10th International Winter Meeting on Coagulation
Basic, Laboratory and Clinical Aspects of Venous and Arterial Thromboembolic Diseases
Bormio, April 10-16, 2011

With the auspices of
ALT (Associazione per la Lotta alla Trombosi e alle malattie cardiovascolari – Onlus)
FCSA (Federazione Centri per la diagnosi della trombosi e la Sorveglianza delle terapie Antitrombotiche)
SISET (Società Italiana per lo Studio della Emostasi e della Trombosi)

Scientific Committee
Armando D’Angelo (Milano, Italy),
Antonio Girolami (Padova, Italy),
Charles T. Esmon (Oklahoma City, OK, USA),
Marco Cattaneo (Milano, Italy),
Alexander Spyropoulos (Hamilton, ON, Canada)
Franco Piovella (Pavia, Italy)

Organizing Secretariat
N.L. Congressi s.r.l.
10th International Winter Meeting on Coagulation
Basic, Laboratory and Clinical Aspects of Venous and Arterial Thromboembolic Diseases
Bormio, April 10-16, 2011

TABLE OF CONTENTS

Primary Hemostasis I ........................................................................................................................1
Celebration Lecture .............................................................................................................................1
Primary Hemostasis II.......................................................................................................................2
Arterial Thrombosis ........................................................................................................................4
Mechanisms in Hemostasis and Thrombosis I.................................................................................5
Not Only Deep Vein Thrombosis ....................................................................................................6
Mechanisms in Hemostasis and Thrombosis II .............................................................................11
Cancer and Venous Thromboembolism .........................................................................................13
Mechanisms in Hemostasis and Thrombosis III ..........................................................................14
Perioperative Antithrombotic Management and Pulmonary Embolism ......................................16
Thromboprophylaxis in the Acutely-ill Medical Patient ..............................................................18

Index of authors.................................................................................................................................21
PRIMARY HEMOSTASIS I

CALCIUM SIGNALING AND NO PRODUCTION IN PLATELETS UNDER FLOW

L. De Marco
Centro Riferimento Oncologico, Aviano PN, Italy

Activation of platelets leads to an increase in the concentration of cytosolic calcium (Ca++) which is a major component of the signaling mechanism regulating platelet function. The source of Ca++ can be either intracellular or extracellular. Intracellular Ca++ is released from the sarcoplasmatic reticulum (SR) by inositol 1,4,5 triphosphate (IP3), the major mechanism for entry is store operated Ca++ entry (SOCE), a process controlled by the Ca++ concentration in the SR. Recently CalDAG-GEFI has been identified as a critical Ca++ sensor that links increases in intracellular Ca++ to integrin activation, TXA2 formation and granule release in stimulated platelets. In addition, two other molecules, STIM 1 and ORAI 1 have been identified as key regulators of store operated Ca++ entry. By using Fluo-3 AM labelled platelets, a flow chamber and a videoimaging apparatus, we have investigated platelet cytosolic Ca++ responses during shear dependent platelet adhesion and aggregation on different substrates and found that platelet receptors act synergistically in generating intracellular Ca++ signalling and that sustained Ca++ elevations are necessary to induce an effective and withstanding platelet adhesion and subsequent aggregation. By using a real time confocal microscopy equipped with a dual laser diode illumination and platelets labelled with both X-RHOD-1, AM and DAF-AM, for the simultaneous measurement of Ca++ signalling and NO production, we found that collagen-induced Ca++ elevations and platelet NO production depend on shear rates. In addition, we have observed that NO production is subsequently stimulated, after a lag time interval, by Ca++ oscillations. The functional implications of Ca++ mobilizing properties for NO production are currently under investigation.

CELEBRATION LECTURE

MYOCARDIAL INFARCTION IN HEMOPHILIA PATIENTS

A. Girolami
University of Padua Medical School, Department of Medical and Surgical Sciences, Padua, Italy

It was and still is commonly thought that clotting defects may protect from thrombotic events, both arterial and venous. During the last decade this assumption has undergone an extensive revision, specially for arterial thromboses. This was based on the observation that several cases of myocardial infarction and other acute coronary syndromes have been reported in hemophilia patients and also in other congenital hemorrhagic disorders. This has been attributed to the increasing age of Hemophilia patients thanks to the widespread use of FVIII or FIX concentrates. The role of known risk factors such as hypercholesterolemia, hypertension, diabetes, smoking, reduced physical exercise had to be re-evaluated and emphasized even in these patients. Substitution therapy may be dangerous in the presence of these common risk factors. At least about 60 patients with either Hemophilia A or B have been reported so far to have had a myocardial infarction. In addition several other patients have suffered from Unstable Angina and other coronary syndromes and undergone invasive re-canalization procedures. The need to use antiplatelet drugs in patients with a bleeding disorder has created several problems concerning the best approach to be followed. No final conclusion has been reached. The occurrence of myocardial infarctions, angina and other arterial diseases in Hemophilia patients casts doubts on the role of Factor VIII or Factor IX on atherogenesis. The protective effect seems more evident for venous thrombosis but is not absolute even in this case. The condition of the arterial wall seems crucial in the pathogenesis of coronary disease despite the existence of a clotting defect. The study of thrombotic events in patients with bleeding disorders represents a unique model for the understanding of the relation existing between vessels and circulating blood.
PRIMARY HEMOSTASIS II

INSIGHT OF MEGAKARYOCYTE-MATRIX INTERACTIONS IN THE BONE MARROW ENVIRONMENT

A. Balduini

Department of Biochemistry, University of Pavia, Pavia, Italy; and Department of Biomedical Engineering, Tufts University, Medford, Mass., USA

Background. The mechanisms by which megakaryocytes (Mks) proliferate, differentiate, and release platelets into circulation are not well understood. Mk maturation and platelet generation occur in the bone marrow and is consequent to Mk migration from the osteoblastic to the vascular niche, where Mks extend proplatelets and newly generated platelets can be released into the bloodstream. Growing evidence indicate that a complex regulatory mechanism, involving megakaryocyte-matrix interactions, may contribute to the quiescent or permissive microenvironment related to Mk differentiation and maturation within the bone marrow. It has been demonstrated that interactions of primary human Mks with matrices supposed to fill the vascular niche, such as fibrinogen or von Willebrand factor, is able to sustain Mk maturation and proplatelet formation, while type I collagen, in the osteoblastic niche, totally suppresses these events and prevents premature platelet release. The negative regulation of proplatelet formation by type I collagen is mediated by the interaction with integrin α2β1, and involves the Rho/ROCK pathway. Hypothesis. The dynamic interaction of Mks with different extra-cellular matrices, that fill the bone marrow spaces, may orchestrate their maturation in specific sites. Despite the improvement in knowledge of biochemical niche, little is known about the mechanical force that regulate Mk-niche interactions. Therefore, in this work, we correlated activation of signaling cascade with generation of contractile force to understand the influences of bone marrow environment on Mk function. Methodology/Principal Findings. To address this hypothesis, we first demonstrated that human Mks express and synthesize cellular fibronectin (cFN), with a predominance of the EDA isoform, and transglutaminase FXIII-A. Thereafter, we proposed that these two molecules are involved in a new regulatory mechanism of Mk-type I collagen interaction in the osteoblastic niche. We propose that Mk adhesion on type I collagen promotes Mk spreading through a mechanism that involves FN, membrane receptors and FXIII-A activity. This mechanism seemed to be mediated by the exposure of cFN to the cell membrane and maintained by FN polymerization catalyzed by FXIII-A. These data address a new role to FN that, upon specific activation, could be released and thereby modulate Mk interaction with extracellular matrices. In this context FXIII-A catalyzes FN cross-linking at cellular sites, stabilizes FN assembly and promotes the organization of extracellular matrix. Consistently, the same mechanism regulated the assembly of plasma FN (pFN) by adherent Mks to type I collagen. Most importantly, our results demonstrated that only Mks adherent to type I collagen, and not to fibrinogen, were able to promote FN assembly. As a result, we observed that Mk adhesion to type I collagen promoted Mk spreading over-time, while Mks on fibrinogen showed a shortened spreading that was replaced by proplatelet formation in sixteen hours of adhesion. Thus, FN assembly regulate the anchoring of Mks to type I collagen with consequent activation of biochemical signalling and generation of contractile force that may prevent proplatelet formation. Conclusion/Significance. In conclusion, this study provides important new elements in the understanding of the regulatory pathways for Mk-matrix interactions within bone marrow environment. In particular, our results demonstrate that fibronectins and FXIII-A modulate Mk spreading on type I collagen by promoting matrix assembly. This work opens new prospective in the study of illnesses, such as primary myelofibrosis or MYH9-related thrombocytopenia, related to defect of Mk-matrix interactions within the bone marrow environment, whose origin is still matter of debate.

