Thrombosis & Haemostasis Research Review™

Making Education Easy

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In this issue:

Abbreviations used in this issue:

AAV = adeno-associated vector; **AML** = acute myeloid leukemia; **CI** = confidence interval; **CTEPH** = chronic thromboembolic pulmonary hypertension; **DOAC** = direct-acting oral anticoagulant; **DVT** = deep vein thrombosis; **ELISA** = enzyme-linked immunosorbent assay; **FVIII** = factor VIII; **HIT** = heparin-induced thrombocytopenia; **HR** = hazard ratio; **IPSS** = International Prognostic Scoring System; **IRR** = incidence rate ratio; **ITP** = immune thrombocytopenia; **LKB1** = liver kinase B1; **LMWH** = low-molecular-weight heparin; **MDSC** = myeloid-derived suppressor cells; **PE** = pulmonary embolism; **PF4** = platelet factor 4; **PVO** = pulmonary vascular obstruction; **rh-tPA** = recombinant human tissue plasminogen activator; **RR** = relative risk; **sPAP** = systolic pulmonary arterial pressure; **VITT** = vaccine-induced immune thrombotic thrombocytopenia; **VTE** = venous thromboembolism; **VWF** = von Willebrand factor.

Welcome to the first issue of Thrombosis & Haemostasis Research Review for 2023.

We begin this issue with results from the international, phase 4 HIGHLOW study that aimed to elucidate the optimal intensity of anticoagulant therapy in pregnant women with a history of venous thromboembolism (VTE). We also discuss data from the Italian RIDTS double-blind trial comparing six versus 12 weeks of rivaroxaban treatment in patients with symptomatic isolated distal deep vein thrombosis (DVT). In other research, adeno-associated vector (AAV)-mediated gene therapy proves safe over the long-term with durable efficacy in canine models of haemophilia A; a novel test utilising cryopreserved platelets and an enzyme-linked immunosorbent assay (ELISA) with a thrombospondin-1 endpoint demonstrates proof-of-concept for definitive heparin-induced thrombocytopenia (HIT) diagnosis; and based on results from the Italian APIDULCIS study, D-dimer test results cannot be used in isolation to make decisions regarding extended anticoagulation treatment to prevent recurrent VTE. Finally, pre-clinical research from the Netherlands indicates that the von Willebrand factor (VWF)-targeting plasminogen activator Microlyse may be an attractive novel thrombolytic agent for acute ischemic stroke and further assessment is eagerly awaited.

We hope you enjoy this update in Thrombosis & Haemostasis research, and we welcome your comments and feedback.

Kind Regards,

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Intermediate-dose versus low-dose low-molecular-weight heparin in pregnant and post-partum women with a history of venous thromboembolism (Highlow study): an open-label, multicentre, randomised, controlled trial

Authors: Bistervels I et al., for the Highlow Block writing committee and Highlow Investigators

Summary: Once-daily low-dose low-molecular-weight heparin (LMWH) remains the gold standard for VTE prophylaxis in at-risk pregnant women with results from the international, phase 4 HIGHLOW study (ClinicalTrials.gov Identifier: NCT01828697) published in *The Lancet* failing to demonstrate improved efficacy or safety for weight-adjusted intermediate-dose LMWH. A total of 1,110 adult women (mean age 32 years; contraceptive hormone therapy, 38%) in their first trimester of pregnancy (≤14 weeks of gestational age) with prior unprovoked, or provoked by hormonal or minor risk factors, VTE were accrued to the trial from sites in the Netherlands, France, Ireland, Belgium, Norway, Denmark, Canada, the USA and Russia. Women were randomised to receive a combined antepartum/postpartum course of either once-daily low-dose LMWH (n=555) or weight-adjusted intermediate-dose LMWH (n=555) with choice of LWMH (nadroparin, enoxaparin, dalteparin or tinzaparin) varying by country. Treatment was paused during delivery and ceased at six weeks postpartum. Rates of both the primary efficacy and safety outcomes of VTE (DVT, pulmonary embolism [PE] or unusual site venous thrombosis) and major bleeding (antepartum, early post-partum plus late post-partum major bleeding), respectively, were comparable between treatment arms (2% vs 3%; relative risk [RR] 0.69; 95% confidence interval [CI], 0.32-1.47; p=0.33 and 4% vs 4%; RR 1.16; 95% CI, 0.65-2.09). The incidence of antepartum VTE was 1% in both trial arms but a non-statistically significant lower incidence of postpartum VTE was found with intermediate-dose versus low-dose LWMH (1% vs 2%).

