Thrombosis & Haemostasis Research Review™

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In this issue:

Abbreviations used in this issue:

4F-PCC = 4-factor prothrombin complex concentrate; **AHA** = acquired hemophilia A; **ALL** = acute lymphoblastic leukaemia; **APTT** = activated partial thromboplastin time; **ARDS** = acute respiratory distress syndrome; **CI** = confidence interval; **COVID-19** = coronavirus disease 2019; **COX-1** = cyclooxygenase-1; **ET** = essential thrombocythemia; **FVIII** = coagulation factor VIII; **HMB** = heavy menstrual bleeding; **ICH** = intracranial haemorrhages; **ICU** = intensive care unit; **ISTH** = International Society on Thrombosis and Haemostasis; **L-ASP** = L-asparaginase; **PCR** = polymerase chain reaction; **QoL** = quality of life; **SARS-CoV-2** = severe acute respiratory syndrome coronavirus 2; **sTXB$_2$** = serum thromboxane B$_2$; **TTP** = thrombotic thrombocytopenic purpura; **VTE** = venous thromboembolism; **VWF** = von Willebrand factor.

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Welcome to the latest issue of Thrombosis and Haemostasis Research Review.

We begin this issue with an autopsy series from the US that contrasted pulmonary vascular injury in patients who died of severe coronavirus disease 2019 (COVID-19) versus acute respiratory distress syndrome (ARDS) secondary to influenza A H1N1 infection. The research found three features of vasculopathy that distinguished COVID-19 infection by their scale and extent including endothelial injury with intracellular virus, widespread vascular thrombosis and vascular angiogenesis, all of which occurred to a far greater magnitude than that seen with an equally serious influenza infection. A single-center study from New Haven, Connecticut, USA, is the first to provide biochemical evidence that endotheliopathy is an important feature in the coagulopathy of COVID-19. This study finds a potential prognostic role for the measurement of endothelial markers in COVID-19 patients, with elevated plasma von Willebrand factor levels and soluble P-selectin both increasing with disease severity and significantly associated with mortality. We also look at the latest research in thrombotic thrombocytopenic purpura (TTP); an Italian study of patients successfully treated for acquired acute disease found high rates of neuropsychologic sequalae years after the acute disease phase. Patients with neuropsychologic manifestations at the TTP's first presentation were particularly susceptible to long-term deleterious effects. Laboratory studies by a group in Belgium delineating the interaction between autoantibodies and conformational forms of the metalloprotease ADAMTS13 in immune-mediated TTP found that antibodies to ADAMTS13 change its configuration from the active open form to a closed inactive form and therefore prevent its physiological function in von Willebrand cleavage. This may lead to clinical applications as a biomarker to monitor and identify subclinical immune-mediated TTP disease and the ability to tailor subsequent therapy as required.

We hope you find these and the other selected studies interesting, hope that they help you improve the lives of your patients living with these diseases and look forward to receiving any feedback you may have.

Kind Regards,

Professor Harshal Nandurkar

harshal.nandurkar@researchreview.com.au

Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19

Authors: Ackermann M et al.

Summary: This autopsy series published in the *New England Journal of Medicine* examined the histologic and pathologic characteristics in peripheral lungs of patients who died from coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The researchers used seven-colour immunohistochemical analysis, micro–computed tomographic imaging, scanning electron microscopy, corrosion casting and direct multiplexed measurement of gene expression to compare lungs obtained during autopsy from patients with died from COVID-19 (n=7), acute respiratory distress syndrome (ARDS) secondary to influenza A H1N1 infection (n=7) and age-matched uninfected controls (n=10). Pulmonary vascular angiogenesis, thrombosis and endothelialitis were all evident with COVID-19 infection with significantly more pulmonary alveolar capillary thrombosis (nine-times more; $p<0.001$) and angiogenesis (2.7-times greater; $p<0.001$) compared to an equally severe influenza infection. Intracellular SARS-CoV-2 was correlated to severe endothelial cell injury. The researchers also found an increase in the number of angiogenesis-related genes with disparate expression in COVID-19 versus influenza lungs (69 vs 26, respectively).

