcinogenesis in mice.

Methods / Materials: We used the azoxymethane/dextran sulphate sodium (AOM/DSS) mouse model that leads to tumor development within 65 days. Clopidogrel was continuously administered via the drinking water. Temporal changes in blood, and spleen cell composition, and colon infiltration by immune cells were assessed by flow cytometry. Platelet activation and reactivity were monitored throughout the protocol. The number and size of the tumors were measured. Colorectal tumor proliferation rate, and infiltration by myeloid and T cells were analysed by immunohistochemistry. Phenotype and immunosuppressive activity of sorted myeloid cells were analysed by RT-qPCR and mixed lymphocyte reaction assays, respectively.

Results: AOM DSS provoked profound changes in platelet and immune cell counts at the inflammatory stages preceding the apparition of tumors. Platelet count increased while immunosuppressive immature myeloid cells accumulated very rapidly in the spleen, blood, and in the colon of the mice. The number of circulating activated platelets significantly

increased when tumor developed and microthrombi were observed in tumor microvasculature. Clopidogrel efficiently inhibited platelet activation in tumor-bearing mice. It limited diarrhea and, importantly, did not worsen bleeding. It significantly reduced dysplasia and the numbers of adenocarcinoma observed at 15 and 80 days, respectively (tumor number per colon: AOM DSS: 12,64 ± 2,064 versus Clopidogrel: 7,650 ± 1,019, P=0,0213). It did not affect the expansion of immunosuppressive myeloid cells in the spleen, but efficiently prevented their accumulation in tumors. Accordingly, numbers of cytotoxic CD8 T cells in tumors were augmented. The immature myeloid cells isolated from the spleen of clopidogrel-treated mice were unable to inhibit T cell proliferation ex vivo, which was compatible with reduced mRNA expression of TGF-β. Clopidogrel treatment decreases the expansion of pro-tumoral regulatory T cells in mouse blood.

Summary / Conclusions: The antiplatelet drug clopidogrel may inhibit colitis-induced carcinogenesis by affecting functional interactions of platelets with immunosuppressive cells, thereby improving antitumor immunity.

A-140
Staphylococcus aureus and Staphylococcus lugdunensis bind von Willebrand Factor to overcome shear stress and cause endocarditis

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Background / Introduction: Both Staphylococcus aureus and Staphylococcus lugdunensis are feared causes of infective endocarditis, a dreaded disease with a mortality of 30-40%. The pathogenesis of this complex disorder remains insufficiently understood, explaining why neither its incidence nor its mortality improved over the last decades. In order to cause endocarditis, bacteria must first attach to the heart valves. However, they are hampered by the high blood flow that gushes over these valves. This high blood flow creates tremendous shear stress, which bacteria need to overcome to bind and colonize the heart valves. By unraveling how bacteria withstand shear stress we can interfere with their initial adhesion and develop new ways to prevent endocarditis.

Aims: In this study we explored how S. aureus and S. lugdunensis overcome shear stress and cause endocarditis. Since blood platelets use von Willebrand factor (VWF) to adhere under shear stress, we investigated weather this was also the case for S. aureus and S. lugdunensis.

Methods / Materials: Using flow chamber technology, we measured the adhesion of S. aureus and S. lugdunensis to VWF and endothelial cells under shear stress. To study the adhesion of bacteria to the vessel wall in vivo, we used a mesenteric microvascular perfusion mouse model. In this model we could visualize the interaction between fluorescently labeled bacteria and the vasculature in real time. Finally, we also developed a new inflammatory endocarditis mouse model. In this model we inserted a small catheter in the carotid artery and advanced it beyond the aortic valve. By infusing histamine via this catheter, we could locally activate the aortic valve endothelium. After activating the endothelial cells, we injected fluorescently labeled bacteria. Using confocal imaging of the heart valve, we could for the first time study the initial adhesion of S. aureus and S. lugdunensis to the heart valves. Using VWF knockout mice, we investigated the role of VWF in early bacterial adhesion to heart valves.

Results: Similar to platelets, both S. aureus and S. lugdunensis were able to bind to VWF under flow. This in contrast to other coagulase negative staphylococci that do not cause endocarditis. VWF is normally released when endothelial cells are activated by blood vessel inflammation or damage. By binding to VWF, S. aureus and S. lugdunensis could adhere to these activated endothelial cells despite opposing shear stress. Also, in our in vivo microvascular perfusion model we showed that these bacteria do not adhere to resting blood vessels. However, when the vascular endothelium is activated and VWF was released bacteria, readily bound to the vessel wall. In consequence, vascular adhesion of S. aureus and S. lugdunensis was significantly reduced in VWF knock out mice. Similarly, in our new inflammatory endocarditis model we could show that these bacteria hardly bound to resting valve endothelium. However, by inducing local inflammation and VWF release, we could make bacteria stick to heart valves and induce endocarditis. In VWF knockout mice binding of both S. aureus and S. lugdunensis to heart valves was significantly reduced.

Summary / Conclusions: Just like platelets S. aureus and S. lugdunensis overcome shear stress by binding directly to VWF. This allows them to bind and infect the hearth valves and explains why S. aureus and S. lugdunensis are so effective in causing endocarditis, compared to other staphylococci.


VAUBAN

A-103
Validation of a chromogenic assay for FVIII: C and FIX: C (CSA) measuring and comparison with a chronometric assay (OSA).

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Background / Introduction: Two types of laboratory tests are available for diagnostic and treatment monitoring of hemophilia A and B: the chronometric method derived from the aPTT one stage clotting assay (OSA).The chromogenic method is based on a "two stages clotting assay" (CSA) with a first stage involving FXa generation and a second stage where generated FXa cleaves a specific chromogenic substrate resulting in a color intensity directly proportional to the amount of FVIII or FIX present in the sample. Currently, the SSC of FVIII and FIX of the ISTH recommends measuring FVIII:C or FIX:C by both one stage and chromogenic assays and treating patients on the basis of the lowest measured plasma values.

Aims: The aim of our study was to evaluate a CSA for the dosage of FVIII: C and FIX: C and to compare the results with the OSA, the unique test routinely used in our laboratory.

Methods / Materials: "Biophen FVIII: C" and "Biophen Factor IX" (Hyphen Biomed®) CSA were evaluated. OSA reagents were Cephascreen® aPTT reagent (Stago) and CRYOcheck® FVIII and FIX deficient plasmas (Precision BioLogic). Linearity, limits of quantification and detection, repeatability, reproducibility, stability, carry-over of CSA were evaluated using "low" and a "high" CSA curves. Determination of usual values was established for both CSA and OSA on plasmas from 46 normal subjects. Sixty patient plasmas (hemophiliacs or not) were analyzed with both OSA and CSA. Results were compared using the Deming regression and the Bland Altman method.

Results: Quantification limit of CSA was 0.33% for FVIII: C and 0.25% for FIX: C. Precision, linearity and carry-over were excellent. The calibration curve for FVIII was stable at least 24 days for "low curve" and 32 days for "high curve" and 15 days and 21 days respectively for FIX. Usual values were lower for CSA than for OSA. Bland Altman method shows a proportional positive bias of OSA compared with the CSA of 21.6% (-1.15%-44.3%) for the FVIII and of 26.1% (-12.5%–64.7%) for FIX (see figure 1).

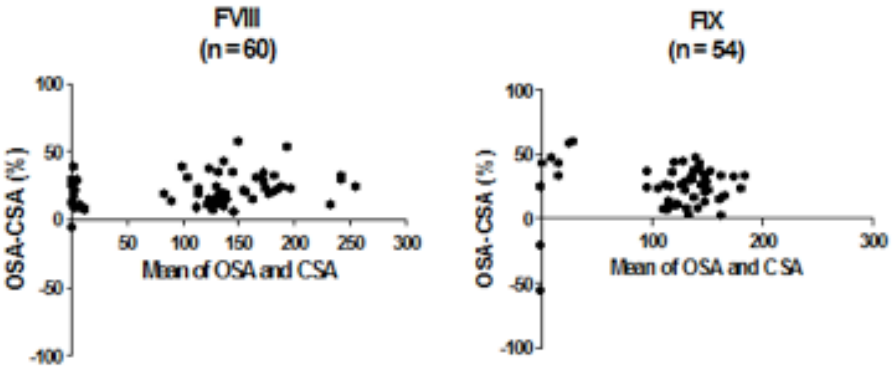

Figure 1 : Bland Altman plot for FVIII *(to the left)* and FIX *(to the right)*

Summary / Conclusions: The CSA for both FVIII: C and FIX: C showed excellent performance. However, our results highlight that the use of different tests raises some challenge in FVIII:C and FIX: C testing. For example, there are some well-known discrepancies in case of diagnosis of minor hemophilia or for the monitoring of new recombinant FVIII and FIX products. This illustrates the fact that to implement the optimal test for monitoring will be a challenge for clinical laboratories. Periodic assessment of performance of these assays through proficiency testing is critical in plasma of treated patients.