Comment: Pregnancy creates an increased risk of VTE during its course and postpartum. Women with prior VTE are at an even higher risk of VTE recurrence. The question that remains is about the intensity of anticoagulation to prevent VTE. In this open-label study, women were randomised to either an intermediate-dose LMWH regimen which was approximately half of a therapeutic dose, categorised by actual bodyweight, fixed low-dose regimen based on body weight of <100 kg or ≥100 kg. Both treatments were once a day until six weeks postpartum. Despite accepting enrolment in nine countries, the study randomised only 1,110 women over seven years and there were considerable protocol deviations. The summary is that there was no statistically significant difference in the primary efficacy outcome of objectively confirmed VTE from randomisation until six weeks postpartum in both interventions. However, subgroup analysis revealed that the intermediate-dose LMWH cohort had a lower incidence of post-partum pulmonary embolism (one [<1%] of 555 women vs seven [1%] of 555 women) and of superficial thrombophlebitis (none vs 11 [2%]) than the cohort receiving low-dose LMWH. However, this study is inadequate to recommend changes specific to postpartum anticoagulation and the overall summary is that the outcomes from both protocols are similar. There was no difference as well in the primary safety outcome of major bleeding.

Reference: Lancet 2022;400(10365):1777-87
Abstract

Rivaroxaban treatment for six weeks versus three months in patients with symptomatic isolated distal deep vein thrombosis: randomised controlled trial

Authors: Ageno W et al., on behalf of the RIDTS study group

Summary: The Italian RIDTS double-blind, placebo-controlled clinical trial (NCT02722447) enrolled adult patients within three days of first acute symptomatic isolated distal DVT of the leg to evaluate the optimal duration of rivaroxaban thromboprophylaxis. A total of 402 adults, accrued from 28 specialist outpatient clinics, were randomised to receive six (n=202) or 12 weeks (n=200) of 20 mg rivaroxaban with blinding maintained through the use of placebo. The doubling of rivaroxaban treatment duration from six to 12 weeks reduced the incidence of recurrent VTE (a composite outcome comprised of isolated or recurrent distal DVT, proximal DVT and symptomatic or fatal PE) from 19% to 11% (RR 0.59; 95% CI, 0.36-0.95; *p*=0.03). Most of the benefit derived from reductions in recurrent isolated DVT (15% vs 8%; *p*=0.02) rather than proximal DVT or PE (4% vs 3%; *p*=0.80). It was estimated that the number needed to treat to prevent one clot was 13. Extending the duration of rivaroxaban thromboprophylaxis was not associated with increased risk of major bleeding with no major bleeds reported in either trial arm over two years of follow-up.