Comment: Features of ARDS are non-specific and are observed in advanced pulmonary disease in several viral pneumonias that include COVID-19 as well as influenza. This paper identifies differences in COVID-19 lung versus influenza-ARDS by analysing post mortem specimens of seven lungs from each viral illness and comparison with normal lungs. The clue that there could be coagulation-related findings in COVID-19 come from other independent observations of disseminated intravascular coagulation, raised D-dimers, micro- and macrovascular thrombosis in COVID-19. Lungs were analysed using sophisticated imaging and histological techniques and also gene expression analysis. Lungs from both COVID-19 and influenza showed similar upregulation of angiotensin-converting enzyme 2 in alveolar epithelial cells and endothelial cells as compared to normal lungs. Higher number of CD4 T cells and fewer neutrophils were noted in COVID-19 versus influenza lungs. Key findings were in the microvasculature: the lungs in the COVID-19 group had a distorted vascularity with structurally deformed capillaries. There were three distinctive angiocentric features of COVID-19. The first feature was severe endothelial injury associated with intracellular SARS-CoV-2 virus and disrupted endothelial cell membranes. Second, the lungs from patients with COVID-19 had widespread vascular thrombosis with microangiopathy and occlusion of alveolar capillaries. Third, the lungs from patients with COVID-19 had significant new vessel growth through a mechanism of intussusceptive angiogenesis. Transmission electron microscopy of the COVID-19 endothelium showed ultrastructural damage to the endothelium, as well as the presence of intracellular SARS-CoV-2. This report and others that have also been published identify features of 'vasculopathy' in COVID-19 that differentiate it from influenza and possibly other viral ARDSs.

Reference: N Engl J Med 2020;383(2):120-28

Abstract

Evaluation of andexanet alfa and four-factor prothrombin complex concentrate (4F-PCC) for reversal of rivaroxaban- and apixaban-associated intracranial hemorrhages

Authors: Barra M et al.

Summary: In a retrospective, single-center case series published in the *Journal of Thrombosis and Haemostasis*, Megan Barra and colleagues from Massachusetts General Hospital, Boston, USA, found that andexanet alfa compared to 4-factor prothrombin complex concentrate (4F-PCC) resulted in higher rates of better than good haemostasis and good functionality on discharge but was associated with a longer time to administration and a significantly higher cost per patient in patients treated for oral factor Xa inhibitor-associated intracranial haemorrhages (ICH). A total of 29 patients treated for oral factor Xa inhibitor-associated ICH with either andexanet alfa (n=18) or 4F-PCC (n=11) between 2016 and 2019 were included in the analysis. Outcomes between patients treated with andexanet alfa compared to 4F-PCC, respectively, are as follows: ≥ good haemostasis (88.9% vs 60%), good functional outcome on discharge (55.6% vs 9.1%), thrombotic complications (16.7% vs 9.1%), median time of medication order-to-administration (1.1 vs 0.5 hours) and median cost of therapy per patient (US$29,970 vs US$6,925).