A-115
Acquired von Willebrand syndrome after left ventricular assist device implantation

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Background / Introduction: Acquired von Willebrand syndrome (aVWS), characterized by the loss of high molecular weight (HMW) von Willebrand factor (VWF) multimers, contributes to a bleeding diathesis observed in patients treated with left ventricular assist device (LVAD) implantation for end-stage heart failure. Remarkably, VWF size and activity seem to quickly recover upon LVAD removal and heart transplantation.
In classical von Willebrand disease (VWD), the decrease in HMW VWF multimers is accompanied by a decreased ratio of collagen binding activity (VWF:CB) over VWF antigen (VWF:Ag) (VWF:CB/VWF:Ag <0.7) and a decreased VWF ristocetin cofactor activity (VWF:RCo) over VWF:Ag (VWF:RCo/VWF:Ag <0.7). Although LVAD patients have lower HMW VWF multimers and a decrease in VWF:CB/VWF:Ag ratio, whether or not together with a reduced VWF:RCo/VWF:Ag ratio, these ratios rarely become less than 0.7. Hence, studying aVWS in LVAD patients is important to clarify the differences between classical VWD and LVAD induced aVWS.

Aims: To follow the aVWS laboratory characteristics in patients after LVAD implantation.

Methods / Materials: Plasma samples were collected from 14 LVAD patients at time points ranging from 1 month to 3 years during LVAD therapy and from 2 patients before LVAD implantation. Four out of 14 patients received a heart transplantation after LVAD removal. In these patients, plasma samples were also collected immediately after surgery. VWF multimer pattern, VWF:Ag, VWF:CB and VWF:RCo were determined. Classical VWD phenotype was defined by decreased HMW VWF multimers, together with reduced VWF:CB/VWF:Ag (<0.7) and VWF:RCo/VWF:Ag (<0.7) ratios.

Results: Patients demonstrated a significant loss of HMW VWF multimers at 3 (17.0 ± 1.34%), 6 (19.3 ± 1.61%) and 9 (17.1 ± 3.69%) months after LVAD implantation compared to normal human plasma (30.3 ± 1.00%, P<0.05). Ten out of 14 patients demonstrated a decreased VWF:CB/VWF:Ag ratio at 3 and/or 6 months during LVAD therapy (0.71 ± 0.15% and 0.72 ± 0.19% respectively compared to 1.01 ± 0.03% in normal human plasma, P<0.05). In contrast, VWF:RCo/VWF:Ag ratios were decreased in only five out of 14 patients (0.83 ± 0.06% compared to 1.03 ± 0.03% in normal human plasma, P<0.05) within the first 6 months after LVAD implantation but not in the other 5 patients (1.34 ± 0.23% compared to 1.04 ± 0.03% in normal human plasma).
Three of the 14 patients were followed up from one year onwards after LVAD implantation and also have a decreased VWF:CB/VWF:Ag ratio (0.87 ± 0.05% compared to 1.01 ± 0.03% in normal human plasma, P<0.05). VWF:RCo/VWF:Ag ratio was also decreased in one patient that was investigated from 15 to 18 months after LVAD implantation (0.76 compared to 1.04 in normal plasma).
Four out of 14 patients received a heart transplantation and immediately after surgery, HMW VWF multimers increased (from 17.1 ± 1.43% during LVAD implantation to 27.7 ± 5.88% after heart transplantation). Two out of 4 patients with a heart transplantation demonstrated a significant increase in VWF:CB/VWF:Ag ratio and one of these two patients had a significantly increased VWF:RCo/VWF:Ag ratio.

Summary / Conclusions: During LVAD therapy, patients have decreased HMW VWF multimers but their mean VWF:CB/VWF:Ag and VWF:RCo/VWF:Ag ratios were never below 0.7. Heart transplantation resulted in an immediate rise in the HMW VWF multimers and was associated in a subgroup of these patients with a restoration of VWF activity.

A-137
Coagulation Factor IX inhibitor determination revisited: practical evaluation of the anti-FIX Bethesda assay.

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Background / Introduction: Likewise inhibitor development in factor (F) VIII deficiency, an inhibitor is suspected in patients with FIX deficiency when their FIX level fails to increase in response to factor IX infusion therapy. The presence of a FIX-inhibitor can be confirmed using a Bethesda assay for FIX. The Nijmegen modification of the classic Bethesda assay is an effective method for monitoring and quantifying inhibitor titres and is the standard recommended by the FVIII/FIX Scientific & Standardization Committee of the International Society on Thrombosis & Haemostasis (SSC-ISTH) (1).

Aims: The goal of the study was to re-evaluate an anti-FIX inhibitor Bethesda assay routinely used, by evaluating the influence of the diluent; imidazol buffer versus FIX deficient plasma. Additionally, the limit of detection (LOD) and the imprecision was evaluated.

Methods / Materials: FIX one-stage clotting assay with STA® IMMUNODEF IX and C.K. Prest® as FIX deficient plasma and aPTT reagent respectively, was used. STA® - Unicalibrator was used as a known source of FIX (buffered normal pool plasma pH 7.47). The diluent study was performed on five samples with increasing FIX inhibitor concentrations (range 0.58 – 4.60 Bethesda units (BU)/mL) by making a dilution series of a commercial control sample (Mild FIX Inhibitor plasma, Affinity Biologicals) with imidazol buffer pH 7.35 or STA® IMMUNODEF IX . LOD was performed by measuring normal pool plasma after denaturation at 56°C for 1 hour (n=6). Analytical performance was evaluated by between-run imprecision and calculating Bias% and Total error (TE). Between-run imprecision experiment (n=6) was performed using 2 levels of control material (Mild FIX Inhibitor plasma, Affinity Biologicals).

Results: Diluent study showed no statistical difference between imidazol buffer and Immunodef IX (Wilcoxon test: P=0.3125) prediluted samples. Furthermore we observed a good correlation of 0.9928 and 09994 to the expected theoretical values for imidazole buffer and Immunodef IX diluted samples, respectively. Deviation from the expected values was <13%. A negative mean value was obtained for LOD detection. The coefficient of variation (CV) was 6.14% and 6.49% for the low (target value 2.3 BU/mL) and high (target level 4.6 BU/mL) control material, respectively. Bias and TE were 1.96% and 12.08% for the low level control material and 2.72% and 13.43% for the high level control material.

Summary / Conclusions: Our evaluation showed no apparent difference between results obtained with both diluents. Although the use of a factor deficient plasma is recommended, our results suggest imidazol buffer can be used as a diluent. This leads to a more cost efficient approach.
Analytical performance of between-run imprecision, bias and total error is good.

(1) Giles A.R. et al. A detailed comparison of the performance of the standard versus the Nijmegen modification of the Bethesda assay in detecting factor VIII:C inhibitors in the haemophilia A population of Canada. Association of Hemophilia Centre Directors of Canada. Factor VIII/IX Subcommittee of Scientific and Standardization Committee of International Society on Thrombosis and Haemostasis. Thrombosis and haemostasis, 1998, 79, 4, 872 -875.

A-139
Comparison of 4T-score and laboratory tests in the diagnosis of HIT.

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Background / Introduction: Heparin induced thrombocytopenia (HIT) is a life-threatening complication of heparin exposure. It is a rare disease characterized by thrombosis and thrombocytopenia. It is hard to diagnose because of the broad differential diagnosis of thrombocytopenia and the comorbidities which are often present. The 4T-score is a clinical scoring system to differentiate patients with HIT from those with other causes of thrombocytopenia. The scoring system is based on the degree and onset of thrombocytopenia after starting heparin therapy, presence of thrombosis and possible other causes of thrombocytopenia.

Aims: Comparison of the 4T-score with different immunoassays for HIT.

Methods / Materials: Samples for HIT testing were tested with a rapid test (STic Expert® HIT, Stago or D-PaGIA® Heparin/PF4 Antibody Test, Diamed) and the clinician is contacted for the 4T-scores. Samples with a suspicion of HIT (4T-score ≥ 4 and/or positive immunoassay) were also tested with the Bioflash (AcuStar HIT-Ab(PF4-H), HemosIL®). Where available, patient records were checked whether heparin was stopped and platelet counts were noted.

Results: Thirty-three samples were tested with a rapid test and Bioflash. Twenty samples had a 4T-score ≥ 4 of which 1 had a 4T-score of 8. There was also 1 sample with an unknown 4T-score. The rapid tests were often hard to interpret and user-dependent, those results were noted as weak positive. The rapid tests had 19 samples positive, 7 samples

weak positive and 7 samples negative. However only 4 out of 33 samples were positive with Bioflash (cut-off 1 U/ml). Those 4 samples had 4T-scores of 5 and 6 and were clearly positive with the rapid test. Thirteen samples (out of 16 samples) had a doubling of platelet count within 3-5 days after testing. Of 8 of those 13 samples we are sure that heparin (or derivate) was stopped. The 4T-score of these 16 samples were ≤3 (4 samples),4-5 (8 samples) and ≥ 6 (4 samples).

Summary / Conclusions: Rapid tests have a high number of false positive samples. Some are caused by the difficult interpretation. Bioflash had a very low number of positive samples. We think it is possible that there were false negative results with Bioflash, especially one sample with a 4T-score of 8 and with platelet elevation after heparin stop. Although this is a small study, we can conclude that HIT is still hard to diagnose and there is no ideal test. It is important to contact the clinician, determine the 4T-score and when it is suggestive for HIT, advice the clinician to stop heparin, independent of the test results. Disadvantage of this study is the fact we didn't compare with a functional test.