NEUTROPHIL EXTRACELLULAR TRAPS GENERATION IS A REGULATED OUTCOME OF THE NEUTROPHIL/PLATELET INTERACTION

N. Maugeri,1 A. D’Angelo,1 A.A. Manfredi2

1Thrombosis Research Unit & 2Autoimmunity and Vascular Inflammation Unit. University Vita-Salute San Raffaele and San Raffaele Scientific Institute, Milan, Italy

As the first line of innate immune response, neutrophils are recruited at sites of infection and inflammation. They rely on at least three strategies to eliminate invading hosts, such as phagocytosis, secretion of antimicrobial proteases and the recently described formation of Neutrophil Extracellular Traps (NETs).1,2 NETs were initially considered as a protective mechanism involved in the innate response to pathogens. However, it is now accepted that NETs are also generated in response to sterile stimuli; in several acute or chronic inflammatory disorders aberrant NET formation and/or decreased NET degradation1 seem to correlate with the disease outcome.1 The presence of NETs was observed in patients with pre-eclampsia1,7 and in autoimmune small-vessel vasculitis,8 systemic lupus erythematosus.2,9 NETs formation may contribute to tissue damage associated with placental hypoxia,10 multiorgan failure in sepsis10 and even, possibly, to thrombus formation.10 Platelets act as intermediaries in the pathogenesis of sepsis-sensing bacteria and committing neutrophils to release fibrous traps that remove bacteria from the bloodstream. This response may also contribute to tissue injury, by a mechanism that depends on the platelet recruitment by neutrophil adherent to small capillaries and platelets recognition of the bactericidal surface molecule lipopolysaccharide through Toll-like receptor 4. The signals involved in the cross-talk between platelets and neutrophils have been only partially characterized. Platelets contain high mobility group B1 (HMGB1),10 a prototypic endogenous signal known to activate both leukocytes and endothelial cells,11,12 which acts at least partially through the TLR4 receptor. Here we charac-
sterize the mechanisms involved in the release of HMGB1 by activated platelets, the pathways involved in platelets’ ability to modulate neutrophil fate, inclusive of the activation state, degranulation, phagocytic potential and death, and eventually NET formation. The results indicate that this pathway may be involved in the thrombogenic action of neutrophils in human diseases.

References

PLATELET-ASSOCIATED TISSUE FACTOR: STILL AN ONGOING CONTROVERSY?
Dept. of Pharmacological Sciences, University of Milan and Centro Cardiologico Monzino IRCCS Milan, Italy

The first data demonstrating the presence of tissue factor (TF) in platelets date back to early 2000; nevertheless, the scientific community occasionally questions this evidence by publishing opposite findings. Indeed, if the dynamics of the studied events are not properly taken into account, this could bring to misleading results. Data from our and other groups support the evidence that platelets, upon activation, do express TF as a result of a rapid and dynamic process. Platelet activation plays a key role in atherothrombosis and coronary artery disease (CAD). We have recently shown that the levels of TF mRNA and protein in resting platelets are significantly higher in patients with non-ST elevation acute coronary syndrome (NSTE-ACS) than in patients with stable angina (SA) or in healthy subjects. This results in a higher thrombin generation capacity of platelets, which in turn may contribute to the prothrombotic phenotype of ACS. These findings further support the pro-thrombotic potential of platelets from NSTE-ACS patients, especially considering the platelet biosynthetic capacity. Recent findings highlighted that platelet activation, through the signalling events evoked by engagement of integrin α2bβ3 (GPⅡb/Ⅲa) or collagen receptor α2bβ1 (GPⅠa/Ⅰla) ligation, not only result in thrombus formation, but also lead to de novo protein synthesis through rapid and highly regulated translation of pre-existing megakaryocyte-derived mRNAs. This ability to perform new protein synthesis in response to cellular activation allows them to modify their phenotype and, as a consequence, to modulate their functions. Identification of disease-associated platelet-specific transcripts is of particular relevance in platelet pathophysiology, since it may lead to the discovery of novel therapeutic targets. We have recently completed a study aimed to prove the concept that platelets from patients with NSTE-ACS have differential mRNA expression profiles, in the hypothesis that this may influence their thrombogenicity. Microarray analysis identified transcripts with a significant ±2.0-fold difference in expression between NSTE-ACS and SA platelet pools. Thus, gene expression profiles at least partially discriminate unstable from stable CAD. Validation confirmed a significant over-expression of at least 3 genes in NSTE-ACS at both mRNA and protein level. Of note, the glycoprotein βIb-platelet-derived growth factor (GPⅠBB) was increased in NSTE-ACS also in comparison with healthy subjects. This study provides evidence that NSTE-ACS platelets are potentially preconditioned to a higher degree of reactivity on the transcriptional level. Our data suggest that a different composition of the mRNA pool might mediate an increased platelet prothrombotic potential in NSTE-ACS patients.
Several biomarkers, that have a pathophysiologic role in plaque instability and rupture were recently identified and proposed as soluble markers of vascular activation in acute coronary syndromes (ACS). These markers include cellular adhesion molecules (CAM) such P- and E-selectins, which are able to mediate the interaction between the endothelium and leukocytes – thus playing a crucial role in the localization of the inflammatory response – and others circulating pro-inflammatory molecules. Inflammatory processes are essential for atherogenesis, complication, and rupture of atherosclerotic plaque and pro-inflammatory molecules have been proposed as circulating markers of plaque instability. CD40 ligand (CD40L) is another of these potential candidates. This mediator is expressed by activated T helper lymphocytes, smooth muscle cells, macrophages, basophils and activated platelets. In the coronary atheroma and, particularly, in the shoulder region of the plaque, it interacts with CD40 receptors thus enhancing endothelial dysfunction and inflammation within the plaque. CD40L/CD40 binding is also able to induce procoagulant tissue factor and matrix metalloproteinase (MMP) synthesis within atherosclerotic lesions which, respectively, induce an increase in thrombogenicity of the plaque and in the degradation of the fibrous cap that separates the inner necrotic core of the lesion from circulating blood. The final step of this process is plaque rupture and thrombus formation within arterial lumen. Another molecule which can be potentially useful as soluble marker of plaque vulnerability is pregnancy-associated plasma protein A. This mediator is an MMP which is abundantly expressed in unstable but not in stable atherosclerotic lesions. Increasing circulating levels of all these substances have been shown in patients with ACS and for some of them a correlation was found with complex coronary lesions as demonstrated by angiography. Thrombospondin-1 is a glycoprotein which plays an important role in the pathogenesis of atherosclerosis. It impairs endothelial regeneration and promotes vascular smooth cell proliferation and migration. Elevated plasma levels of this substance have been observed in patients with ACS and its increase is associated with platelet activation. Finally, another protein, osteoprotegerin (OPG), has recently received much attention as potential soluble marker of atherosclerotic plaque activity. This substance is a soluble member of the TNF receptor super-family and acts by binding with high affinity to the TNF super-family member activator of NF-kB ligand and, by acting as neutralizing receptor, to another member of the TNF super-family, the TNF-related apoptosis inducing ligand (TRAIL). Recent studies demonstrated high circulating OPG levels in patients with ACS, with particularly increased concentrations in those who subsequently developed heart failure. These data suggest that OPG is a soluble biomarker of ACS and that high circulating levels of this substance might have a negative prognostic significance in ACS patients.