Comment: The availability of andexanet alpha has provided an Xa inhibitor-specific reversal strategy, but its availability is still limited. This study compares the effectiveness and complications associated with andexanet alpha in comparison with 4F-PCC. This is of relevance to the clinical practice in Australia as andexanet alpha is not yet available for routine use. But a note of caution that 3F-PCC is commonly used here (lacking coagulation factor VII). ICH is less frequent with direct oral anticoagulants as compared with warfarin, but it still remains the most difficult bleeding complication to manage. It is already known that off-label use of 4F-PCC for ICH suggests effective haemostasis can be achieved in 65% to 94.7% of patients with apixaban and rivaroxaban-associated ICH. Connolly et al (N Engl J Med 2019;380:1326-35) have reported that in 171 patients with ICH, andexanet alpha achieved excellent or good haemostatic efficacy in 80% of patients and the 30-day thrombosis event rate was 10%. In this real-world retrospective review of 18 patients treated with andexanet alpha and 11 managed with 4F-PCC, primary outcome was haemostatic efficacy as judged by imaging estimates of ICH volumes, subdural haematoma thickness and subarachnoid haemorrhage thickness. Functional outcomes at discharge were assessed by Glasgow Outcome Score. Thromboembolic complications occurring up to 30 days were enumerated. Good or excellent haemostasis on follow-up imaging occurred in 88.9% (16/18) of patients in the andexanet alfa group and 60% (6/10) of patients in the 4F-PCC group. There were no differences in baseline coagulation parameters or other baseline characteristics between patients with good or excellent haemostasis and those with poor haemostasis. While functional outcomes were better in the andexanet alpha cohort compared to 4F-PCC (see above), the use of andexanet alpha was associated with higher thromboembolic episodes (all were venous thrombosis, no acute myocardial infarction). Three patients in each cohort required neurosurgical intervention. The caveat to this study is that the median pre-reversal ICH volume was higher in the 4F-PCC cohort (37.4 mL) than the andexanet alpha group (20.6 mL) that may have influenced haemostatic response.

Reference: J Thromb Haemost 2020;18(7):1637-47
Abstract

COVID-19 and acute coagulopathy in pregnancy

Authors: Koumoutsea E et al.

Summary: Evangelia Vlachodimitropoulou Koumoutsea and colleagues present two cases of maternal COVID-19 infection during the third trimester of pregnancy associated with early organ dysfunction and progressive coagulopathy, both of whom recovered post -delivery. Both patients were admitted to hospital with cough and pyrexia (one patient [40 years old; secundigravida; 35 + 3 weeks' gestation] to Mount Sinai Hospital, Toronta, Canada and one [23 years old; primigravida; 35+ 2 weeks' gestation] to a peripheral hospital prior to being moved to Antoine Béclère Hospital, France). SARS-CoV-2 infection was confirmed by polymerase chain reaction (PCR) analysis of a nasopharyngeal swab. Notable medical history in case one included gestational diabetes and neutropenia and asthma and obesity in case two. Deterioration in coagulation parameters and platelet indices were observed in both patients including prolonged activated partial thromboplastin time (APTT; 30.3 and 51 s for case 1 and case 2, respectively in the days prior to delivery) and thrombocytopenia (98 and 63 x 10^9/L platelet counts, respectively). Both patients underwent emergency caesarean section delivery after which coagulation parameters stabilised. The authors suggest that in pregnant patients with COVID-19 infection, APTT and fibrinogen levels be monitored in addition to the regular parameters.

Comment: This is a first report highlighting a possible link between third-trimester maternal COVID-19 infection and rapid maternal deterioration, with progressive coagulopathy, improving shortly after delivery. The International Society on Thrombosis and Haemostasis (ISTH) has an interim guideline that addresses coagulopathy in COVID-19 highlighting D-dimer elevation, thrombocytopenia and low fibrinogen as poor prognostic indicators of mortality risk. The first case was a forty-year-old gravida 2 para 1, with familial neutropenia diagnosed in infancy and with an uncomplicated course in adulthood. She was admitted with fever and neutropenia and noted to be COVID-19 positive on PCR but with normal chest X-ray. Over 48 hours, there was progressive thrombocytopenia, declining fibrinogen, and rising APTT. While HELLP (haemolysis, elevated liver enzymes, low platelet count) syndrome was a possibility it was considered less likely in the absence of hypertension, haemolysis, or proteinuria. Clinical consequence of coagulopathy was postpartum haemorrhage post caesarean section. Coagulopathy improved post- delivery and after appropriate blood products. The second case was a twenty-three-year-old gravida 1 para 0 admitted at 37 weeks' gestation with cough and fever. Nasopharyngeal swab demonstrated SARS-CoV-2. Progressive thrombocytopenia, prolonged APTT and transaminitis triggered an emergency caesarean section and blood factor support with improvement post-delivery. Both women were administered prophylactic low-molecular weight heparin. Routine assessment of D-Dimers, international normalized ratio, APPT and other markers of disseminated intravascular coagulation should be performed on all pregnant women with COVID-19.