A-138
Comparison of activated clotting time measured by I-STAT, Sonoclot and ACTPlus and correlation with anti-Xa during cardiopulmonary bypass procedures

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Background / Introduction: Activated clotting time (ACT) measurements are used to monitor anticoagulation therapy with high heparin concentrations during cardiopulmonary bypass procedure. Various point-of-care instruments are available for measuring ACT. However, as baseline values and therapeutic ranges are method dependent, these instruments cannot be used interchangeably.

Aims: In the present study we evaluated ACT measurements by three different point-of-care instruments [I-STAT (Abott), ACTPlus (Medtronic) and Sonoclot (Sienco, Inc)], and their correlation with anti-Xa values during cardiopulmonary surgery.

Methods / Materials: One ACTPlus instrument (which measures in duplicate) was compared with simultaneous, parallel measurements on two I-Stat and two Sonoclot instruments. For 30 adult patients who received heparin during surgery, ACT was measured on the different instruments at 4 time points: before heparin administration (baseline), 3 minutes following administration, and 15 and 45 minutes after starting cardiopulmonary bypass. In accordance with manufacturers' guidelines, acceptance criteria for duplicate (intra-assay, ACTPlus) and parallel (inter-assay, I-Stat and Sonoclot) measurements were set as ≤12% spread error. Citrated plasma was collected at each time point for determination of anti-Xa by a chromogenic assay (BioPhen Heparin LRT). Method comparison was performed using Passing and Bablok regression analysis.

Results: Results per type of instrument are displayed in the table. Overall, only 51,2% of ACTPlus measurements fulfilled the 12% acceptance criteria; large differences were observed in cases with > 12% spread error (mean difference 143,1%). In comparison, 8,4% of parallel measurements on I-STAT and 32,8% on Sonoclot differed >12%. Correlation between anti-Xa and ACT values was only moderate for the three instrument types. Method comparison showed no proportional or systematic error between I-STAT and ACTPlus. However, we observed a proportional and systematic error when comparing Sonoclot with both I-STAT and ACTPlus. For each time point, ACT values were significantly lower on Sonoclot in comparison with ACTPlus and I-STAT.

	ACTPlus	**I-STAT**	**Sonoclot**
Type of measurement	Duplicate (D)	Parallel (P)	Parallel (P)
Number of measurements	119	119	120
D/P with valid result for both measurements	100 (84,0%)	112 (94,1%)	113 (94.2%)
Measurement <=12% spread error	61 (51,2%)	102 (85,7%)	74 (61,7%)
Measurement >12% spread error	39 (32,8%)	10 (8,4%)	39 (32.5%)
Mean spread error between all D/P	143,1%	5,2%	30,6%
Minimum – maximum spread error between D/P	0,0 – 1781,5%	0,0 – 34,8%	0,0 – 800,0%
Correlation between anti-Xa and ACT	R^2 = 0.57 (0.38 – 0.71)	R^2 = 0,62 (0,50 – 0,72)	R^2 = 0,45 (0,31 – 0,58)

Results per type of instrument for ACT measurements

Summary / Conclusions: Overall, I-STAT performed better than ACTPlus and Sonoclot for measurement of ACT. When using 12% spread error as criterium, only 51,2% of measurements performed on ACTPlus and 61,7% on Sonoclot passed. These results are unacceptable in clinical practice and require further investigation.

A-116
Evaluation of the clinical performance of two automated immunoassays in the diagnosis of thrombotic antiphospholipid syndrome

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Background / Introduction: Detection of antiphospholipid antibodies (aPL) is mandatory for the diagnosis of antiphospholipid syndrome (APS). Despite efforts for standardization of aPL testing, inter-assay variability continue to be observed and sensitivity and specificity may differ between assays.

Aims: To evaluate the clinical performance of a fully automated assay system for simultaneous detection of anticardiolipin (aCL) and anti-β2 glycoprotein I (aβ2GPI) antibodies in the diagnosis of thrombotic APS (TAPS) and to compare its performance to an automated chemiluminescent immunoassay, routinely used in our laboratory.

Methods / Materials: Plasma samples from 354 patients, including 66 patients with thrombotic APS (TAPS), 97 patients with non-APS thrombosis, 91 patients with autoimmune disease and 100 healthy controls, were tested for aCL (IgG, IgM) and aβ2GPI (IgG, IgM) using the BioPlex® 2200 multiplex immunoassay system and the HemosIL® AcuStar chemiluminescence immunoassay system. The cut-off values for positivity were set based on recommendations by the manufacturer: 20 U/mL for aCL and aβ2GPI IgM and IgG.
Comparison of HemosIL AcuStar to BioPlex 2200 was performed based on 2 x 2 contingency tables for test results against definite TAPS and against thrombosis (classification of patients in clinically/ non-clinically affected). Sensitivity, specificity, odds ratios (OR) and P-values (two-sided Fisher's exact test) were calculated. Differences between sensitivity and specificity of the test systems were evaluated with the McNemar chi-square test. Cohen kappa agreement test and Spearman correlation test were performed to analyze the qualitative and quantitative agreement.

Results: Regarding TAPS, sensitivity of BioPlex aCL IgM was significantly lower compared to AcuStar aCL IgM (P=0,004), while the specificity was significantly higher with BioPlex (P<0,001). A small but significant difference in specificity could be observed for aCL IgG (P=0,031) and aβ2GPI IgG (P=0,031). Regarding thrombosis, sensitivity of BioPlex aCL IgM and aβ2GPI IgG was significantly lower compared to AcuStar aCL IgM (P=0,004) and aβ2GPI IgG (P=0,004). However, specificity of BioPlex aCL IgM was significantly higher (P<0,001).
OR for TAPS and thrombosis were comparable for both BioPlex and AcuStar for all parameters. With respect to TAPS, P Fisher and OR demonstrated good clinical performance for IgG and IgM on both test systems. Regarding thrombosis, OR and P Fisher were not significant for IgM, while they were for IgG.

Moderate qualitative agreements were observed for aCL IgG (κ=0,722), aβ2GPI IgG (κ=0,785) and aβ2GPI IgM (κ=0,738), while weak agreement was found for aCL IgM (κ=0,553). Significant quantitative correlations were identified for aCL (IgG, IgM) and aβ2GPI (IgG, IgM) (P<0,001), with the rho value ranging from 0.425 to 0.695.

A. Results of aCL and aβ2GPI antibodies related to TAPS diagnosis (n=66) in overall population (n=354)

	aCL IgG		aβ2GPI IgG		aCL IgM		aβ2GPI IgM	
	BioPlex	AcuStar	BioPlex	AcuStar	BioPlex	AcuStar	BioPlex	AcuStar
sens (%)	19,7	19,7	18,2	24,2	12,1*	25,8*	13,6	10,6
spec (%)	96,6*	99,0*	97,2*	92,4*	97,2*	92,4*	96,9	97,6
p Fisher	<0,001	<0,001	<0,001	<0,001	0,004	<0,001	0,002	0,007
OR	7,6	23,3	7,8	6,3	4,8	4,2	4,9	4,7
95% CI	3,1-18,7	6,4-84,6	3,0-19,9	2,9-13,6	1,7-13,4	2,1-8,5	1,9-12,9	1,6-14,1

B. Results of aCL and aβ2GPI antibodies related to thrombosis (n=163) in overall population (n=354)

	aCL IgG		aβ2GPI IgG		aCL IgM		aβ2GPI IgM	
	BioPlex	AcuStar	BioPlex	AcuStar	BioPlex	AcuStar	BioPlex	AcuStar
sens (%)	10,4	8,6	8,6*	14,1*	6,1*	11,7*	6,8	4,3
spec (%)	97,4	99,0	96,6	96,3	96,9*	89,5*	96,3	96,3
p Fisher	0,003	0,001	0,036	<0,001	0,205	0,736	0,228	0,791
OR	4,3	8,9	2,9	4,5	2,0	1,1	1,9	1,2
95% CI	1,6-12,0	2,0-39,7	1,1-7,2	1,9-10,8	0,7-5,7	0,6-2,2	0,7-5,0	0,4-3,4

aCL, anticardiolipin; aβ2GPI, anti-beta2 glycoprotein I; sens, sensitivity; spec, specificity; TAPS, thrombotic antiphospholipid syndrome; OR, odds ratio; 95% CI, 95% confidence interval for OR.

* P<0,05 significantly different (comparison between BioPlex and AcuStar)

"Table 1: Clinical performance characteristics for aCL and aβ2GPI measured by BioPlex and AcuStar"

Summary / Conclusions: In our study population, the BioPlex® 2200 assays performed well and comparable with the HemosIL® AcuStar assays. In combination with its high throughput and multiplex technology, BioPlex 2200 may be a useful tool in the diagnosis of TAPS with a simultaneous detection of multiple aPL autoantibodies from one sample.

A-141
Interference of CRP in lupus anticoagulans testing: is CRP the only cause?