Comment: There is a perception that isolated distal DVT is a more benign condition than proximal DVT and asymptomatic distal DVT may not need anticoagulation. In this study, patients with symptomatic isolated distal DVT (i.e., no proximal extension or PE) were randomised (double-blind) to ongoing rivaroxaban (20 mg daily) or placebo after the initial six weeks of full anticoagulation and followed for 24 months. DVT was unprovoked in approximately 40% in each arm and 13%-16% had a history of previous VTE. At the time of randomisation, mandatory ultrasonography showed complete clot resolution in approximately 50% in each cohort. The primary efficacy outcome of recurrent VTE during follow-up after randomisation was more frequent in the placebo arm, 39 (19%) patients as compared to 23 (11%) patients (*p*=0.03) with relative risk of 0.59 and number needed to treat of 13. The incidence rate of the primary efficacy outcome was 6.6 (rivaroxaban) versus 12.2 (placebo) per 100 patient years. Isolated distal DVT comprised the higher recurrence while the incidence of proximal DVT and PE was the same on both arms. The risk of bleeding was similar in this study. In summary, this study shows efficacy benefit with continuing rivaroxaban beyond six weeks and it reflects the conclusions of a meta-analysis that anticoagulation for isolated distal DVT led to a statistically significant 50% reduction of recurrent VTE without an increase in the risk of major bleeding, compared with no treatment.

Reference: BMJ 2022;379:e072623
Abstract

Long-term follow-up of liver-directed, adeno-associated vector-mediated gene therapy in the canine model of haemophilia A

Authors: Batty P et al.

Summary: Dr Paul Batty and colleagues from the Queen's University in Ontario, Canada, present 10-year follow-up data from their evaluation of the recombinant AAV gene therapy platform for haemophilia A treatment in a preclinical canine model. The trial, conducted in collaboration with the American biotechnology company BioMarin Pharmaceutical Inc, involved a single infusion of a recombinant AAV vector containing a liver-targeted canine factor VIII (FVIII) transgene (median dose 1.25 x 10^{13} vg/kg) into eight dogs with severe haemophilia A (four hemizygous and four homozygous). In the three-quarters of dogs who responded to therapy (n=6/8) responses were durable with detectable FVIII expression at up to 12 years (range, 8.2-12 years) and an almost complete resolution of the bleeding phenotype (3.9 vs 0.3 annual bleeding events; p=0.003). Post-mortem examinations revealed that the FVIII transgene was exclusively expressed in the liver. Capsid-directed cellular immune responses were persistent over the study period, precluding repeat dosing. The treatment had a safe long-term profile with no acute or chronic hepatic toxicity (fibrosis or cirrhosis) or malignancy. The authors noted that differences in the durability of gene expression between canines and humans requires elucidation in order for the therapeutic to be curative.

Comment: While gene therapy for haemophilia is becoming increasingly available (at a very high cost) questions remain about longer-term efficacy, safety and transgene expression with the use of an AAV vector. This is a long-term (median 10.8 years) follow-up of eight dogs with severe haemophilia A treated with a single portal vein infusion of a B-domain–deleted (BDD)-canine FVIII AAV vector (using AAV2, AAV6, or AAV8 capsids). In the 6/8 responding dogs, median FVIII: C activity (chromogenic 7.2%, on-stage 12.7%) was observed over a median follow-up of 10.8 years that is also reflected in the normalisation of the whole blood clot time. The clinical correlation was with a significant reduction in mean annualised bleeding rates from 5.4 to 0.6 events per year (p=0.014). Seven of the eight dogs demonstrated an asymptomatic rise in liver enzymes (ALP and ALT). However, it seems that this is par for course for canines and untreated dogs also routinely show age-related elevations in liver enzymes. There was one episode of development of an FVIII inhibitor which resolved. A strong humoral response was seen toward the dosed AAV capsid, which may mean that a different capsid may have to be used if second dosing is necessary. Post-mortem liver histology showed hepatocyte nodular hyperplasia present in six dogs. There was no cirrhosis or malignancy. Further analysis of organs by real-time PCR and immunohistochemistry confirmed that long-term FVIII production was exclusively from the liver. These studies are reassuring in the absence of long-term data in human subjects.

Reference: Blood 2022;140(25):2672-83
Abstract

Off-the-shelf cryopreserved platelets for the detection of HIT and VITT antibodies

Authors: Kanack A et al.