Reference: J Thromb Haemost 2020;18(7):1648-52
Abstract

Endotheliopathy in COVID-19-associated coagulopathy

Authors: Goshua G et al.

Summary: This single-centre, cross-sectional study published in the *Lancet Hematology* provides evidence that COVID-19 infection causes endotheliopathy which is associated with severe illness and mortality. Goshua et al compared markers of endothelial cell and platelet activation between subsets of adult patients with laboratory-confirmed COVID-19 hospitalised between April 13 and April 24, 2020; patients in the intensive care unit (ICU; n=48), patients not in intensive care (n=20) and non-hospitalised, asymptomatic controls (n=13). Elevated markers of endothelial activation including von Willebrand factor (VWF) antigen levels and soluble P-selectin increased with disease severity (ICU versus non-ICU patients; mean VWF antigen, 565% vs 278%; $p<0.0001$. Soluble P-selectin, 15.9 vs 11.2 ng/mL; $p=0.0014$) and were significantly associated with mortality ($r=0.38$; $p=0.0022$ and $r=0.38$; $p=0.0078$, respectively). Concentrations of soluble thrombomodulin higher than 3.26 ng/mL were associated with lower rates of hospital discharge (low concentrations versus high concentrations; 88% vs 52%; $p=0.0050$) and an almost six-times higher risk of mortality (hazard ratio 5.9; 95% confidence interval [CI], 1.9-18.4; $p=0.0087$).

Comment: This study is the first to provide biochemical evidence that endotheliopathy is an important feature in the coagulopathy of COVID-19. D-dimers, VWF-antigen and activity, and factor 8 levels were higher in COVID-19 patients in ICU (n=48) versus not in ICU (n=20) in a single centre in New Haven, CT, USA. There was no change in protein C, S and α2-antiplasmin activity. Endothelial activation was reflected in elevated levels of P-selectin and soluble thrombomodulin. A particularly interesting observation was that high levels of soluble thrombomodulin correlated with inferior survival and lower rates of discharge from the hospital. Thrombomodulin is an endothelial cell surface-anchored protein that is critical for the ability of thrombin to generate activated protein C, which has important anti-inflammatory and anticoagulant actions. Hence, it is intuitive to hypothesise that loss of endothelial thrombomodulin by shedding or cell death (reflecting as higher levels of circulating soluble thrombomodulin) leads to a thrombotic phenotype in the vasculature.

Reference: Lancet Haematol 2020;7(8):e575-82
Abstract

Long-term neuropsychological sequelae, emotional wellbeing and quality of life in patients with acquired thrombotic thrombocytopenic purpura

Authors: Riva S et al.

Summary: Silvia Riva and colleagues assessed the long-term neuropsychological consequences in patients successfully treated for acquired thrombotic thrombocytopenic purpura (TTP) with immunosuppressive therapy and plasma exchange. A total of 35 patients (77% females; median age at onset 41 years) with TTP who were seen regularly at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy, between December 2015 and October 2016, underwent psychological evaluation at a minimum of three months after their last TTP event (interquartile range, 17-54). Compared to the general Italian public, patients recovering from acute acquired TTP had reduced direct, indirect and deferred memory functioning and reduced health-related quality of life (QoL; mean difference 58.43, 95% CI, 71.49–45.37). Persisting anxiety was reported in 20% of TTP patients and depression in 43%. Almost half of TTP patients (49%) self-reported neurological impairments such as disorientation, dizziness, headache or diplopia.