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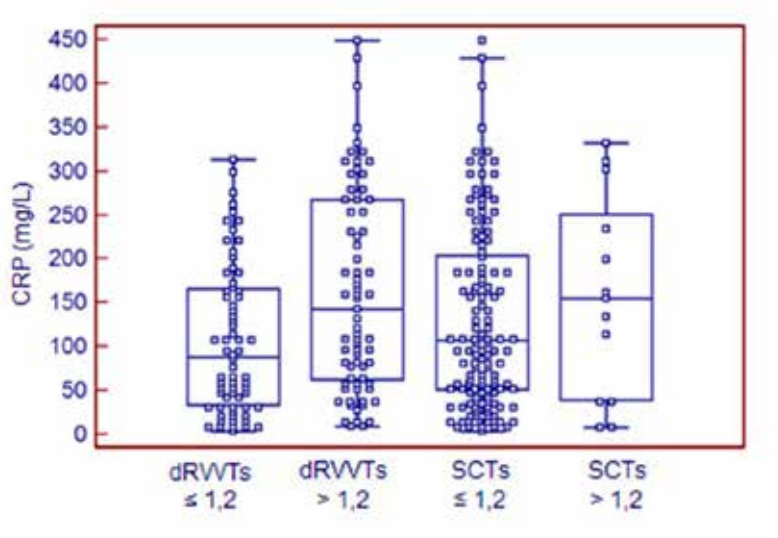
Background / Introduction: Interference of CRP in lupus anticoagulans (LAC) testing has been previously described. Most studies are retrospective or performed with spiking experiments. As far as we know, no studies with ACL-TOP (IL®, Werfen) and/or with normal patient samples have been performed.

Aims: How does CRP interfere with LAC testing on ACL-TOP500.

Methods / Materials: Patient samples with variable CRP values and normal aPTT and PT were selected and tested on ACL-TOP500 according to the ISTH guidelines: screening, mixing and confirming tests in 2 test systems: silica clotting time (SCT) (HemosIL®) and diluted Russell's viper venom test (dRVVT) (LAC screen/confirm, HemosIL®). We examined consecutive samples of the same patient to see any evolution. Patients with aberrant results that didn't normalize with lower CRP were retested after 12 weeks as ISTH criteria prescribe.
We also performed a laboratory information system (LIS) query of patients with LAC and CRP testing from May 2015 till July 2016.

Results: One hundred forty-one samples with CRP values between 3.8 and 448.6 mg/L have been analysed for LAC and 1550 samples were studied by LIS query. Influence of CRP was mainly seen in dRVVT (73 out of 141 samples). All tests of the dRVVT system (screening, mixing, confirming) were prolonged, which made the interpretation of the test doubtful or not interpretable. This was also visible in the LIS query: the highest CRP values had doubtful (median CRP: 33.7 mg/L) and not interpretable (26.0 mg/L) as result. The true positive and negative results had a median CRP value of 14.2 and 10.3 mg/L respectively.
CRP values of positive dRVVT screening ratio (cut-off 1.2) ranges from 9.2 to 448.6 mg/L, mean and median value of 162.5 and 142.7 respectively. The negative dRVVT screening ratio had a CRP range of 3.8 till 312.6 mg/L, mean value 107.3, median value 87.9mg/L. Mann-Whitney U test showed a P-value of 0.0035 ($P<0.05$). Although there is a significant difference between the 2 groups, a clear cut-off is hard to make (see fig). Noticeable are 2 patients with consecutive samples. They had a negative dRVVT in higher CRP values than 1 of their positive samples..
The results of SCT screen ratio showed no difference between aberrant (mean and median CRP value: 133.5 and 107.3 mg/L) and negative SCT screen ratio (156.4 and 155.2 mg/L) with a P-value of 0.5306. Only 13 samples were aberrant for SCT screen.



Summary / Conclusions: Laboratories that perform their testing on ACL-TOP have to be aware of the influence of CRP on dRVVT testing, which mostly causes doubtful or not interpretable results. The differences in interfering CRP levels suggest that it is not necessarily the CRP itself, but may be due to other factors in the inflammatory process. More studies have to be performed f.i. to see any influence of interleukins or other factors of the inflammatory process.

A-102
Performance evaluation of the new point-of-care coagulation analyzer Xprecia stride for measuring INR in VKA-treated patients – comparison with local laboratory automated reference method

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Background / Introduction: Vitamin K antagonist (VKA) therapy requires frequent monitoring of prothrombin time (PT), expressed as International Normalized Ratio (INR), for optimal anticoagulation. INR testing is routinely performed on laboratory automated hemostasis analyzers but over the past few years, portable coagulometers were developed to be used as point-of-care (POC) devices. The Xprecia stride (Siemens Healthcare) is a new POC coagulation analyzer designed to monitor INR on fingerprick blood samples.

Aims: The aim of our study was to compare the performances of the Xprecia Stride coagulation analyzer to our laboratory automated reference method (Thromborel S®/BCS XP system).

Methods / Materials: 83 consecutive patients on VKA therapy were enrolled over a 9 months period (august 2015 - april 2016). 20 of these patients were seen twice during the period of inclusion. The cohort consisted in 59% male/41% female, of age ranging from 23 to 86 years old (median: 55 y).
Blood sampling consisted in two separate fingerprick blood drops for duplicate INR testing using Xprecia stride, and an additional venous sample was collected on 3.2% sodium citrate anticoagulant for standard laboratory INR determination. Venous blood samples were centrifuged 10 min at 2500 g and the platelet-poor plasma was immediately assessed on a BCS XP system using Thromborel S® reagent (reference method).
Xprecia stride employs single-use reagent test strips. Its PT reagent is a recombinant human tissue thromboplastin, similar to the Dade® Innovin® reagent.
The two methods were compared using Pearson correlation test and Deming linear regression analysis using GraphPad Prism® software. Bias versus the reference method was also calculated. Repeatability was assessed on 102 pairs of capillary blood samples. Coefficient of variation (%CV) was calculated across four INR ranges : 1-2; 2-3, 3-4 and >4.
In addition, normal values were verified on 30 healthy donors using Xprecia stride and reported as percentile 2.5% and 97.5%.

Results: INRs measured with the Xprecia stride correlated strongly with the results obtained with our laboratory reference method, with a Pearson coefficient of 0.92. The Deming regression analysis yielded a slope of 1.15 (95% confidence interval: 1.07 – 1.22) and an intercept of -0.37 (95% CI: -0.54 - -0.19), demonstrating a slight systematic proportional bias. The absolute difference in INR measurements between the two methods was 0.03 units, the results obtained with the Xprecia stride being overall 1.5% lower. Across INR ranges, the minimum bias was -0.10 for INR 1-2 and the maximum bias was +0.43 for INR >4.
Repeatability study showed that the mean CV was 3.6%, with a lowest CV of 2.7% for INR range 2-3 and a highest CV of 5.5% for INR 3-4. These results fulfilled the desirable specifications published by the French society of Thrombosis and Hemostasis, that is a CV < 5.3% for INR 1-2 and < 7.5% for INR > 2.
The expected normal values, as determined by the manufacturer, were 0.9 – 1.1. These reference values were verified on 30 healthy subjects: the INR ranged from 0.9 to 1.3. Although the upper limit of the interval was slightly higher, the reference range was nearly identical to that expected.

Summary / Conclusions: Overall, INR testing with the Xprecia Stride coagulation analyzer showed strong correlation with the laboratory automated reference method and good analytical performances in term of repeatability. These results confirm that the Xprecia Stride may be used at the point of care for reliable INR measurements.

A-121
Pharmacokinetic results of two studies with a new fibrinogen concentrate in subjects with afibrinogenaemia.

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Background / Introduction: Fresh-frozen plasma and cryoprecipitate have been the mainstay of replacement therapy for many years for treatment of bleeding episodes in congenital fibrinogen disorders. A first fibrinogen concentrate (1.5 g/100 mL) manufactured from cryoprecipitate was used in France between 1988 and 2009 with a good efficacy/safety profile. In order to enhance both the biological safety and the recovery from plasma donors, a new generation of plasma-derived human fibrinogen concentrate triple secured was developed, named FibCLOT®/ CLOTTAFACT® (1.5 g/100 ml). The product recovered from the supernatant fraction of cryoprecipitate using an ethanol fractionation, includes 3 viral safety steps: solvent-detergent treatment, nanofiltration and dry heat (80° C during 72 hrs).

Aims: Two prospective clinical trials (French and multinational) were conducted consecutively including a total of 26 patients with afibrinogenaemia (6 + 20). Among them, 19 patients with an average weight of 68 kg participated in the pharmacokinetic parts of the studies (5 children or adolescents of more than 40 kg and 14 adults). This abstract focuses only on the pharmacokinetic outcome of these two studies.

Methods / Materials: In the two studies, PK assessment was performed after a single infusion of 0.06 g/kg of FibCLOT®/ CLOTTAFACT®. Concentration of fibrinogen expressed in antigen and activity were followed during 14 days after infusion. PK parameters values are presented using geometric mean values.

Results: Pharmacokinetic studies showed that the maximum concentration was observed at the first available time-point (at about 1 hour) and was followed by a slow mono-exponential decrease, reaching the critical haemostatic plasma fibrinogen level of 0.5 g/l in about 3 to 4 days, with no notable differences when the data were stratified by age or by gender. In both studies, incremental recovery was 23.5 g/l per g/kg fibrinogen infused.
In addition the PK profiles of fibrinogen antigen and activity are superimposed showing that the FibCLOT®/ CLOTTAFACT® manufacturing process preserves the functional properties of fibrinogen.