LIPOPROTEIN(A): READY TO TREATMENT?
D. Prisco, G. Degl’Innocenti, R. Marcucci
Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Careggi Hospital, Florence, Italy

Lipoprotein(a) was first discovered in 1963 by Berg and is essentially a cholesterol containing low-density lipoprotein particle covalently bound to a glycoprotein, Apo(a). Apo(a) is structurally very similar to plasminogen and has been shown to interfere with the activation and function of plasmin-inhibiting fibrinolysis. The structure of lipoprotein(a) suggests that it may be able to directly contribute to the development of atherosclerosis and thrombosis and, thus, to ischemic heart disease (IHD) and myocardial infarction (MI). Early cross-sectional studies very consistently demonstrated an association of elevated levels of lipoprotein(a) with risk of IHD and, more recently, with venous thromboembolism (VTE). Lipoprotein(a) levels are predominantly genetically determined and not greatly influenced by lifestyle factors and may vary up to 1000-fold between individuals. The LPA gene on chromosome 6 codes for the Apo(a) moiety of lipoprotein(a). Variation in this gene has a profound effect on plasma levels of lipoprotein(a). Of a particular importance is the so-called kringle IV type 2 (KIV-2) size polymorphism or copy-number variant defined by a 5.6-kb large sequence that may occur between two-and more than 40-times per allele, dependent on the specific genotype. This polymorphism determines the size of the expressed Apo(a) protein and explains at least 25% of the total variation in plasma concentration of lipoprotein(a) in the general population. Genetic studies of polymorphisms influencing levels of lipoprotein(a) and risk of IHD have provided a strong argument for a casual association of elevated levels of lipoprotein(a) with increase risk of IHD. However, final proof of causality in the form of randomized, controlled trials demonstrating that lowering of lipoprotein(a) levels leads to decreased risk of IHD or VTE are presently lacking. High dose of nicotinic acid are known to reduce levels of lipoprotein(a). Unfortunately nicotinic acid at high dose is not well tolerated, and also may not be effective enough in lowering levels of lipoprotein(a). Unfortunately nicotinic acid at high dose is not well tolerated, and also may not be effective enough in lowering levels of lipoprotein(a). Furthermore, a randomized controlled trial of the effect of lowering lipoprotein(a) levels by nicotinic acid on the risk of IHD would also not be easily interpreted as nicotinic acid has pleiotropic effect on the lipid profile. We need randomized, controlled trials to explore the benefits of lowering levels of lipoprotein(a).
MECHANISMS IN HEMOSTASIS AND THROMBOSIS I

REDUCING CONGENITAL BLEEDING AND COAGULOPATHY THROUGH SELECTIVE INHIBITION OF ACTIVATED PROTEIN C ANTICOAGULANT ACTIVITY

M. Cohen,1 J. Xu,2 C. Esmon2-4

1San Francisco Surgical Research Laboratory, University of California, San Francisco, CA; 2Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 3Howard Hughes Medical Institute, Oklahoma City, OK; 4Departments of Pathology and Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Activated protein C serves as a natural anticoagulant, but it has anti-inflammatory and cytoprotective activities and helps maintain vascular barrier function. Protein C is activated best in the microcirculation where thrombomodulin, a critical player in protein C activation, is at the highest concentration. In trauma, massive injury, especially of the microcirculation, leads to high systemic thrombin generation and the thrombin-thrombomodulin complex then activates protein C excessively. In trauma patients and experimental animals, protein C consumption and elevated levels of activated protein C are readily detected. This gives rise to an increase in the APTT. When endogenous APC is blocked in a mouse trauma model, the elevation in the APTT is prevented. Blockade of all activated protein C functions leads to death, but selective blockade of the anticoagulant activity while preserving the cytoprotective functions results in maintenance of organ function without the APTT elevation. As coagulopathy is a major cause of death in trauma, this approach may protect patients’ uncontrollable blood loss during surgical procedures to correct the traumatic injury. Hemophilia, especially patients with factor VIII inhibitory antibodies, is another area where blocking activated protein C may prove useful. Conceptually, as activated protein C is a major inhibitor of the prothrombin activation complex, inhibiting activated protein C should stabilize whatever activation complexes are formed and increase hemostatic potential. Indeed, based on the literature, patients with hemophilia who co-inherit factor V Leiden (APC resistance) appear to have decreased bleeding complications. Blockade of protein C is a much more effective method to inhibit the pathway than factor V Leiden. In mouse models, selective inhibition of activated protein C appears to improve hemostasis and may offer an additional approach to treating patients with coagulation inhibitors.