Comment: Recovery in TTP, even if complete, is associated with a risk of relapse and occurrence of persistent neurologic, cardiac and renal abnormalities. After recovery, despite normal physical examination and laboratory data, many patients complain of difficulties with memory, headache, loss of concentration and endurance. This study is a longer term follow up of 35 acquired (immune) TTP patients, conducted in Milan. Patients underwent a comprehensive neuropsychological evaluation including memory and attentional functions, emotional wellbeing and health-related QoL assessments. At the time of neuropsychological evaluation, 10 (29%) of 35 patients had suffered from recurrent TTP bouts. 22 patients (63%) presented with neurological signs and symptoms at presentation of the first acute TTP episode (including coma [n=2], focal neurological signs [n=12], personality changes [n=2], transient ischemic attack [n=4], seizures [n=1], stroke [n=3]). There was significant underperformance (compared to the normal Italian population) in various aspects of tests conducted for memory and cognition. There was higher degree of neurocognitive impairment in patients who had neurological signs and symptoms at presentation of the first acute TTP episode. Emotional parameters such as depression, loss of concentration and impaired mood were also more frequent, leading to inferior health-related QoL. Interestingly, no differences in neuropsychological assessments were found between patients with ADAMTS13 activity levels during remission below and above 45%. This has important impacts in the longer-term management of patients with immune TTP with extra attention to be focussed on neuropsychological domains rather than just assessments of laboratory parameters.

Reference: Haematologica 2020;105(7):1957-62
Abstract

Bleeding disorders in adolescents with heavy menstrual bleeding in a multicenter prospective US cohort

Authors: Zia A et al.

Summary: This US multicenter prospective cohort study found high levels of bleeding disorders including Von Willebrand disease and qualitative platelet dysfunction in adolescents with heavy menstrual bleeding (HMB). Researchers analysed a total of 200 adolescents with either anovulatory (cycle length, < 21 or > 45 days) or ovulatory bleeding referred for HMB and diagnosed bleeding disorders *a priori*. A bleeding disorder was diagnosed in 67 patients with HMB the sole manifestation in almost half (43%). The prevalence of bleeding disorders was similar between cohorts (31% vs 36%; p=0.45) but there was a significant lag in diagnosis in patients with ovulatory bleeding (time from onset of first bleed to diagnosis, 2 vs 6 years; log-rank test, p<0.001). Of the patients with blood disorders, low VWF levels were found in 57%, von Willebrand disease was diagnosed in 11% (13 with type 1, and 4 with type 2),13% had a qualitative platelet disorder and two were diagnosed with coagulation factor deficiencies. Multivariable logistic regression analysis showed younger age at first bleed, Hispanic ethnicity, non-presentation to emergency department for heavy bleeding and ISTH Bleeding Assessment Tool score ≥4 to be independent predictors of bleeding disorders (odds ratio, 0.83, 2.48, 0.14 and 8.27, respectively).

Comment: A large body of research has focused on the prevalence of bleeding disorders in adult women presenting with HMB but this data is lacking in adolescents. Significant bleeding disorders in males (e.g. haemophilia) manifest in infancy or childhood. Menarche is often the first exacerbation of bleeding disorders in females. There is also heightened anxiety amongst parents and clinicians to exclude a bleeding diathesis. In this study 200 subjects were followed with median age of 15 years. Participants were classified and compared among two groups: the anovulatory HMB group was defined as having menstrual duration <21 or >45 days; the ovulatory HMB group was defined when this menstrual pattern was not present. HMB was diagnosed based on established criteria. Thirty-three percent presented to the emergency department for evaluation and management of HMB; 25% needed to be hospitalized, of which 19% received packed red blood cells. A family history of bleeding symptoms or established blood disorder was present in 21% and 6.5%, respectively. Family history of gynaecologic or obstetric bleeding was present in 60%. Overall, 33% (n=67) of adolescents were diagnosed with a blood disorder. Among those with a blood disorder, there were no differences in the frequency of blood disorders in the anovulatory and the ovulatory HMB groups. Presentation at a younger age and ISTH bleeding assessment score more than 4, were associated with blood disorders. Almost 80% of those with a blood disorder had a diagnosis of either low VWF or von Willebrand disease. Two participants were found to have coagulation factor deficiencies (one with mild FVIII deficiency (FVIII:C= 29%) and mild FXIII deficiency (FXIII: 40%). Qualitative platelet function defects were uncommon (~4%).