Summary / Conclusions: FibCLOT®/ CLOTTAFACT® shows consistent PK properties in the 19 afibrinogenaemic patients included in two different studies. Our results from two clinical studies conducted separately, demonstrate that an infusion of 3 g of FibCLOT® / CLOTTAFACT® increases plasma fibrinogen level by 1 g/l in a 70 kg person.

A-126
Platelet Acetyl-CoA Carboxylase phosphorylation: a potential marker for atherothrombotic coronary artery disease

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Background / Introduction: Acetyl-CoA Carboxylase (ACC), the downstream specific substrate of the AMP-activated protein kinase, is mainly phosphorylated in response to thrombin compared to other platelet agonists. In clinical situation, such as coronary artery disease (CAD), associated with thrombin generation, ACC phosphorylation (P-ACC) can be an interesting marker of thrombin action on platelets.

Aims: In the current prospective clinical trial (ACCTHEROMA), we sought to investigate platelet P-ACC in patients admitted for coronary angiogram.

Methods / Materials: A total of 188 consecutive patients (65±12 years) were included from March 2015 to February 2016. Blood samples were drawn immediately after sheath insertion at the cath lab. Platelets were isolated and extracts were stocked frozen for further analysis by immunobloting and electrochemiluminescence (ECLIA test). CAD was assessed in all patients by coronary angiogram. Global atherosclerotic burden was evaluated by coronary (CAC) and extra-coronary artery calcification on thoraco-abdominal scanner with prospective ECG-gating in a randomly selected subgroup of patients (n=70).

Results: Patients with demonstrated CAD (CAC score>100 and/or at least 1 vessel disease on the angiogram) have higher platelet P-ACC compared to non-CAD (0,51±0,02 versus 0,30±0,03 respectively; p<0,001). Moreover, most of the patients with unstable CAD have the highest P-ACC (p=0,01). After adjusting for established cardiovascular risks factors, platelet P-ACC was an independent predictor of CAD (odds ratio: 2,96, p=0,004). Likewise, ECLIA test shows significant correlation with immunobloting for platelet P-ACC analysis (r=0,574, p<0,001).

Summary / Conclusions: Platelet P-ACC is a potential marker for screening patients with CAD at high ischemic risk. Optimizing ECLIA test for P-ACC analysis seems to be a promising tool for future clinical studies.

A-128
Prevalence of heterozygous type 2N Von Willebrand disease mutations in Belgium.

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Background / Introduction: Von Willebrand Disease (VWD) is an inherited bleeding disorder caused by a quantitative (type 1 and 3) or qualitative (type 2) defect of Von Willebrand factor (VWF). VWD type 2N is caused by a homozygous or compound heterozygous mutation within the D'/D3 area (exon 18-24) of VWF. Most frequent well-documented causal mutations of VWD type 2N are reported within exons 19-21.

Aims: In this study, we analyze the prevalence of type 2N heterozygous VWD mutations within the Belgian population. As the most frequent causal mutations of type 2N VWD are located in exons 19-21, these regions have been analyzed in selected samples.

Methods / Materials: 929 blood samples were collected from three university hospitals spread throughout the country. Patients were selected for not having visited the hospital for bleeding-related problems. Extracted DNA was used to amplify exons 19-21 by polymerase chain reaction (PCR) and sequenced where the three most common VWD type 2N mutations are situated; p.R816W/c.2446C>T, p.R854Q/c.2561G>A, p.R924Q/c.2771G>A.

Results: So far, 421/929 (45,3%) of all collected samples (respectively 183/421 UZA, 238/421 UZB) were completely analyzed. A heterozygous variant of p.R854Q/c.2561G>A (exon 20) and p.R924Q/c.2771G>A (exon 21) has been found in respectively 5/421 (1.18%) and 11/421 (2.61%) of all analyzed patients. No other mutation has yet been found in this population. Molecular analysis is still ongoing for the remainder samples.

Summary / Conclusions: These preliminary results show a significant prevalence of the heterozygous mutations of VWD type 2N of 3.79% of the Belgian population, of which the largest part is p.R924Q/c.2771G>A with 2.61%. The prevalence of the p.R854Q/c2561G>A variant of 1.18% is in accordance with previous estimations made by Casonato et al. in Italy and by Eikenboom et al. in the Netherlands. Further analysis should provide a more comprehensive overview of the prevalence of heterozygous variants of VWD type 2N in the Belgian population.

A-113
Preventability of serious adverse drug reactions related to the use of direct oral anticoagulants: a prospective study

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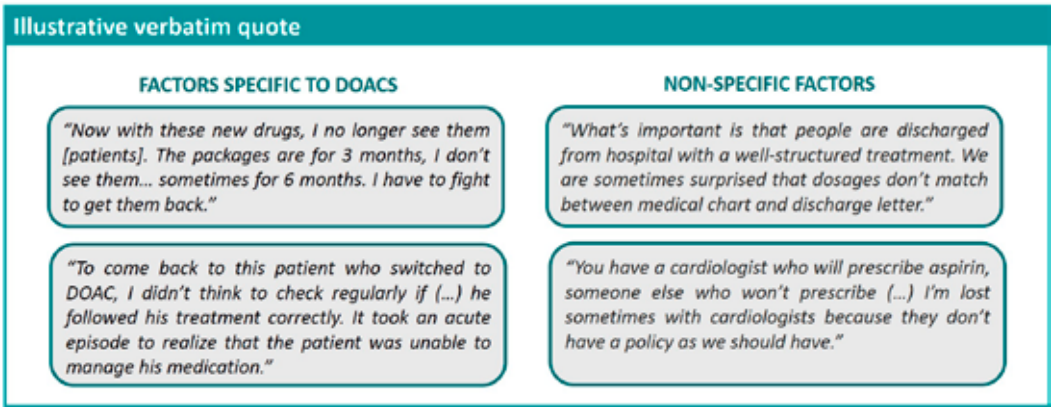
Background / Introduction: Direct oral anticoagulants (DOAC) were developed to address the drawbacks of vitamin K antagonists (VKA). However, current data suggest that ensuring appropriate use of DOACs remains challenging.

Aims: To determine the preventability of serious adverse drug reactions (ADRs) related to the use of DOACs and to explore underlying causes of preventable ADRs. Results were compared with VKAs.

Methods / Materials: We conducted a prospective observational study in the emergency departments of two teaching hospitals from July 2015 to January 2016. Patients admitted with a thrombotic or bleeding event while under DOAC or VKA were included. Two pharmacists and two haematologists assessed causality, seriousness and preventability of ADRs, using pilot-tested scales. For cases of serious and potentially preventable ADRs, semi-structured interviews were performed with general practitioners (GPs) to identify factors contributing to ADRs. The primary outcome was the proportion of serious ADRs that were potentially preventable. The study was approved by the Ethics Committees. Written

informed consent was obtained from each patient.

Results: The analysis included 46 DOAC- and 43 VKA-treated patients (median age 79 years, 54% male). Rivaroxaban (n=29) and acenocoumarol (n=40) were the most prescribed DOAC and VKA respectively. We observed 19 thromboembolic and 70 bleeding events. Adverse events were mainly gastro-intestinal (n=44) and intracranial (n=16) bleedings. **Among the 38 DOAC- and 41 VKA-related serious ADRs, 53% and 61% were (potentially) preventable respectively.** Medication errors occurred mainly at stages of prescribing for DOACs (n=15) and monitoring for VKAs (n=17). When interviewed, GPs reported that DOACs are more comfortable for themselves and for patients, considering the fixed-dose and the lack of INR monitoring. However, some GPs considered that DOAC prescribing results in a reduced clinical follow-up of patients. The risk of unnoticed non-adherence was also mentioned. **Most preventable factors contributing to ADRs were common to both classes of oral anticoagulants (e.g. discontinuity of care, pharmacodynamics drug interactions).**



Summary / Conclusions: More than half of serious ADRs were potentially preventable for both DOACs and VKAs. Contributing factors such as discontinuity of care or inappropriate antiplatelet use should be targeted to reduce the occurrence of ADRs related to the use of oral anticoagulants.

A-120
The importance of a low limit of detection for coagulation factor assays: FIX, an example in daily practice

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Background / Introduction: Inherited deficiencies of factor VIII (FVIII) and factor IX (FIX), Hemophilia A and B respectively, are X-linked recessive diseases that present in male children of carrier females. Hemophilia is characterized as mild (factor activity level ≥5 % and <40 % of normal), moderate (factor activity level ≥1 % and <5 %), or severe (<1 % factor activity), based on the residual factor activity level which is expressed as a percent of normal. Factor levels typically correlate with the degree of bleeding symptoms. Given this classification, it is of the utmost importance to accurately measure low factor activity levels, and in this respect a limit of detection (LOD) below 1% of normal is highly desirable. For this reason, it is important to evaluate the limit of detection of the FVIII and FIX activity assays.

Aims: The goal was to determine and evaluate the LOD with different commercial FIX deficient plasmas used for the FIX one-stage clotting assay on different STA® platforms.