THE PROTECTIVE EFFECT OF ANTITHROMBIN THROUGH INTERACTION WITH HSPGS ON ENDOTHELIUM

A. Rezaie

St. Louis University School of Medicine, MS, USA

Antithrombin (AT, also called ATIII) is a serine protease inhibitor of the serpin superfamily which regulates the proteolytic activities of the procoagulant proteases of both intrinsic and extrinsic pathways. However, AT is a relatively weak inhibitor of coagulation proteases unless it binds to either pharmaceutical heparin used for prophylaxis and treatment of venous thrombosis or specific heparan sulfate proteoglycans (HSPGs) lining vascular endothelial cells. Heparin binds to a basic exosite of AT to induce a conformational change on the reactive center loop of the serpin, thereby facilitating its optimal interaction with the coagulation proteases. In addition to its essential anticoagulant function through the direct inhibition of procoagulant coagulation proteases, recent results have indicated that AT also possesses potent antiinflammatory properties when it binds to vessel wall HSPGs, thereby eliciting protective signaling responses in vascular endothelial cells. Numerous studies using different animal models have established a protective antiinflammatory role for AT in reducing mortality from severe sepsis. Nevertheless, it has been noted that the protective activity of AT requires very high doses, thus bleeding remains a serious drawback of the AT-therapy. To circumvent this problem, we have constructed AT mutants which exhibit highly reduced anticoagulant activities due to mutations either in the reactive center loop (RCL) or in the heparin-binding site. We have evaluated the protective signaling activities of these mutants in both cellular and animal models. In cellular models, both wild-type and an RCL mutant with no detectable reactivity with thrombin (AT/Proth-2) exhibited similar potent barrier protective activities in response to LPS and inhibited the adhesion of neutrophils to endothelial cells via inhibition of the NF-κB pathway. However, the mutants lacking ability to bind heparin (AT-K114E and AT-K125E) did not exhibit any protective activity in either one of these assays. Further studies revealed that the interaction of AT with syndecan-4 is required for the prostacyclin-dependent protective activity of the serpin through a pertussis-sensitive Gi-protein coupled receptor. The antiinflammatory and cardioprotective properties of wild-type and the non-anticoagulant AT/Proth-2 were also evaluated in a mouse left anterior descending coronary artery ischemia/reperfusion injury model. Both wild-type and AT/Proth-2 exhibited similar potent protective activities in this acute injury model. These results suggest that AT/Proth-2 may potentially be developed as a safer therapeutic drug for treating antiinflammatory disorders without increasing the risk of bleeding.
MATERNAL-FETAL HEMOSTASIS: MECHANISMS AND IMPLICATIONS

B. Brenner,1,2 Y. Nadir,1,2 A. Aharon
1Department of Hematology, Rambam Health Care Campus; 2Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

The placenta is a highly vascularized organ receiving blood supply from fetal and maternal circulations. Maternal blood flows in the inter-villous spaces, while fetal blood is confined in the intra-villous blood vessels. This may result in potential haemostatic problems, mainly the risk of hemorrhage or placental vascular complications. Low Molecular Weight Heparins (LMWHs) stimulate expression, synthesis and release of TFPI in endothelial cells and may exert their effect in pregnant women at risk for GVC, by modulating local hemostasis at the placental syncytiotrophoblast surface. Heparan sulphate proteoglycans are abundant in the extracellular matrix (ECM) of placenta and decidua. Heparanase up-regulates TF expression and interacts with TFPI on endothelial cells, resulting in increased cell surface coagulation activity. The regulatory effect of heparanase on TFPI and TFPI-2 in trophoblasts suggests a potential involvement of heparanase in early miscarriages. Microparticles (MPs) obtained from healthy pregnant women demonstrate high procoagulant activity compared to non-pregnant females. The procoagulant activity further increases in MPs of women with GVC without a change in the TF expression, but with reduction in TFPI. The balance between the procoagulant TF and the physiologic anticoagulants is crucial for pregnancy and influences the placenta, embryo and maternal cross talk. The placental haemostatic balance has a major role in normal pregnancy and the balance disruption may result in pregnancy complications.

NOT ONLY DEEP VEIN THROMBOSIS

TESTING FOR INHERITED THROMBOPHILIA AND PREDICTIVE VALUE FOR VENOUS THROMBOEMBOLISM

V. De Stefano, T. Za, A. Ciminello, S. Betti, E. Rossi
Institute of Hematology, Catholic University, Rome, Italy

Venous thromboembolism (VTE) susceptibility genes are present in 5 to 10% of the general population and in at least 40% of patients with VTE. An association with VTE has been firmly established for antithrombin (AT), protein C (PC), and protein S (PS) deficiency, as well as for factor V Leiden (FVL) and prothrombin (PT) 20210A. There is consistent evidence for a risk gradient for VTE, which is higher in carriers of AT, PC, PS deficiency and those homozygous or carriers of multiple defects, and moderate in heterozygous carriers of FVL or PT20210A. However, many experts consider testing for thrombophilia to be of little utility in the clinical management of the large majority of patients with VTE. The association of inherited thrombophilia with arterial thrombosis or obstetric complications has been reported to be weaker and equivocal such that that laboratory investigation in this setting is not warranted or should be conducted in selected patients. Despite such limitations, testing for inherited thrombophilia is common in clinical practice. A partial survey carried out in 2004 in Italy (60 million inhabitants) recorded 15,000 genetic tests each for FVL and PT20210A; in 2007 in Australia (20 million inhabitants) 20,378 genetic tests for FVL were recorded. In current practice, the reason of testing for inherited thrombophilia is VTE in 42% of the checked patients, arterial thrombosis in 15-23%, and an obstetric complication in 13-17%. Asymptomatic individuals account for 12-16% of testing because there is a known history of thrombophilia in a relative or there is a positive family history of VTE. Despite unanimous recommendation against indiscriminate screening, a number of women are tested prior to the prescription of oral contraceptives or hormone replacement therapy or before planning a pregnancy; in a survey conducted in a tertiary hospital, 15% of the young women tested for FVL were referred before prescribing oral contraception.

Testing for thrombophilia in patients with venous thromboembolism and consequences for secondary antithrombotic prophylaxis. After a first VTE the duration of secondary prophylaxis with oral anticoagulants (INR target 2 to 3) should be established weighing the risk for major hemorrhagic complications against the risk for a novel spontaneous VTE event. The risk of recurrent VTE is as high as 40% after 10 years from the first event, being low in patients having had VTE in association with circumstantial risk factors (surgery, trauma, pregnancy and puerperium, use of oral contraceptives) and maximal in patients with first spontaneous VTE. Prediction of recurrence should allow to select patients candidates to long-term (indefinite) duration of anticoagulation. Unfortunately the factors associated with a clinically relevant increase in risk for recurrence are not fully understo-
od so far, being the final likelihood the resultant of clinical circumstances, features of early treatment, genotypes, laboratory global phenotypes (such as D-Dimer assay), and clinical global phenotypes (such as vein recanalization); the complexity of interactions and differences in study methodologies generates discrepancies of results and uncertainty in making decisions on thrombophrophylaxis. Inherited thrombophilia has been reported to have little impact on the risk for recurrence in two prospective studies. As expected, in such investigations the most common gene polymorphisms associated with thrombophilia are FVL and PT20210A, present in nearly one third of the patients with VTE. Studies specifically aimed to investigate the risk for recurrence in carriers of either mutations gave conflicting results. The risk for recurrent VTE among heterozygous carriers of either FVL or PT20210A has been recently revised by at least three meta-analyses. The former estimated that patients with first VTE and FVL or PT20210A have a 1.4-fold or 1.7-fold significant increase in the risk of recurrence, respectively. In a second meta-analysis restricted to prospective studies, the risk for recurrent VTE conferred by heterozygous FVL was 1.4-fold increased, whereas the risk found among heterozygotes for PT20210A was lower. A more recent systematic review found that heterozygosity for FVL was associated with a 1.6-fold increase in risk for recurrent VTE in probands, whereas heterozygosity for PT20210A was not predictive of recurrence. However the magnitude of the risk is modest and the hemorrhagic risk related with indication for long-term anticoagulation could be not justified in the majority of cases. In a prospective cohort of 599 patients with first VTE inherited thrombophilia was associated with a 1.8-fold increase in risk for recurrence; measurement of D-dimer levels was demonstrated to identify among patients with inherited thrombophilia a subset with low risk for recurrence (4.2% after 1.4 years of follow-up in the presence of normal D-dimer levels) and a subset with high risk for recurrence (27.1% in the presence of altered D-dimer levels) with a hazard ratio of 8.3-fold in comparison with the subset with low risk. Those findings give evidence that thrombophilia can not be considered as a whole and that further efforts are needed to clarify the role of mild thrombophilia in the interaction with other predictors of recurrent VTE and to identify subsets of patients at higher risk for recurrence. Recent recommendations consider patients with AT, PC, or PS deficiency or multiple gene alterations not different from all the other patients with inherited thrombophilia as regards the duration of anticoagulant treatment. Yet it can be expected that in most studies the risk of recurrent VTE for the rare patients with deficiency of a natural anticoagulant is difficult to pick out since it is diluted by the weak effect of the much more frequent polymorphisms FVL and PT20210A. In a prospective cohort of unselected patients those with AT deficiency had a 2.6-fold increase in risk for recurrence, yet not significant likely for the low number of cases. In a retrospective controlled investigation we found that in the absence of anticoagulation AT deficiency is associated with a 1.9-fold significant increase in risk for recurrence in comparison with patients with no thrombophilia. Moreover in probands and their deficient relatives belonging to the EPCOT prospective cohort the incidence of recurrent VTE was 10.5% patient-years in patients with AT deficiency and 3.5% patient-years in carriers of FVL. In a retrospective investigation on proband patients with deficiency of natural anticoagulants and their deficient relatives the incidence of recurrent VTE was confirmed to be high, resulting 7.7% patient-years (10% for AT deficiency, 6% for PC deficiency, and 8.4% for PS deficiency). There is convincing evidence that patients with multiple defects are more prone to recurrent VTE. A retrospective study demonstrated that homozygotes for factor V Leiden show a higher risk for recurrent VTE than heterozygotes. In a systematic review homozygosity for FVL was estimated associated with a 2.6-fold increased risk for recurrent VTE. In conclusion, although the quality of the evidence in this area is low and does not allow firm recommendations, patients with AT deficiency, homozygosity for FVL, multiple defects, and perhaps PC or PS deficiency should be considered potential candidates for long-term oral anticoagulation after a first spontaneous VTE. It should be underlined that the conditions above listed are present in a not negligible portion of patients with VTE, being identifiable in at least 10% of them.