Reference: Haematologica 2020;105(7):1969-76
Abstract

Bleeding and response to hemostatic therapy in acquired hemophilia A

Authors: Holstein K et al.

Summary: This analysis of the prospective GTH-AH 01/2010 study examined bleeding risk and hemostatic therapy in acquired hemophilia A (AHA). Coagulation factor VIII (FVIII) activity was monitored weekly in a total of 102 patients (289 bleeds) with AHA treated with a uniform immunosuppressive therapy protocol. Prior to achieving partial remission, the mean rate of bleeds per patient-week was 0.26. The GTH-AH 01/2010 study found that partial remission (restoration of FVIII activity to >50 IU/dL) was achieved by 83% of patients after a median of 31 days of treatment. This analysis expanded on that research to show that the risk of bleeding was not eradicated until a greater than 50% activity of FVIII was restored. Haemostatic treatment was effective in 96% of bleeds.

Comment: In contrast to congenital haemophilia, the measured plasma level of FVIII at diagnosis is not predictive of the risk or severity of bleeding in AHA. Spontaneous, catastrophic bleeding may occur in patients with FVIII levels that would be considered of mild severity in congenital haemophilia. The GTH-AH 2010 study, a prospective observational study in patients with AHA, was conducted in 29 centres in Germany and Austria between 2010 and 2013. Patients were enrolled consecutively and treated with immune suppression therapy immediately after diagnosis. Clinical data and FVIII activity were collected once weekly until complete response or death. A total of 102 patients with AHA were enrolled in the study and a total of 289 bleeds (2.8 bleeds per patient) were documented. Interestingly, baseline factor 8 activity (<1 or higher) or Bethesda inhibitor titre (<20 or higher) did not change the cumulative incidence of new bleeds after diagnosis. Bleeding risk differences observed between FVIII levels up to 20% are minimal. Bleeding rates decrease with factor 8 levels >20% but only achieving an FVIII level ≥50% abolishes the risk of bleeding. Hence focus should be on instituting immunosuppressive therapy rapidly to hasten inhibitor elimination.

Reference: Blood 2020;136(3):279-87
Abstract

Thromboembolism prophylaxis in adult patients with acute lymphoblastic leukemia treated in the GRAALL-2005 study

Authors: Orvain C et al.

Summary: This large descriptive study provides results for thromboembolism prophylaxis in adult patients with acute lymphoblastic leukemia (ALL) treated in the GRAALL-2005 study (Group for Research on Adult Acute Lymphoblastic Leukemia 2005; ClinicalTrials.gov #NCT00327678). The paediatrics-inspired prospective GRAALL-2005 study enrolled 813 adult patients (aged 18-60 years) with *de novo* Philadelphia Chromosome-negative ALL between 2006 and 2014. A total of 784 patients were included in this analysis. Venous thromboembolism (VTE) occurred in 16% of patients, predominantly during the induction phase (69%). An increased risk of VTE was seen with the administration of fibrinogen concentrates and heparin prophylaxis and developed despite antithrombin replacement. The authors recommend that the use of fibrinogen concentrates be restricted to rare patients with hypofibrinogenemia-induced haemorrhage.