Methods / Materials: STA® - C.K. Prest® (Diagnostica Stago, Asnières, France) was used as aPTT reagent in the FIX one-stage clotting assay with STA® ImmunoDef IX (Stago) as FIX deficient plasma. Three different commercial FIX deficient plasmas were evaluated, STA® ImmunoDef IX (Stago), STA® - Deficient IX (Stago) and HemosIL® Deficiënt IX (Werfen, Barcelona, Spain). If available, different lots of FIX deficient plasmas were tested. A 7-point calibration curve was used for FIX activity assays. 6 consecutive measurements of FIX activity using each FIX deficient plasma under evaluation were done. LOD was determined as the mean of the 6 measurements (residual activity) + 3.3 times the standard deviation. A fourth commercial FIX deficient plasma, VisuDep-F Frozen Factor IX Deficient Plasma (Affinity Biologicals inc., Ancaster, Canada) was additionally tested in the FIX activity assay.

Results: Table 1 displays the results with different FIX deficient plasmas – coagulation platform combinations. LOD results for STA Deficient IX and HemosIL® FIX deficient plasma were lower compared to STA ImmunoDef IX. There are no large differences when LOD's were determined on multiple platforms. Although manufacturer's criteria were achieved for all tested FIX deficient plasmas on all platforms with all tested lotnumbers, LOD results of STA ImmunoDef IX were not satisfactory as the clinical decision point of 1% FIX activity of normal could not be measured accurately. LOD cannot exceed 1% and needs to be far enough below 1% to accurately determine the 1% FIX activity, taking into account at least a coefficient of variation (CV) of 5% . Therefore, an independent FIX deficient plasma (VisuDep) was tested with the STA® ImmunoDef IX + STA C.K. Prest reagent combination resulting in LOD values of 0.45% and 0.39% on two tested platforms.

FIX deficient plasma (used as LOD test sample)	STA® - ImmunoDef IX plasma Lot #1			STA® - ImmunoDef IX plasma Lot #2		STA® - ImmunoDef IX plasma Lot #3	VisuDep-F FIX deficient plasma		STA® - Deficient IX plasma			HemosIL® FIX deficient plasma
Coagulation platform	STA R Max®	STA Compact Max®	STA R Evo®	STA R Max®	STA Compact Max®	STA R Evo®	STA R Max®	STA R Evo®	STA R Max®	STA Compact Max®	STA R Evo®	STA R Evo®
Mean measured residual activity (%)	0,93	0,94	0,96	0,81	0,75	0,82	0,39	0,34	0,16	0,13	0,14	0,32
SD (%)	0,04	0,11	0,03	0,05	0,07	0,04	0,02	0,01	0,02	0,01	0,008	0,02
LOD (%)	1,08	1,31	1,04	0,97	0,98	0,96	0,45	0,39	0,24	0,16	0,16	0,37
LOD (%) manufacturer				n/a			n/a			<1,5		n/a
Maximum residual activity specified by manufacturer (%)				<1			n/a			n/a		<1

n/a: not available

Summary / Conclusions: Manufacturer's criteria for lower limits of factor dosages are expressed as maximum residual activity of the deficient plasma. The LOD determined on the deficient plasmas used in the one-stage clotting assay are not always below 1%. Use of an independent factor deficient plasma may be necessary to investigate the lower measurement range. Results for LOD determinations on different lotnumbers indicate there is substantial lot-to-lot variability and therefore, LOD testing with every lot transition is warranted.

A-133
Acquired Haemophilia A

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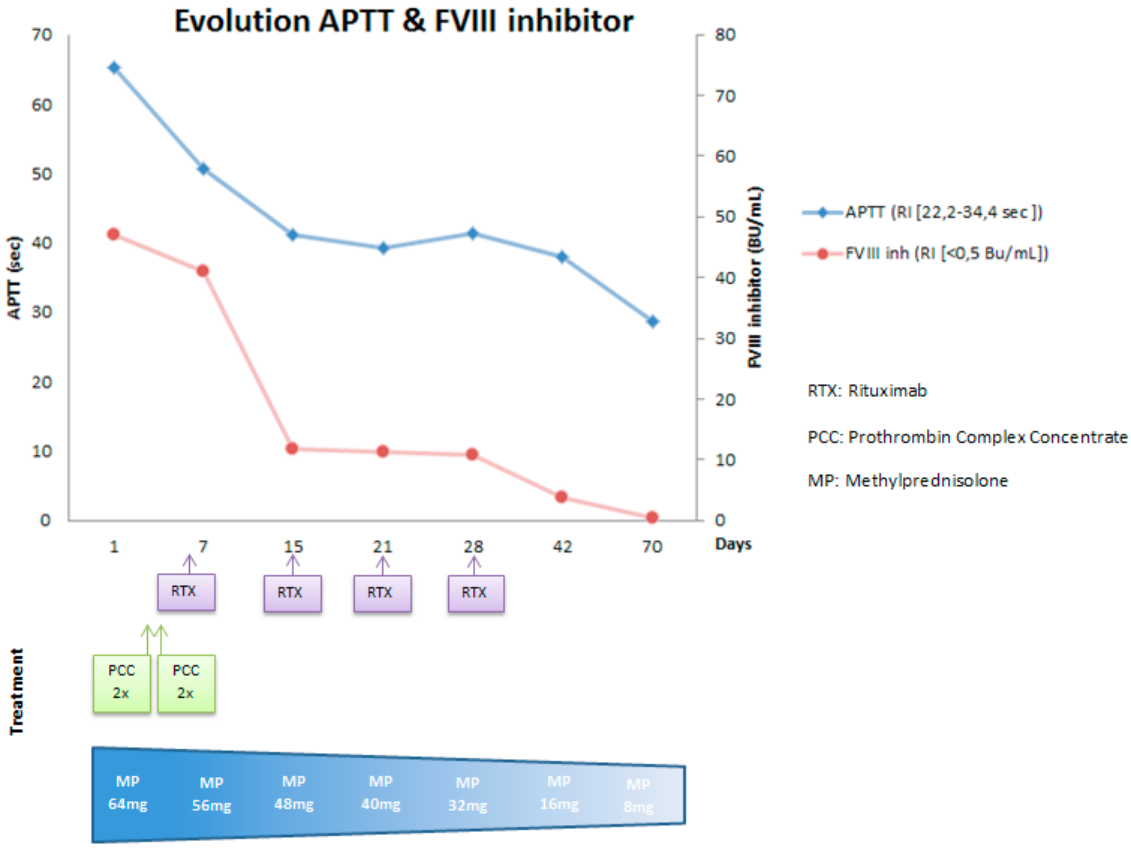
Background / Introduction: A 55 year old woman presented to the emergency department with a swollen, painful and warm calf. She reported minor trauma to her leg three weeks earlier with formation of a hematoma, worsening since 10 days. She also complained of having frequent ecchymoses. She was discharged with the suspicion of a torn soleus muscle and analgetics were prescribed. Three months later, she presented with purpura and extensive ecchymoses, covering more than 40% of her body. Initial laboratory investigations showed normal platelet count, normal PT and a prolonged APTT. As there was no previous personal or known family history of bleeding diathesis, an acquired coagulopathy was suspected. Coagulation factors were determined and revealed a FVIII:C-activity of 1%. The FVIII inhibitor titer was 47 BU/mL, measured by the Nijmegen method, confirming the diagnosis of acquired haemophilia A (AHA). Autoimmune diseases and malignancy were excluded as were drugs known to be associated with AHA. In order to control acute bleeding, infusion of the bypassing agent prothrombin complex concentrate (PCC) was started twice daily. Immunosuppressive therapy, aiming to reduce the autoantibodies, consisted of 64 mg of methylprednisolone daily, reduced by 8 mg per week, associated with off-label use of rituximab once weekly during 4 weeks. The inhibitor titer gradually decreased and the APTT normalized. One month later she complained of a swollen face, amyotrophy and troubled alertness. Steroids were reduced to a lower weekly dose. 2.5 months after diagnosis, a normal APTT and a FVIII:C-activity of 138% were obtained and all clinical manifestations disappeared.


Evolution APTT & FVIII inhibitor
APTT (sec)
FVIII inhibitor (BU/mL)
Days
APTT (RI [22,2-34,4 sec])
FVIII inh (RI [<0,5 Bu/mL])
RTX: Rituximab
PCC: Prothrombin Complex Concentrate
MP: Methylprednisolone
Treatment
RTX
PCC 2x
MP 64mg
MP 56mg
MP 48mg
MP 40mg
MP 32mg
MP 16mg
MP 8mg

A-129
Sustained inhibition of acetyl-CoA carboxylase decreases platelet dense granules secretion and aggregation.

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Background / Introduction: Acetyl-CoA carboxylase (ACC) regulates fatty acids synthesis and oxidation. Our preliminary results showed that ACC is phosphorylated in platelets of patients with coronary artery disease, potentially due to persistent thrombin generation. ACC phosphorylation results in its inhibition.

Aims: Given the primary roles of lipids in platelets structure, energy storage and signalling, we hypothesized that a sustained inhibition of ACC could have consequences on platelets bioenergetics and activation.

Methods / Materials: To test our hypothesis, platelets were treated with 30 µM TOFA, an ACC inhibitor, for 2 hours before thrombin stimulation. We measured lipogenesis via 14C-acetate incorporation into fatty acids. Platelet functions were assessed by aggregometry and flow cytometric studies.