Testing for thrombophilia in asymptomatic individuals and consequences for primary antithrombotic prophylaxis. VTE is a common complex (multifactorial) disease, being the resultant of gene-gene and gene-environment interaction. Unfortunately, a simple model due to the presence or the absence of two dichotomous factors (high-risk allele and exposure to an environmental risk factor) is not reliable in most of the cases. This is due to incomplete clinical penetrance of genotypes, since not all carriers develop VTE during life, and to variable expressivity of severity and age of onset of the disease; moreover, the onset of disease is modulated also by gene-gene interactions, in the large majority of cases still obscure, and by multiple effects of various environmental risk factors, acting on the genotype by additive or multiplicative way. The above limitations render so far of little or null clinical utility indiscriminate genetic testing of populations for VTE-susceptibility genes and unlikely to compete for resources with other medical interventions. Universal screening before exposure to environmental risk circumstances such as oral contraceptive intake or pregnancy has been estimated not cost-effective too. Moreover, individuals labeled as carriers by random screening could experience insurance discrimination or feel undue anxiety receiving no real benefit in terms of prevention. In conclusion, general population screening is discouraged because of doubtful utility and potential detrimental effect on the carriers. Targeted screening in the siblings of the index patients with VTE is obviously more fruitful than in the general population, with a diagnostic yield of 50%, being such traits genetically dominant. The primary argument for screening asymptomatic relatives of patients with thrombophilia is the possibility of reducing the occurrence of provoked VTE by offering advice concerning primary antithrombotic prevention during circumstances that could potentially lead to VTE and that are not usually covered with prophylaxis (e.g. low-risk surgery or pregnancy and puerperium), and counseling...
carrier women about the use of hormone therapies. However, this type of counseling should be weighed against potential detrimental effects in the carriers, such as emotional burden due to an overestimated perception of risk. The presence of a family history of VTE may be a way to engage in the targeted case-finding of carrier relatives who may be at higher risk. In fact, family history of VTE has been consistently reported to be a risk factor for VTE independent of the presence of known thrombophilic abnormalities. Moreover, the carriers of thrombophilia with a family history of VTE have been reported to be more prone to VTE than those without. Several family studies have investigated the risk for VTE among relatives of individuals with inherited thrombophilia. In both prospective and retrospective studies, the incidence of VTE among relatives was higher in carriers of AT, PC, or PS deficiency, with a range between 0.36 and 4.0% individual-years. The highest incidence was consistently observed among carriers of AT deficiency, with 1.0 to 4.0% individual-years. In studies using unaffected relatives as the reference group, the risk for VTE among carriers of AT, PC, or PS deficiency was 4 to 30 times greater than that in non-carriers. On the other hand, a lower incidence of VTE was reported among the relatives of FVL and PT20210A, consisting of 0.19 to 0.58% individual-years for FVL, and between 0.11 and 0.37% individual-years for PT20210A. The low absolute incidence of VTE reported in relatives of patients with FVL or PT20210A has prompted many experts to consider familial screening for inherited thrombophilia to be unwarrented in this setting, as it is without high clinical utility. However, it should be kept in mind that among the relatives of probands, some asymptomatic individuals could be carriers of multiple abnormalities and, therefore, could receive a benefit from diagnosis.

References


THE ROLE OF FAMILY PHYSICIANS IN THE PREVENTION OF VENOUS THROMBOEMBOLISM

P. Prandoni
University of Padua, Italy

As patients are on average discharged from the hospital much earlier than in the past and are often not hospitalized at all, family physicians are expected to play an increasing role in the management of venous thromboembolism. Once patients are discharged from orthopedic departments, family physicians are expected to prolong thromboprophylaxis at home for a few additional weeks at least in those who have undergone elective or emergency hip surgery, even if this conduct has not been recommended by specialists. The same is true of selected cancer patients who have undergone major abdominal or pelvic surgery. Family physicians are expected to implement prophylaxis in medical patients at high-risk of VTE who are not hospitalized. They should not prolong prophylaxis beyond the period of acute medical disease in the large majority of medical patients. Family physicians are expected to administer VTE prophylaxis in patients with lung or pancreas cancer while undergoing chemotherapy if they do not have contraindications to anti-thrombotic drugs; in patients with other types of cancer the Khorana score can be used to identify those requiring prophylaxis. They should not administer LMWH for prolonging survival in cancer patients. Family physicians are expected to administer prophylaxis in a number of situations where guidelines offer no or weak recommendations whenever the personal and/or the family history of patients suggest that this is the case, for example in pregnant women, in the case of leg injuries below the knee, injuries of the arm and other minor risk factors, such as long travels by plane or coach, knee arthroscopy for surgical or non-surgical purposes, laparoscopic surgery, day-
surgery for minor indications, ligation or stripping of superficial leg veins). Finally, family physicians are expected to dissuade their patients from adopting measures that have been incorrectly recommended by specialists.