Summary: This research from a group at the Rochester Mayo Clinic and Retham Technologies demonstrated proof-of-concept for a rapid and easily performed test for the definitive diagnosis of HIT and vaccine-induced immune thrombotic thrombocytopenia (VITT). The researchers developed a thrombospondin-1-release assay in an ELISA format and optimised trehalose-based cryopreservation of platelets to improve length of viability while maintaining antibody activation potential following platelet factor 4 (PF4) or heparin treatment. The assay positively identified known HIT and VITT cases from HIT/VITT-negative cases, concordant with conventional assay results including the radioactive serotonin release assay and the platelet excitation assay, utilising either PF4 or heparin-treated cryopreserved platelets or PF4-treated cryopreserved platelets, respectively. With the obvious hurdles still in place to commercial roll-out including resolution of interindividual platelet response variability, expense of recombinant human PF4 and standardisation, this assay may provide a novel method to diagnose HIT in standard laboratory settings and reduce current delays.

Comment: These advances have significant impact for the laboratory testing for suspected HIT and VITT. While ELISAs for antibodies reacting to PF4 are simple to perform and sensitive, they are relatively non-specific. Hence the use of functional assays that measure platelet activation and release. Traditionally, this is cumbersome and limited to specialised laboratories. In this work, the authors report a methodology for cryopreserving platelets. These platelets were thawed and incubated with PF4 or heparin and exposed to patient sera. Platelet activation was detected by the measurement of the platelet α-granule protein, thrombospondin-1. The summary is that these cryopreserved platelets were able to accurately detect both HIT and VITT sera. In concordance with now established data, sera from VITT activated the cryopreserved platelets which were only treated with PF4-treated, but not those treated with heparin. Thus, this technology will broaden the availability of confirmatory assays for both HIT and VITT.

Reference: Blood 2022;140(25):2722-29
Abstract

Abnormal metaphase cytogenetics predicts venous thromboembolism in myeloma: derivation and validation of the PRISM score

Authors: Chakraborty R et al.

Summary: Chakraborty et al report the development and validation of a novel risk prediction model – the PRISM score – that combines patient-, disease- and treatment-specific factors for assessment of VTE risk in patients with newly diagnosed multiple myeloma. Retrospective data from a derivation cohort comprised of 783 patients treated through the Cleveland Clinic in an 11-year period spanning 2008 to 2018, inclusively, was evaluated in a multivariable model to identify and weigh risk factors for VTE in the first year of treatment. The model, consisting of five factors - prior VTE event, prior surgery within 90 days, immunomodulatory drug use in induction therapy, abnormal metaphase cytogenetics and Black race – was subsequently validated in an external cohort. Three levels of VTE risk were identified - low, intermediate and high – with 12-month cumulative incidences of VTE of 2.7%/6.4%, 10.7%/10.8% and 36.5%/23.8% in derivation and validation cohorts, respectively. An approximate 23% to 28% increased risk of VTE was associated with each one-point increase in the PRISM score (subdistribution hazard ratios [HR] of 1.28 and 1.23 per one-point PRISM score increase; both p<0.005). The discrimination power (c-statistic) of the model was 0.622.

Comment: Both the presence of myeloma and its treatment increase the risk of VTE. Hence there are guidelines for risk prediction and appropriate use of thromboprophylaxis. The established models have the drawback of being based on data from patients using a much higher dose of dexamethasone than that is in common use now and also by the use of doxorubicin, which is now rarely used to initiate treatment. This scoring system was derived using patient data from 783 consecutive patients treated at the Cleveland Clinic. The PRISM score (prior history of VTE, race, immunomodulatory drug use in induction, surgery within 90 days, and abnormal metaphases) stratified the patients into three risk groups with the cumulative incidence of VTE at 12 months being: low risk (score 0), intermediate risk (scores of 1 to 6) and high risk (scores ≥7). The PRISM score was independently validated with patients from Columbia University Irving Medical Centre. The cumulative incidence of VTE for the derivation and validation cohort respectively per risk group was: low risk (2.7% and 6.4%), intermediate risk (10.8% and 10.7%) and high risk (36.5% and 23.8%). Additional validation is necessary to test the discriminatory power of this prediction model.