Comment: Patients who undergo treatment of ALL are at risk of thrombosis caused by the disease itself, by the use of steroids, and by the use of L-asparaginase (L-ASP). L-ASP inhibits the hepatic synthesis of L-asparagine–dependent haemostatic proteins, such as antithrombin, and may induce endothelium activation. Previous reports showed that patients with VTE have a lower event-free survival that may be related to early discontinuation of L-ASP. Antithrombin replacement could decrease the rate of thrombosis and thus prevent L-ASP discontinuation. This study monitored 784 patients with ALL undergoing the GRAALL-2005 protocol. FFP or fibrinogen concentrates were recommended if fibrinogen levels were <0.5 g/L and antithrombin concentrate substitution therapy was recommended to maintain antithrombin levels >60%. Unfractionated heparin at 100 IU/kg per day via continuous infusion was recommended during induction. The 1-year cumulative incidence of first VTE was 17%. Risk factors at diagnosis for thrombosis included older age, female sex, high body mass index, and a high platelet count at diagnosis. Oral contraceptives before diagnosis and a history of smoking were not associated with thrombosis. Despite extensive antithrombin supplementation (87%), many patients experienced VTE, possibly as a substantial proportion of patients receiving antithrombin supplementation still had antithrombin levels lower than the cut-off target of 60%. Fibrinogen concentrates effectively increased fibrinogen levels but were associated with an increased risk for thrombosis (odds ratio, 2.2). Hence, ibrinogen concentrates should be restricted to severe but rare bleeding associated with hypofibrinogenemia. Surprisingly, heparin thromboprophylaxis was associated with higher risk of VTE, mechanisms unclear.

Reference: Blood 2020;136(3):328-38
Abstract

Open ADAMTS13, induced by antibodies, is a biomarker for subclinical immune-mediated thrombotic thrombocytopenic purpura

Authors: Roose E et al.

Summary: Elien Roose and colleagues from the Laboratory for Thrombosis Research, IRF Life Sciences, Kortrijk, Belgium, performed a pre-clinical delineation of the interaction between autoantibodies and conformational forms of the metalloprotease ADAMTS13 in immune-mediated TTP. The researchers expanded on their previously published finding that the acute phase of immune-mediated TTP is characterised by the presence of ADMATS13 in an open conformation (Roose E et al. An open conformation of ADAMTS-13 is a hallmark of acute acquired thrombotic thrombocytopenic purpura. J Thromb Haemost. 2018;16(2):378-388) by showing that immunoglobulin G autoantibodies to ADAMTS-13 isolated from patients in the acute phase of immune-mediated TTP induced a conformational change of ADAMTS-13 molecules in healthy donor plasma from the closed to the open conformation (14/18 samples changed conformation; 78%). They also provided evidence that disease monitoring could be possible using the open ADAMTS13 conformation as a biomarker by showing that the open conformation was detectable in patients with acute disease, patients in remission with ADAMTS13 activity levels <50% and in patients in remission with ADAMTS13 activity levels >50%.

Comment: ADAMTS13 is a multidomain enzyme consisting of a metalloprotease domain, a disintegrin-like domain, a first thrombospondin type-1 repeat, a cysteine-rich domain, a spacer domain, seven additional thrombospondin type-1 repeats, and two CUB domains. Under normal circumstances, ADAMTS13 adopts a closed conformation in which the spacer domain interacts with the C-terminal CUB domains. Interaction with VWF or activating murine antibodies to ADAMTS13 uncouples the spacer-CUB interaction, resulting in an open ADAMTS13 conformation. It is already established that in immune-mediated TTP, ADAMTS13 activity < 10% and/or presence of anti-ADAMTS13 autoantibodies during remission indicate a higher risk for relapse and these parameters are often used as a trigger to recommence immunosuppression. The biological significance of an 'open' confirmation is unknown and it is hypothesised that it may trigger further immune response against ADAMST13. In this study, the authors establish a murine antibody that is able to specifically recognise the open confirmation of ADAMTS13 by binding to the spacer region and quantify this variant of ADAMTS13 by ELISA. Autoantibodies purified from immune-mediated TTP patients also bind exclusively to the spacer region. An important question addressed here was whether presence of open ADAMTS13 is of clinical relevance. Analysis of 197 immune-mediated TTP patients both with acute disease and in clinical remission, showed that in acute patients with ADAMTS13 activity levels <10%, the open ADAMTS13 conformation was detected in >90% of samples. In the clinical remission cohort, there was increasing transition to the native 'closed' conformation that correlated with increasing ADAMTS13 activity. There was a significant association between decreased ADAMTS13 activity (<50%) and an open ADAMTS13 conformation. Is an open ADAMTS13 conformation a more sensitive biomarker for subclinical disease than is ADAMTS13 activity? Evidence in favour was presented with serial monitoring over six years of a patient with immune-mediated TTP. The patient was treated with pre-emptive rituximab whenever the activity decreased below 10%. An open conformation was noted just prior to every relapse and even when autoantibodies were mostly not detectable. This observation raises the possibility that open ADAMTS13 conformation is a sensitive biomarker for subclinical disease and monitoring it may permit better control of the disease.