ANTIPATELET TREATMENT COMBINED WITH OLD OR NEW ORAL ANTICOAGULANT DRUGS: NEED FOR AN EVIDENCE-BASED APPROACH

M. Moia
Angelo Bianchi Bonommi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Milan, Italy

Oral anticoagulants and antiplatelets are used in the treatment and prevention of thromboembolic diseases. For more than 50 years, vitamin K antagonists (VKA) have been the only available oral anticoagulants, but new oral anticoagulant drugs will soon be available. Aspirin and thienopyridines are the currently used antiplatelet drugs, but new molecules are coming. VKA are used in the primary and secondary prevention of venous thromboembolism, in the prophylaxis of cardioembolic events in patients with mechanical or biological prosthetic heart valves, in patients with atrial fibrillation, and in ischemic myocardial diseases. Antiplatelets are used mainly to prevent and/or to treat myocardial infarction, stroke, and peripheral arterial diseases. Combined anticoagulant–antiplatelet treatment is often attempted in order to increase the efficacy of the antithrombotic treatment. However, for both antiplatelets and antiplatelets the efficacy cannot be dissociated from an increased risk of bleeding. Overall, the evidence of benefit of combined treatment is limited and the increased risk of bleeding is proven. Therefore, the combination of these drugs should be considered with caution. A critical review of the available studies suggests that the indications for combined anticoagulant–antiplatelet treatment are limited. VKA and aspirin is the only combined anticoagulant–antiplatelet treatment which was extensively investigated. There is evidence that this association increases the risk of bleeding. The benefit was proven only in (some) patients with mechanical prosthetic heart valves or with coronary artery disease, and also among these patients a selection is recommended in order to assess the individual risk–benefit profile. However, in clinical practice an aggressive therapeutic approach seems currently to be more attractive and popular than an evidence-based one, so that combined anticoagulant and antiplatelet treatment is frequently prescribed. An example is atrial fibrillation. Even if no clear advantage of the combined treatment has been shown in patients with atrial fibrillation, the proportion of patients treated also with aspirin enrolled in phase III trials of new anticoagulants, vs warfarin, has progressively increased in the last ten years. Antiplatelet drugs different from aspirin, namely clopidogrel, could cause even higher risk of severe bleeding when combined with VKA, as shown in a recent retrospective analysis of a nationwide registry in Denmark. New anticoagulants may offer a better safety profile than warfarin, when combined with aspirin. For example, preliminary data suggest that combined treatment with dabigatran and aspirin would not significantly increase the risk of major bleeding, if compared with dabigatran alone. However, this hypothesis needs to be carefully tested within specific studies with different anticoagulant and/or antiplatelet drugs. Waiting for these studies, a more careful evidence-based approach in prescribing combined anticoagulant-antiplatelet treatment is advisable.

HEPARINS AND BLEEDING COMPLICATIONS: SIZE OF THE PROBLEM AND RISK DETERMINANTS

M. Pini, C. Pattacini, D. Dazzi
Medicina II, Ospedale di Fidenza, Italy

Background. Prompted by the occurrence of several major bleeding episodes observed in our patients with venous thromboembolism (VTE) or acute coronary syndrome (ACS) treated with anticoagulant drugs and in particular with therapeutic doses of low-molecular-weight heparin (LMWH), we checked if bleeding rates reported in observational studies - that more closely reflect what is going on in the real world setting - were higher than the ones reported in randomized clinical trials (RCT), and which were the main determinants of bleeding risk. Methods. We made a medline search for recent large observational studies and RCT reporting on hemorrhagic complications of anticoagulant therapy in patients with VTE and ACS, and compared the cumulative major bleeding rates observed in the two types of clinical studies. Reports on determinants of bleeding risk were also assessed. The chi square test was used for statistical analysis. Results. In patients with VTE treated with heparin/LMWH and vitamin K antagonists, the rate of major bleeding reported in the Worcester Venous Thromboembolism study was > 6%, while it was 3% in the RIETE Registry and about 1.5% in recent RCT. In patients with ACS, although a reliable comparison is hampered by the different criteria for major bleeding and by the different observation period in the various studies, on average the bleeding rate was much lower in the RCT than in the Registries (odds ratio 0.40 [0.39-0.42], p <0.0001). Major bleeding correlates with an increase risk of death in a number of studies, and determinants of bleeding risk include baseline risk factors – among which impaired renal function, anemia, female sex, and advanced age emerge for consistency in the statistical models developed in the various studies – and treatment-related factors which differ between VTE and ACS patients. In patients with venous thromboembolism, fixed dose LMWH appears to be safer, as well as more effective, than adjusted dose unfractionated heparin (UFH), while in patients with ACS, treatment related factors include excessive heparin/LMWH dosage, LMWH versus UFH, heparin/LMWH versus bivalirudin, and enoxaparin versus prophylactic dose fondaparinux. Convincing evidence exists from observational and randomized studies that enoxaparin dosage should be halved in patients with estimated glomerular filtration rate < 30 ml per minute and that fon-
daparinux 2.5 mg once daily has a better efficacy/safety profile than enoxaparin in patients with ACS. Conclusions. In the real world setting, the risk of bleeding associated with anticoagulant treatment, and particularly with heparin/LMWH given in therapeutic dosage, is much higher than the one reported in RCT, both in patients with VTE and ACS. Established bleeding risk factors such as impaired renal function, anemia, female sex, and advanced age should be considered when heparin/LMWH dosage is chosen, especially in patients with ACS, who also receive other antithrombotic drugs and often undergo invasive procedures.

MECHANISMS IN HEMOSTASIS AND THROMBOSIS II

SEPSIS AND CELLULAR TRAUMA LEAD TO HISTONE MEDIATED ORGAN FAILURE AND DEATH


Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; Howard Hughes Medical Institute, Oklahoma City, OK; Departments of Pathology and Biochemistry & Molecular Biology, Oklahoma City, OK; San Francisco Surgical Research Laboratory, University of California, San Francisco, CA; Division of Transplantation, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Nucleosomes containing histones are released in severe sepsis and trauma. These are quite toxic and can lead to direct tissue injury, trigger platelet activation leading to thrombosis, stimulate inflammatory cytokine production, and lead to leukocyte deposition in the tissues. In endotoxin, tumor necrosis factor or cecal ligation and puncture models of SIRS/sepsis, inhibiting the cytotoxic activity of histones protected against organ failure and death. Reperfusion injury and chemical toxicity also lead to histone release. The organ injury that ensues is blocked when the histones’ cytotoxicity is inhibited. Examination of the underlying mechanisms indicate that the histones trigger several of the toll like receptors resulting in the inflammatory cascade and tissue injury. Not surprisingly, histones are also released in trauma at levels similar to those seen in other models and are therefore likely to contribute to the inflammatory response seen in sterile trauma. These results suggest that histones play a major role in the pathogenesis of many serious disease processes.