Reference: Blood 2022;140(23):2443-50
Abstract

D-dimer and reduced-dose apixaban for extended treatment after unprovoked venous thromboembolism: the Apidulcis study

Authors: Palareti G et al.

Summary: APIDULCIS, an Italian, prospective, phase 4 study (NCT03678506) conducted by the Arianna Anticoagulazione Foundation, assessed the efficacy of a management strategy consisting of serial D-dimer testing to identify patients for whom extended anticoagulant therapy beyond the conventional 12 months following a first episode of unprovoked VTE is unnecessary. Adult patients (n=732; median age 59 years; 63.1% male) who had received at least 12 months of oral anticoagulant therapy after a first episode of DVT or PE were recruited from 49 hospitals and stratified into an extended anticoagulant treatment cohort (18 additional months of 2.5 apixaban twice-daily; 60.9%) or no further anticoagulant cohort (39.1%) based on D-dimer testing results. After a formal planned interim analysis revealed a more then eight-fold increased risk of recurrent VTE or major bleeding (symptomatic proximal DVT and/or PE, death from VTE or major bleeding) in patients with serial negative D-dimer tests who did not receive extended anticoagulation versus patients who received extended anticoagulation (7.3% vs 1.1%; HR 8.2; 95% CI, 3.2-25.3), exceeding the prespecified stopping-rule threshold, recruitment was prematurely terminated by an independent data monitoring and safety board committee. It was concluded that D-dimer testing cannot be used in isolation to make decisions regarding extended anticoagulation treatment to prevent recurrent VTE in this population.

Comment: This is a clinically relevant study as it studies the use of D-dimer to predict VTE recurrence in the current era of direct-acting oral anticoagulant (DOAC) use. In this APIDULCIS study, patients treated for a first episode of unprovoked VTE completed 12 months of anticoagulation and D-dimer was quantified first during anticoagulation and then (if the first test turned out negative) at 15, 30, and 60 days after anticoagulation was stopped. At the first positive D-dimer result, patients were commenced on apixaban 2.5 mg twice-daily for 18 months. Patients who were persistently negative were kept off anticoagulation. Patients were followed in the clinic or by phone every three to six months and those with either symptoms of DVT or PE or bleeding were evaluated further. The index event was proximal DVT with or without PE in 72%-79% and isolated PE was 20%-27%. The proportion of patients with an initial unprovoked VTE was approximately 75%. Almost 90% of patients were initially anticoagulated using a DOAC. The study was stopped after a pre-specified interim analysis identified a higher risk of VTE in the cohort off anticoagulation: 19 VTEs occurred in patients with negative D-dimer (6.2 per 100 person years) versus 3 (0.9 per 100 person-years) in those on apixaban. There were two episodes of major bleeding in both cohorts. There are some points worth noting: the incidence of VTE in those with negative D-dimers is higher in this study and the reasons are not clear. Patients were enrolled after being on anticoagulation for 12 months rather than the conventional three-six months. This raises the question that the patients may have had a higher risk of recurrence as per the treating physician. This study also overlapped with the COVID pandemic that may have impacted the risk of VTE and also D-dimer results. Approximately 25% of patients were on lower doses of DOAC for initial therapy. The only realistic conclusion is that extended use of apixaban 2.5mg twice-daily is effective in VTE prevention without an increased bleeding risk (data that was already quite established!).

Reference: Blood Adv 2022;6(23):6005-15
Abstract

Independent commentary by Prof Harshal Nandurkar

Prof Harshal Nandurkar, is the Director of the Alfred Cancer Program and Clinical Haematology, Head of the Australian Centre for Blood Diseases. He is the past President of the Thrombosis and Haemostasis Society of Australia and NZ and past Vice President of the International Union of Angiology (Australian Chapter).