Reference: Blood 2020;136(3):353-61
Abstract

A randomized double-blind trial of 3 aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia

Authors: Rocca B et al.

Summary: This Italian multicenter, randomised, double-blind trial, published in a recent edition of *Blood*, assessed various aspirin dosing schedules to optimise platelet cyclooxygenase-1 (COX-1) inhibition for the purpose of cardiovascular prophylaxis in patients with essential thrombocythemia (ET). A total of 245 patients with ET (median platelet count, 521×10^9/L; 59% JAK2-mutated; 4% prior ET-associated thrombosis) were randomised to one of three low-dose aspirin dosing arms for two-weeks: 100 mg once per day (n=85), 100 mg aspirin twice per day (n=75) or 100 mg aspirin three times per day (n=75). The primary surrogacy efficacy end point to assess platelet COX-1 activity was serum thromboxane B_2 ($sTXB_2$) change from randomisation to the end of the clinical trial. More frequent aspirin administration (both 12- and 8-hourly) resulted in improved antiplatelet responses compared to the once-daily regimen ($sTXB_2$ levels, 4 vs 2.5 vs 19.3 ng/mL, respectively), although the response was not significantly improved in the three-times per day regimen compared to the twice-daily dosing schedule. Safety, as assessed by urinary prostacyclin metabolite excretion was similar between arms. A higher abdominal discomfort score was observed in the cohort in the most frequent dosing arm.

Comment: Aspirin (75-100 mg) once daily is the most commonly used schedule in patients with myeloproliferative neoplasms and has been extrapolated from its use in non- myeloproliferative neoplasms subjects and a single trial in polycythemia vera. Aspirin exerts its antithrombotic and cardioprotective effects by irreversibly inhibiting platelet COX-1 and blocking TXA2 biosynthesis. Only 10% of the platelet pool is replenished every 24 hours in healthy individuals with almost complete suppression of TXB2 levels. However, there is increased platelet generation in ET, which results in accelerated renewal of COX-1 and once a day dose may lead to less complete COX-1 inhibition. In this study a comparison of enteric coated aspirin 100mg once a day was compared with twice daily dosing and a thrice daily schedule. The end points were laboratory assessments of TXA_2 metabolites in serum ($sTXB_2$) and urine (TXM) and urinary marker of protective prostaglandin I2 levels (PGIM; which should ideally be not suppressed). Almost 80 patients were included in each of the three arms and approximately 60% were on myelosuppression with median platelet count of 520×10^9/L. Data revealed enhanced COX-1 inhibition with the twice a day dose as compared to daily dosing with no further gain in three times a day schedule. Importantly, PGIM levels were not affected with twice daily and thrice daily dosing. Patients experienced symptoms of gastrointestinal disturbance more frequently with thrice daily dosing. There were no clinical endpoints reported but the twice daily dose will now be compared against daily dosing in the longer-term phase of the ARES (The Aspirin Regimens in Essential Thrombocythemia) study.

Reference: Blood 2020;136(2):171-82
Abstract

Thrombosis & Haemostasis Research Review™

Independent commentary by Prof Harshal Nandurkar

Professor 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).