Results: We show that a preincubation of platelets with TOFA significantly decreased lipogenesis (Control: 6 pmol/min/109 platelets ± 0.8; TOFA: 1 pmol/min/109 platelets ± 0.3; $P<0.05$). This effect was accompanied by a significant defect in dense granules secretion and aggregation in response to low thrombin concentrations, whereas α -granules secretion was not affected, suggesting that the default in aggregation likely resulted from a lower autocrine and paracrine roles of ADP. PKC activity is essential for granule secretion. Accordingly, we show that TOFA significantly decreased PKC substrates phosphorylation in baseline and after thrombin stimulation. Since PKC-δ has been shown to play a role in dense granules regulation, the effects of TOFA on its activity was evaluated through the analysis of VASP phosphorylation. Indeed, inhibition of PKC-δ has been shown to promote hyperphosphorylation of its Ser157 in platelets. Treatment with TOFA led to a drastic increase of Ser157 phosphorylation, in baseline and after thrombin stimulation, and in a cAMP-independent way.

Summary / Conclusions: This study shows that a sustained inhibition of platelet ACC decreases lipogenesis and affects dense granule secretion and aggregation through a PKC/PKC-δ dependent mechanism. We believe that it could affect thrombus stabilization in atherosclerotic patients and favour acute ischemic events.

A-132
PEAR1 promoter methylation is associated with platelet and white blood cell phenotypes in the Moli-family cohort

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Background / Introduction
Platelet Endothelial Aggregation Receptor 1 (PEAR1) is a cell-cell contact receptor involved in platelet activation and megakaryopoiesis. Genetic variants in the PEAR1 gene have been extensively associated with platelet function, but also, very recently, with white blood cell phenotypes, opening the possibility that PEAR1 is involved in general in the regulation of hematopoiesis. Variations in platelet and white blood cell parameters among individuals may modulate the risk for thrombosis, leading to CVD. We have already characterized PEAR1 regulation during megakaryopoiesis, a complex process in which both genetic predisposition (polymorphisms) and epigenetic status (DNA methylation at the promoter region) play a role. This data led us to hypothesize that PEAR1 methylation could be a better and reliable marker of platelet variability than genetic variants in a larger population.

Aims
The aim of this study is to investigate PEAR1 methylation in the Moli-family cohort and associate it with blood cell count and phenotype measurements.

Methods / Materials
Moli-family is an Italian family-based study designed to identify genetic and epigenetic variability of platelet-related phenotypes, their heritability and association with MI at young age. The study population includes 732 subjects (aged 43±19 SD, 44% men) from 54 extended pedigrees (23 families with familiarity for MI at young age and 31 matched control families). Blood cell count, whole blood platelet function (P-selectin, platelet-PMN and platelet-monocyte mixed aggregates, PFA-100) and VWF plasma levels were recorded.
After exclusion of failing DNA samples, PEAR1 methylation was investigated on a total of 650 individuals by using the Sequenom EpiTYPER Mass-Array. A total of 32 CpG sites were analysed spanning the putative promoter region of PEAR1. Association analyses were performed using regression models, taking into account the family structure (generalized estimating equations, GEE).

Results
While PEAR1 methylation was not associated with CVD, we found a significant negative association with Mean Platelet Volume (MPV) and plateletcrit (Pct) and a positive association with Platelet distribution width (PDW). No significant association was found with platelet count. Interestingly, methylation levels at several CpG sites were associated with both monocyte and granulocyte, but not with lymphocyte cell counts. Associations with red blood cell and red distribution width were also not significant.

Summary / Conclusions
We have performed the first large PEAR1 epigenetic analysis in the framework of the Moli-family epidemiological study. No association between PEAR1 methylation and CVD, but with several platelet and white blood cell count parameters was found, suggesting a possible role of PEAR1 in influencing not only megakaryopoiesis, but hematopoiesis in general. Further analysis will investigate the relationship of PEAR1 methylation with more specific platelet activation and inflammatory markers already available in the Moli-family cohort.

A-123
P2X1 ion channel is critical for vascular integrity in inflammation

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Background / Introduction: In addition to their role in hemostasis and thrombosis, platelets play a key role in inflammation by supporting leukocyte extravasation and maintaining vascular integrity in inflamed tissue. In mice, thrombocytopenia provokes bleeding that is limited to the site of inflammation. In humans, in the absence of injury, some patients show spontaneous bleeding while others do not bleed despite equally low platelet counts. Though the mechanisms controlling vascular integrity are not fully understood, it has been reported that, in thrombocytopenic mice, neutrophils are responsible for inflammation-associated haemorrhage. The platelet ATP-gated P2X1 ion channel has been involved in arterial thrombosis and may represent an attractive target for antiplatelet therapy since P2X1-deficient mice do not show prolonged bleeding time. Recently, we have shown that this ion channel also regulates neutrophil function.

Aims
To investigate whether P2X1 ion channel contributes to maintain vascular integrity in acute inflammatory conditions.

Methods / Materials: We used P2X1-deficient mice in an acute colitis model induced by oral administration of dextran sodium sulfate (DSS). Disease activity index (DAI) was determined to assess colitis severity (diarrhea, bleeding, weight loss). Differential counts of leukocytes and platelets and haematocrit were measured. Crosstalk between platelets, neutrophils and endothelium was evaluated in real time using laser-induced injury of arteriolar endothelia in cremaster muscle.

Results: In the model of acute colitis, P2X1-deficient mice displayed higher intestinal blood loss as compared to control animals, resulting in anemia. This was associated with increased colon infiltration by myeloperoxidase-expressing myeloid cells. In the thrombo-inflammatory model of cremaster muscle microcirculation, DSS-treated P2X1-deficient mice showed a strong increase of neutrophil accumulation at the site of arteriolar wall injury whereas platelet recruitment in the growing thrombi was severely impaired.

Summary / Conclusions: P2X1 deficiency exacerbates bleeding in inflamed intestine, possibly due to increased accumulation of neutrophils in tissues combined with impaired platelet aggregate formation. Hence, P2X1 ion channel is critical for vascular integrity in acute inflammation.

A-134
Itpkb as new platelet-activation regulator.

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Background / Introduction: Inositol 1,4,5-trisphosphate 3-kinase B (ItpkB) is expressed only by lymphocytes, some neurons, splenocytes and platelets. This enzyme converts Ins(1,4,5)P3 into inositol 1,3,4,5-tetrakisphosphate (IP4) in the cytosol. Ins(1,4,5)P3 triggers the release of Ca2+ from intracellular stores. ItpkB inhibition or ItpkB knockout has been recently demonstrated that elevates intracellular Ca2+ in T cells. Ca2+ is a very important mediator in platelet activation. Therefore, we hypothesize that ItpkB can play a role in platelet activation by regulating Ca2+ flux.

Aims: This project has to main objectives:
1. Analyze platelet activation genetic inactivation of ItpkB in a murine model.
2. Correlate murine model with human data by ItpkB inhibition in human platelets.

Methods / Materials: Mouse wash platelets preparation.8- to 12-week-old mice were bled under sodium pentobarbital anesthesia from the retro-orbital plexus. Mouse blood was collected ACD solution containing 1 U/mL apyrase. PRP was obtained by centrifugation a 800g for 30 seconds followed by 5 minutes at 150g. PRP from 3 animals were pooled, and resuspended in Ca2+-free Tyrode buffer containing 0.35% (wt/vol) bovine serum albumin and 1 U/mL apyrase, at a density of 2.5x105ptls/L.
Platelet aggregation. Light transmission during mouse platelet aggregation was recorded using apyrase-treated washed platelets in the presence of 2 mM CaCl2 on an ELVI 840 aggregometer.
Intracelullar Ca2+ measurement. Fura-2-loaded platelets (1x108/ml) in Tyrode buffer. Ca2+ responses in the loaded platelets were measured by ratio fluorometry.
Static adhesion assay. Coverslide were coated with collagen I or fibrinogen for 2h, and bocked with BSA. Washed platelet from ItpkB -/- or wild type mice were adjusted to 200000 ptl/ul and 400 ul was added per well. Platelets were allowed to adhere for 45 minutes at room temperature in tyrode buffer. After the static assay, coverslips were stained for confocal imaging with phalloidin-TRICT.

Results:
ItpkB chemical inhibition or genetic inactivation increase platelet activation.
Washed platelets from ItpkB-/- or wild type mice were stimulated with thrombin, CRP (collagen related peptide) or ADP (Adenosine diphosphate) at different concentrations. Aggregation was highly increased in ItpkB-/- platelets compared to wild type controls. Moreover, low doses of agonist that do not induce wild type platelets response were enough to induce the aggregation of ItpkB-/- platelets.
These data were confirm in human platelets using a specific ItpkB chemical inhibitor.
We also studied platelet activation by measuring P-selectin surface expression and fibrinogen binding to integrin αIIb β1. In

both cases, ItpkB inhibition enhanced P-selectin expression and fibrinogen binding after thrombin and CRP stimulation. Finally, we measure platelet adhesion to collagen and fibrinogen in static conditions. ItpkB inhibition was also increasing platelet adhesion to these proteins.
ItpkB inhibition result in elevated intracellular Ca2+ upon stimulation.
Thrombin, CRP or ADP stimulation increase intracellular Ca2+ in absence of ItpkB activity. This Ca2+ elevation is due to an increased store-operation calcium entrance (SOCE) function.