Predictors of thrombosis and bleeding in 1613 myelofibrosis patients from the Spanish Registry of Myelofibrosis

Authors: Hernández-Boluda J-C et al., for the Spanish MPN Group (GEMFIN)

Summary: In an effort to develop consensus thromboprophylactic recommendations for patients with myelofibrosis the Spanish Myeloproliferative Neoplasms Group evaluated factors prognostic for vascular complications and possible preventative effect of therapies in a cohort of patients (n=1,613) from the Spanish Registry of Myelofibrosis. Just over 6% of the population experienced one or more thrombotic events after diagnosis and 5.3% a major bleeding episode, translating to an incidence rate of 1.65 and 1.5 events per 100 patient-years, respectively. Risk factors for thrombosis included a prior history of thrombosis, the presence of the *JAK2* mutation and the intermediate-2/high-risk International Prognostic Scoring System (IPSS) categories and the magnitude of risk was not mitigated by anti-thrombotic or cytoreductive therapy. Immunomodulatory agent treatment was found to confer a significantly increased risk of venous thrombosis. Anticoagulation use was associated with an elevated risk of major bleeding.

Comment: Both primary myelofibrosis as well as that progressing from essential thrombocytosis or polycythaemia vera are associated with an increased incidence of thrombosis at the time of diagnosis and its development in the course of the disease. It is known that older age, pre-existent cardiovascular risk factors, leucocytosis, thrombocytosis, prior thrombosis history, and *JAK2* mutational status are variables that increase the frequency of thrombosis. This study uses data from the Spanish Myeloproliferative Neoplasm Registry to understand factors that predict thrombotic and bleeding risk and covers 1,613 patients diagnosed with myelofibrosis between January 2000 and October 2021 in 59 centres. Baseline data reveals that almost 60% of patients were IPSS category intermediate-2 and high-risk. *JAK2* was noted in 71% and *CALR* in 18%. After a median follow-up of 6.7 years, 154 (of 1,613) patients had progressed to acute myeloid leukemia (AML), 148 had undergone allogeneic-stem cell transplant (17 after AML), and 793 (49.2%) patients had died. The median OS of the series was 6.3 years. Vascular complications were the cause of death in almost 10%. Excluding episodes at diagnosis, 115 episodes of thrombosis were noted of which 104 were first episodes (61 arterial and 43 venous thrombosis). Overall, 9.1% *JAK2*$^+$ patients, 5.9% *CALR*$^+$ patients, and 5.4% *MPL*$^+$ patients and 1.3% triple-negative patients developed at least one thrombotic complication. The overall incidence rate of arterial and venous thrombosis was 0.97 and 0.67 events per 100 patient-years, respectively. Base-line factors that increased the incidence of thrombosis during follow-up included prior thrombosis (incidence rate ratio [IRR] 2.6), *JAK2* mutation (IRR 1.93) and intermediate-2 and high-risk IPSS categories (IRR 1.99). Treatment with anticoagulants or anti-platelet drugs did not reduce the IRR for thrombosis while being on immunomodulatory drugs substantially increased the risk (IRR 5.85). Interestingly, older age, male sex, cardiovascular risk factors, myelofibrosis subtype, leucocytosis, thrombocytosis, spleen size, and bone marrow fibrosis grade were not associated with a significantly higher incidence of thrombosis. Approximately 5% of patients had at least one episode of major bleeding, mainly from the gastrointestinal tract. The resulting incidence rate, 1.5 events per 100 patient-years, was as frequent as the thrombosis rate. Risk of bleeding correlated with the use of anti-coagulants (IRR 2.46) and intermediate-2/high-risk IPSS classification at myelofibrosis diagnosis (IRR 2.46) but not with thrombocytopenia or the use of anti-platelet drugs. This real-world registry data will aid in advising patients regarding prognosis and planning treatments.

Reference: Br J Haematol 2022;199(4):529-38
Abstract

VWF-targeted thrombolysis to overcome rh-tPA resistance in experimental murine ischemic stroke models

Authors: van Moorsel M et al.