THE ROLE OF HDL IN REGULATION OF BLOOD COAGULATION

B. Dahlbäck

Lund University, Dept. Laboratory Medicine, Wallenberg laboratory, University Hospital, Malmo, Sweden

The plasma levels of HDL cholesterol correlate inversely with the risk of coronary artery disease, and HDL has several anti-atherogenic and endothelium protective functions. It is a key component in reverse cholesterol transport, it has anti-inflammatory effects and it is important for the integrity of the endothelial barrier function. HDL has also been suggested to have anticoagulant properties, functioning as a cofactor to activated protein C (APC) in the degradation of factor Va (FVa). The primary aim for our study was to elucidate whether there are additional anticoagulant activities associated with HDL. Our results, suggesting an anticoagulant anionic phospholipids scavenging function of HDL, prompted us to re-evaluate the role of HDL as cofactor to APC. Blood coagulation invol-
ves a series of enzymatic protein complexes that assemble on the surface of anionic phospholipids. To investigate whether apolipoproteins, isolated from HDL, VLDL, or LDL, affect these reactions, they were incorporated into anionic liposomes using a detergent solubilization-dialysis method. Apolipoprotein A-I (apoA-I), a major component of HDL, had a pronounced anticoagulant effect, the anionic phospholipids losing their procoagulant properties when the liposomes were prepared in the presence of apoA-I. The phospholipid-apoA-I liposomes were 8-10 nm in diameter and contained around 60-80 phospholipid molecules, thus being similar to reconstituted HDL (rHDL). These liposomes/rHDL particles were unable to support the activation of prothrombin by FX in the presence of FVa. In a Biacore analysis, they were shown to be unable to bind FVa, whereas both prothrombin and FXa could bind. The inability of the liposomes/rHDL to bind FVa, and support the prothrombinase complex, was due to the small surface area of exposed anionic phospholipids. To investigate whether HDL in blood can act as an anticoagulant by incorporating the anionic phospholipid, procoagulant anionic liposomes were added to serum and their procoagulant activity followed over time. The procoagulant activity of the liposomes was lost in a time-dependent manner due to transfer of the anionic phospholipids to HDL, and to a lesser extent to LDL. The phospholipid transfer protein (PLTP), which is present in plasma, was crucial for this transfer. These results demonstrated that apoA-I was able to neutralize the procoagulant activity of anionic phospholipids by arranging the phospholipids in surface areas that are too small to accommodate the prothrombinase complex, similarly to what is described for nanodiscs, which are created by a recombinantly engineered apoA-I and anionic phospholipids. The anionic phospholipid scavenger function of HDL and PLTP may be an important mechanism to control the exposure of such phospholipids to circulating blood and thereby prevent inappropriate stimulation of blood coagulation. The observation that HDL was unable to support assembly of the prothrombinase complex raised doubts regarding the role of HDL as a cofactor to APC in the degradation of FVa and we therefore re-evaluated these effects. In agreement with published data, HDL isolated by ultracentrifugation stimulated APC-mediated degradation of FVa. However, further purification of HDL by gel filtration revealed that the stimulating activity was not a property of HDL. Instead, the stimulating activity eluted separated from HDL in the high molecular weight void volume fractions. These fractions were also able to stimulate FVa degradation by APC and were furthermore found to be able to support the assembly of FXa and FVa into a functional prothrombinase complex. Both the procoagulant and anticoagulant activities were blocked by the addition of annexin V, suggesting that the active principle was negatively charged phospholipid membranes. These results demonstrate that HDL does not stimulate APC/protein S and that the activity previously reported to be a property of HDL rather is caused by contaminating negatively charged phospholipid membranes, possibly microparticles. In conclusion, we demonstrate a novel anticoagulant activity of HDL functioning as a scavenger of anionic phospholipids; the surface area of HDL associated phospholipids being too small to accommodate the prothrombinase reactions. Moreover, we show that the previously claimed APC cofactor function of HDL is not a property of HDL but caused by large anionic phospholipid vesicles contaminating HDL preparations prepared by ultracentrifugation.

References


HOW VITAMIN K-DEPENDENT CLOTTING PROTEINS BIND TO MEMBRANES

J.H. Morrissey
Biochemistry Department, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Almost every step in the blood clotting cascade takes place on membranes with exposed phosphatidylserine (PS). The membrane requirement for clotting reactions is underscored by the fact that releasing blood clotting proteins from membrane surfaces renders them thousands of times less active. The most common membrane-binding motifs in blood clotting are GLA domains, so-called because they contain multiple gamma-carboxyglutamate (Gla) residues. In spite of the critical importance of protein-membrane interactions in blood clotting, a clear picture has yet to emerge of how GLA domains – or indeed, any blood clotting proteins – bind to phospholipid bilayers. We are employing multidisciplinary approaches to provide new insights, at atomic-resolution, into how blood clotting protein complexes assemble and function on membrane surfaces.

EPCR CHANGES THE PAR-DEPENDENT SIGNALING SPECIFICITY OF COAGULATION PROTEASES

A. Rezaie
St. Louis University School of Medicine, MS, USA

We recently demonstrated that the occupancy of endothelial protein C receptor (EPCR) by its natural ligand activated protein C (APC)/protein C switches the protease-activated receptor 1 (PAR-1)-dependent signaling specifi-
city of thrombin from a disruptive to a protective response in cultured human umbilical vein endothelial cells. Thus, the activation of PAR-1 by either thrombin or PAR-1 agonist peptide elicited a barrier protective response if endothelial cells were pre-incubated with protein C. Further studies revealed that EPCR is associated with caveolin-1 in the lipid rafts, however, the occupancy of EPCR by the Gla domain of APC/protein C leads to dissociation of EPCR from caveolin-1 and the recruitment of PAR-1 to a protective signaling pathway. Other recent studies have demonstrated that the activation of PAR-2 by factor Xa on endothelial cells also initiates protective intracellular signaling responses in endothelial cells. To further investigate the PAR-dependent protective activity of coagulation proteases, we decided to examine whether the Gla domain of factor X and other vitamin K-dependent coagulation protease zymogens can modulate PAR-dependent signaling responses in endothelial cells. We discovered that the activation of both PAR-1 and PAR-2 in endothelial cells pretreated with factor FX (FX) or the catalytically inactive FX-S195A, but not with other procoagulant protease zymogens, also results in initiation of protective intracellular responses in endothelial cells. Interestingly, we noted that similar to protein C, FX interaction with endothelial cells also leads to dissociation of EPCR from caveolin-1 and recruitment of PAR-1 to a protective pathway. However, unlike protein C, the Gla domain of FX was not required for its protective activity since a Gla domain deletion mutant of FX also elicited a protective response. Further studies revealed that, FX activated by factor VIIa on tissue factor bearing endothelial cells, also initiates protective signaling responses through the activation of PAR-2 independent of EPCR mobilization. All results could be recapitulated by the receptor agonist peptides to both PAR-1 and PAR-2. These results suggest that a crosstalk between EPCR and an unknown FX/FXa receptor, which does not require interaction with the Gla domain of FX, recruits PAR-1 to protective signaling pathways in endothelial cells. Our results appear to suggest that the activation of both PAR-1 and PAR-2 by coagulation proteases elicits only protective responses in healthy vasculature expressing EPCR.