Summary / Conclusions: In summary, we conclude that the enzyme activity of ItpkB is reducing Ca2+ influx by regulating the SOCE function in human and murine platelets. By this mechanism, ItpkB is modulating platelet activation, aggregation and adhesion.

A-117
GARP deficiency in murine platelets or endothelial cells does neither affect hemostasis nor thrombosis

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Background / Introduction:
Identifying the function of new platelet and endothelial receptors can unravel yet unexplored pathways regulating thrombosis and hemostasis. Glycoprotein-A Repetitions Predominant protein (GARP or LRRC32) is present on among others human platelets and endothelial cells. Evidence for its involvement in thrombus formation was suggested by full knockout of GARP in zebrafish.

Aims: To evaluate the role of GARP in murine platelet physiology and in thrombus formation using platelet and endothelial conditional GARP knock out mice

Methods / Materials: Platelet and endothelial specific GARP knockout mice were generated using the Cre-loxP recombination system. The function of platelets without GARP was measured by flow cytometry, spreading analysis and aggregometry using PAR4-activating peptide and collagen related peptide. Additionally, clot retraction and collagen-induced platelet adhesion and aggregation under flow were analyzed. Finally, in vivo tail bleeding time, occlusion time of the mesenteric and carotid artery after FeCl3-induced thrombosis were determined in platelet and endothelial specific GARP knock out mice.

Results: Platelet specific GARP knockout mice had normal hematological parameters and their platelets had normal surface GPIb, GPVI and integrin αIIb glycoprotein expression. Although GARP expression was increased upon platelet activation, platelets without GARP displayed normal agonist induced activation, spreading on fibrinogen and aggregation responses. Furthermore, absence of GARP on platelets did not influence clot retraction and had no impact on thrombus formation on collagen-coated surfaces under flow. In line with this, neither the tail bleeding time, nor the occlusion time in the carotid- and mesenteric artery after FeCl3-induced thrombus formation in platelet or endothelial specific GARP knock out mice were affected.

Summary / Conclusions: Although previous zebrafish studies have demonstrated an important role for GARP in thrombus formation, the present results provide evidence that platelet and endothelial GARP are not important in hemostasis and thrombosis in mice.

A-135
Clumping factor A, von Willebrand factor-binding protein and von Willebrand factor anchor Staphylococcus aureus to the vessel wall

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Background / Introduction: Staphylococcus aureus (S. aureus) is the leading cause of life-threatening endovascular infections. One of the most feared complications of invasive S. aureus disease is infective endocarditis. Once S. aureus infects the heart valves, almost one in three patients will die, despite aggressive surgery and antibiotics. The dramatic morbidity and mortality of S. aureus endocarditis have remained unchanged over the past decades. This stresses the need for new therapeutic strategies to prevent and treat infective endocarditis. When establishing endovascular infections, S. aureus overcomes shear forces of flowing blood by binding to von Willebrand factor (VWF). Staphylococcal VWF-binding protein (vWbp) interacts with the A1-domain of VWF, however, because vWbp is thought to be a secreted protein not anchoring to the cell wall, it remains unclear how vWbp mediates bacterial attachment. Surface proteins of S. aureus are linked to the bacterial cell wall by sortase A (SrtA). A mutation in this gene leads to an anchoring defect of about 20 S. aureus surface proteins.

Aims: We hypothesized that vWbp interacts with a staphylococcal surface protein, mediating the adhesion of S. aureus to VWF and vascular endothelium under shear stress.

Methods / Materials: We studied the binding of S. aureus to vWbp, VWF and endothelial cells in a micro-parallel flow chamber using various mutants deficient in Sortase A (SrtA) and SrtA-dependent surface proteins, and Lactococcus lactis expressing single staphylococcal surface proteins. In vivo adhesion of bacteria was evaluated in the murine mesenteric circulation using real-time intravital vascular microscopy.

Results: vWbp bridges the bacterial cell wall and VWF, allowing shear-resistant binding of S. aureus to inflamed or damaged endothelium. Absence of SrtA and Clumping factor A (ClfA) reduced adhesion of S. aureus to vWbp, VWF and activated endothelial cells. Selective overexpression of ClfA in the membrane of Lactococcus lactis enabled these bacteria to bind to VWF and activated endothelial cells but only in the presence of vWbp. Absence of ClfA abolished bacterial adhesion to the activated murine vessel wall.

Summary / Conclusions: vWbp interacts with VWF and with the SrtA-dependent staphylococcal surface protein ClfA. The complex formed by VWF, secreted vWbp and bacterial ClfA anchors S. aureus to vascular endothelium under shear stress.

A-112
Absence of Pear1 does not affect murine platelet function in vivo

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Background / Introduction: Platelet Endothelial Aggregation Receptor-1 (PEAR1) is a transmembrane platelet receptor that amplifies the activation of the platelet fibrinogen receptor (αIIbβ3) during platelet aggregation. In man, PEAR1 polymorphisms are associated with changes in platelet aggregability.

Aims: Up to now the exact function of Pear1 in murine platelets was unknown. We characterized Pear1 expression and function in murine platelets.

Methods / Materials: Pear1 phosphorylation and signaling, platelet aggregation, α-degranulation and clot retraction were studied in WT and Pear1-/- platelets. The function of Pear1 in haemostasis and thrombosis was studied in a mouse tail vein bleeding and ferric chloride-induced mesenteric thrombosis model.

Results: Mature murine platelets express Pear1 on their membrane and clustering of Pear1 by anti-Pear1 antibodies triggered platelet aggregation. Pear1 was weakly phosphorylated during collagen-induced murine platelet aggregation and was translocated to the cytoskeleton. Absence of murine Pear1 impaired dextran sulfate-induced platelet aggregation, but did not impact collagen-, AYPGK and ADP-induced platelet aggregation, coupled to a lower Pear1 expression in murine than in human platelets and to weaker Pear1-mediated downstream signaling. Neither clot retraction nor α-degranulation was affected in Pear1-/- mice. Likewise, in vivo tests like the tail vein bleeding time and thrombus formation in mesenteric veins were similar in WT and Pear1-/- mice.

Summary / Conclusions: Murine platelet Pear1 shares a number of characteristics with human platelet PEAR1. Nevertheless, murine Pear1 contributes less to platelet function as does human PEAR1 and does not overtly impact haemostasis and thrombosis in mice.

A-130
AMPKα1 participates in the regulation of thrombin-induced phosphatidylserine exposure in platelets through acetyl-CoA carboxylase phosphorylation

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Background / Introduction: We have previously shown that AMPKα1 is activated in thrombin-stimulated platelets and participates in their activation through phosphorylation of cytoskeletal targets. Acetyl-CoA carboxylase (ACC), the bona-fide substrate of AMPK, is also phosphorylated upon thrombin stimulation but its role in platelets has never been investigated. ACC is a central regulator of lipids metabolism by regulating fatty acids synthesis and oxidation. Its phosphorylation on Ser79 by AMPK inhibits its activity.

Aims: Given the primary roles of lipids in platelets, namely structural, energy storage and signalling, we hypothesized that the inhibitory phosphorylation of ACC, as seen in response to acute thrombin stimulation, could affect platelet functions.

Methods / Materials: To test our hypothesis, we used platelets isolated from a mouse model expressing a genetically modified ACC knock-in (KI) (Ser79Ala) that can no longer be phosphorylated by AMPK and hence inhibited. Proteins phosphorylation was evaluated by western blot. Lipogenesis was measured via 14C-acetate incorporation into fatty acids and mitochondrial respiration analysed using the Seahorse Flux Analyzer. Platelet functions were assessed by aggregometry and flow cytometric studies.

Results: We show that, of the two ACC isoforms, ACC1 is the major isoform present in platelets. Basal ACC1 phosphorylation, lipogenesis and mitochondrial respiration were unchanged in non-stimulated ACC KI platelets relative to control (WT) platelets. Thrombin stimulation led to a rapid and significant increase in ACC phosphorylation in WT, but not in KI platelets. Both types of platelets displayed similar phosphorylation levels of myosin light chains, cofilin and vasodilator phosphoprotein, the cytoskeletal targets of AMPKα1, which play a role in platelets functions. Accordingly, there were no significant defects in platelet aggregation and granules secretion, compared to WT platelets. However, KI platelets treated with thrombin exposed significantly more phosphatidylserine (PS), suggesting that procoagulant activity was increased. A similar result was obtained with AMPKα1-deficient platelets, characterized by a lower level of ACC phosphorylation compared to their WT littermates after activation with thrombin. To test whether this increase in PS exposure had an impact on haemostasis, we measured bleeding time. It was significantly decreased in KI mice (82s ± 41) versus WT mice (188s ± 50).

Summary / Conclusions: This study defines the AMPKα1/ACC1 axis as a new regulator of thrombin-induced PS exposure in platelets with functional consequences on haemostasis. Molecular mechanisms responsible for this effect as well as the impact of ACC phosphorylation on thrombosis are currently investigated.

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