Summary: This Dutch pre-clinical study reports the evaluation of a novel thrombolytic agent for treatment of acute ischemic stroke – Microlyse. The researchers - affiliated with University Medical Centre Utrecht and Utrecht University and TargED Biopharmaceuticals, all in Utrecht, The Netherlands - employed experimentally induced murine models of stroke to assess the efficacy of Microlyse for the lysis of fibrin-rich and platelet-rich thrombi in the middle cerebral artery. Microlyse demonstrated comparable restoration of cortical reperfusion and reduction of cerebral lesion volume as recombinant human tissue plasminogen activator (rh-tPA) in mice with thrombin-induced fibrin-rich thrombi but was superior for cerebral lesion volume reduction in experiments with $FeCl_3$-induced platelet-rich thrombi. It should be noted that neither agent increased cortical reperfusion in platelet-rich thrombi experiments compared to vehicle. The results indicated that VWF-targeted thrombolysis using Microlyse may overcome the problem of rh-tPA treatment resistance associated with platelet-rich thrombi.

Comment: This is a very interesting pre-clinical paper that may potentially add to the repertoire of therapeutics for the treatment of stroke and other thrombotic conditions. The authors have developed a novel drug, 'Microlyse', consisting of a VWF-targeting nanobody and the catalytic domain of urokinase plasminogen activator. Upon binding VWF, Microlyse initiates localised plasminogen activation. The conventional treatment for embolic stroke is fibrinolysis, most commonly with rh-tPA (alteplase), which on binding to fibrin activates plasminogen into plasmin, which then actively degrades fibrin, leading to resolution of the occluding thrombus, increased cerebral perfusion, reduced ischaemic brain volume and neurological recovery. Plasmin also has the capacity to proteolyze VWF, thus enlarging its potential to remove VWF-platelet rich thrombi. The authors pose the question that fibrin is often not fully accessible while most thrombi contain VWF and hence, Microlyse may work as well as or superior to rh-tPA. This hypothesis was tested in two models of stroke; one that is fibrin rich by injection of thrombin and the second that is VWF-platelet rich by a chemical ferric chloride injury. Both rh-tPA and Microlyse worked effectively in the fibrin-rich thrombus model. The data was contrasting in the platelet-rich thrombus wherein none of the agents improved reperfusion but only Microlyse decreased ischaemic volume (the mechanistic rationale is still being worked out). The gaps in clinical translation are that the fibrin-rich model by direct thrombin injection is not quite a model of embolic stroke, typical in human disease. The ferric chloride model is even less representative, but it does open up the potential for Microlyse in other platelet-rich thrombotic conditions. In fact, in earlier work, Microlyse was demonstrated to be effective in a murine model of thrombotic thrombocytopenic purpura. It is not clear why VWF in circulation does not compete to bind Microlyse, thereby decreasing the drugs availability to concentrate at the thrombotic site.

Reference: Blood 2022;140(26):2844-48
Abstract

Low-dose decitabine modulates myeloid-derived suppressor cell fitness via LKB1 in immune thrombocytopenia

Authors: Ni X et al.

Summary: As a follow-on to their earlier work demonstrating that myeloid-derived suppressor cell (MDSC) deficiency and impairment plays a role in the underlying pathophysiology of immune thrombocytopenia (ITP) this Chinese group elucidated the mechanism driving restoration of MDSC function after decitabine treatment. The researchers showed in both *in vitro* experiments and murine models of severe ITP that the therapeutic benefits elicited by low-dose decitabine may be achieved, at least in part, through normalisation of liver kinase B1 (LBK1) levels.

Comment: This work focuses on the capacity of MDSCs to act as natural inhibitors of adaptive immunity. The authors demonstrate that this function is via energy homeostasis through the LBK1 and AMP-activated protein kinase (AMPK) pathway. The function of MDSCs is impaired in ITP, thereby promoting autoimmune-mediated platelet destruction. The authors show elegantly that MDSCs in circulation are lower in patients with ITP and their generation can be increased by treating the patients with low-dose decitabine. MDSCs isolated after patient exposure to decitabine had enhanced aerobic metabolism and the cells were immunosuppressive *in vitro* by their capacity to block cytotoxic T-lymphocyte-mediated platelet apoptosis. Passive transfer of decitabine-treated MDSCs improved platelet count in a mouse model of ITP with concomitant suppression of proinflammatory cytokines. The mechanism of decitabine was shown to be its ability to increase cellular LKB1 by demethylation of its promoter. The authors confirm the role of LKB1 by demonstrating that LKB1-shRNA interference negates the ability of decitabine-treated MDSCs to ameliorate ITP. These strong experimental data lay the path for clinical studies.

Reference: Blood 2022;140(26):2818-34
Abstract

Frequency and predictors for chronic thromboembolic pulmonary hypertension after a first unprovoked pulmonary embolism: Results from PADIS studies

Authors: Fauché A et al., for the PADIS-PE Investigators

Summary: Analysis of data from the French PADIS studies was undertaken to explicate the prevalence of, and risk factors for, chronic thromboembolic pulmonary hypertension (CTEPH) after a primary unprovoked PE event. In a cohort of 371 patients treated with six months of vitamin K antagonist ± 18 months of warfarin and followed for at least eight years the cumulative incidence of CTEPH was 2.8% (n=9), 1.31% of which was deemed to be incident (not prevalent). Cox modelling analysis identified pulmonary vascular obstruction (PVO) > 45% and systolic pulmonary arterial pressure (sPAP) > 56 mmHg at PE diagnosis as predictors of CTEPH, conferring a 33-fold and 12.5-fold increased risk, respectively (HR 33 and HR 12.50; both $p \leq 0.02$).

Comment: This study explores the ability to predict the development of CTEPH after the initial episode of unprovoked PE. CTPEH is a worrying sequalae of PE and is associated with significant morbidity and mortality. This study reports on the incidence of CTPEH during an eight-year follow-up after an initial presentation with PE. The cohort studied was from a previous PADIS-PE (Prolonged Anticoagulation During 18 months versus placebo after Initial 6-month treatment for a first episode of idiopathic Pulmonary Embolism) trial, which included patients with first unprovoked PE confirmed on computed tomography pulmonary angiogram or V/Q lung scan, and initial treatment with vitamin K antagonist during six uninterrupted months. At six months after PE, patients were included and randomly assigned to receive an additional 18 months of warfarin treatment or placebo and were followed during a two-year post-treatment period. Patients were then followed up in the 'PADIS-EXTENSION' study for a further six years. All patients were screened for the presence of CTEPH at six months and five years with V/Q and trans thoracic cardiac echo and residual pulmonary vascular obstruction was quantified on a V/Q lung scan. During the eight-year follow-up nine patients (of 371) were diagnosed with CTEPH, yielding a cumulative incidence of 2.8%. Parameters at the time of PE presentation that impacted on CTEPH included age >65 years (HR 8.78), non-O blood group (HR 0.24), lupus anticoagulant antibodies (HR 5.22). PVO and sPAP values were identified as continuous variables. The most discriminant value of PVO at PE diagnosis for the risk of further CTEPH was 45% (AUC 0.80; 0.75–0.84; HR 33.00) and for sPAP the most discriminant value was 56 mmHg (HR of 12.50). Analysis at six months also demonstrated high PVO and sPAP in those that later developed PE. Other clinically relevant points to stress are that in CTEPH patients, there was no difference in PVO at PE diagnosis, six months or eight years of follow-up, suggesting that failure of thrombus resolution is contributory. While this study was not designed to study the impact of extended anticoagulation, the authors note that there was no significant difference in CTEPH incidence between patients allocated to an additional 18-months of warfarin and those to placebo, suggesting that ongoing anticoagulation does not change vascular modelling.

Reference: J Thromb Haemost 2022;20(12):2850-61
Abstract

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