CANCER AND VENOUS THROMBOEMBOLISM

VENOUS THROMBOEMBOLISM TREATMENT IN CANCER PATIENTS

D. Imberti

Internal Medicine Department, University Hospital of Ferrara, Italy

Venous thromboembolic disease (VTE) is a frequent complication in cancer patients, and represent an important cause of morbidity and mortality. Several studies have recently shown that the presence of malignancy increases the risk of thrombosis to four to six times than in the general population and that the survival of patients with both cancer and VTE is less than that of patients with cancer or VTE alone. The optimal treatment of VTE in patients with malignancy is thus a problem of paramount clinical importance, and differs from the treatment of VTE in the general population for a number of issues. Firstly, there is convincing evidence that demonstrates how antithrombotic treatment is less effective and less safe in such patients than in patients without cancer, since the higher incidence of recurrences and haemorrhagic complications. It is also important to note that in cancer patients the optimal antithrombotic treatment should provide a good quality of life, which is often already compromised, particularly when the cancer is advanced. Moreover, there are still a number of uncertainties about some particular aspects of antithrombotic treatment in patients with both cancer and VTE is less than that of patients in the general population and that the survival of patients increases the risk of thrombosis to four to six times than have recently shown that the presence of malignancy.

CATCH – A RANDOMISED CLINICAL TRIAL COMPARING LONG-TERM TINzaparin VERSUS WARFARIN FOR TREATMENT OF ACUTE VENOUS THROMBOEMBOLISM IN CANCER PATIENTS

R. Bauersachs on behalf of the CATCH investigators

Department of Vascular Medicine, Klinikum Darmstadt GmbH, Darmstadt, Germany

Background: VTE is a major cause of morbidity and mortality in cancer patients. LMWH have been shown to be superior to warfarin in one randomized study, but adequately powered confirmatory studies have not been conducted and warfarin continues to be widely used for treatment of cancer-associated VTE. Methods: We are con-
ducting a randomized clinical trial of tinzaparin versus warfarin in 900 patients with active cancer and symptomatic proximal deep vein thrombosis (DVT) and/or symptomatic proximal pulmonary embolism (PE). Tinzaparin will be administered at full treatment doses (175 IU/kg once daily) for the duration of the study. The control arm will have initial tinzaparin treatment for 5–10 days, and warfarin will be dose adjusted to a target INR level of 2.0–3.0. The primary composite outcome is time to recurrent VTE event per standard diagnostic criteria, and will include incidentally (asymptomatic) diagnosed VTE as well as fatal PE. At randomisation, a scan of legs and lungs will establish the baseline status. Results. The trial is being conducted in Europe, Asia and South America in 26 countries with participation of approximately 160 sites (NCT01130025). As of March 18, 2011 105 sites were activated for study enrolment and 33 sites had initiated enrolment. 59 patients have been enrolled. A series of other baseline characteristics will be captured and analysed for their ability individually or combined to predict the risk for recurrent VTE or bleeding. In particular, the parameters of the Khorana scale and Wells rule will be tested for their usefulness in predicting recurrent VTE. In addition, predictive biomarkers will be tested including D-dimer and Tissue Factor. Presence of post-thrombotic syndrome (PTS) will be evaluated. Evaluation of quality of life and capture of healthcare resource utilization will also be performed on a monthly basis. Conclusions. The results obtained from this study will add significantly to the knowledge not only on the efficacy of LMWH to treat and secondarily to prevent recurrent VTE. Important prospective data on the clinical significance of incidental VTE in patients with active cancer will be generated, and analyses of risk stratification parameters will add important supportive knowledge. The study of PTS, which has not previously been done in this selected patient population, will add to the evidence that tinzaparin significantly reduces the incidence of PTS and leg ulcers (Hull et al, 2009).

MECHANISMS IN HEMOSTASIS AND THROMBOSIS III

WHAT MAKES AN ANTICOAGULANT A GOOD PROFIBRINOLYTIC AGENT

M. Colucci

Department of Biomedical Sciences, University “Aldo Moro”, Bari, Italy

The intensity and duration of thrombin generation is one of the major determinants of clot resistance to fibrinolysis. The higher the concentration of thrombin at the time of fibrin formation and assembly the denser and the more resistant the clot. Moreover, the amount of thrombin generated after fibrin has formed dictates the extent and duration of TAFI activation, a plasma carboxypeptidase that inhibits fibrinolysis by removing the plasminogen binding sites on partially degraded fibrin. For these reasons, the drugs inhibiting thrombin formation and/or activity are expected to promote fibrinolysis by enhancing the clot susceptibility to lysis. There are, however, striking differences in the capability of anticoagulants to stimulate fibrinolysis, at least in in vitro models of clot lysis. Some of the factors shaping the profibrinolytic activity of anticoagulants are the following.

How the anticoagulant affects thrombin generation. To be effective an anticoagulant must be capable to keep thrombin formation in check at the right moment and with the right efficiency. In tissue factor-activated plasma or blood, drugs that delays the lag time of thrombin formation but have little effect on the peak thrombin level should be pointed out, however, that the different behavior of hirudin and fondaparinux does not depend on their target enzyme (thrombin and Factor Xa, respectively), given that other thrombin inhibitors, such as inogatran, dabigatran and argatroban, are strong profibrinolytic agents and affect thrombin generation in a totally different way as compared to hirudin.

Presence and concentration of thrombomodulin. Thrombomodulin (TM) may have different effects on fibrinolysis depending on whether it will favour the activation of TAFI or protein C. There is evidence that low concentrations of TM inhibit fibrinolysis whereas high concentrations stimulate it, and that some anticoagulants (e.g. inogatran and dabigatran) lose their profibrinolytic activity or may even become anti-fibrinolytic when tested in the presence of high TM concentrations (≥ 10 mM). This paradoxical effect appears to be due to a more sustained activation of TAFI which may result either from the slow release of thrombin from the drug-thrombin complex (to equilibrate the slow removal of free thrombin by anti-
thrombin) or from the inhibition of protein C activation by thrombin/TM. Whatever the mechanism, if the same holds true with endothelial-bound TM, the profibrinolytic activity of some anticoagulant drugs may be lost in the microcirculation, where the TM concentration may be higher than 100 nM. 3. Presence of platelets. Platelets enhance clot resistance to lysis in different ways and may heavily interfere with the profibrinolytic activity of some anticoagulants. The typical example is heparin whose activity decreases as the concentration of platelets rises. Two different mechanisms have been proposed to explain this phenomenon: the release of PF4 (anti-heparin factor), and the protection of platelet-bound clotting enzymes towards antithrombin-heparin. Interestingly, platelets have a greater impact on the profibrinolytic than on the anticoagulant activity of heparin. In platelet-rich samples, indeed, heparin fails to stimulate fibrinolysis even at concentrations displaying a clear anticoagulant effect. As expected, the anticoagulants that are insensitive to PF4 and inhibit fairly well platelet-bound clotting enzymes retain most of their profibrinolytic activity in the presence of platelets.

**ISOPROSTANES AND PLATELET ACTIVATION**


*Equally contributed to the study

|Clinica Medica, University of Rome “Sapienza”, Italy; |Department of Internal Medicine, University of Rome “Tor Vergata”, Rome, Italy |

Platelets play a key role in the process of athero-thrombosis via release of inflammatory and pro-thrombotic molecules. Among the activating agonists derived from the metabolism of arachidonic acid, platelet release thromboxane A2 and F2-isoprostanes. 8-iso-PGF2 (PGF2 -III) is a chemically stable compound derived from non-enzymatic oxidation of arachidonic acid whereas Thromboxane A2 derived from the activation of Cyclooxygenase and it is rapidly converted in the inactive metabolite thromboxane B2. We hypothesized that NADPH is implicated in isoprostane formation and in turn in platelet activation. We studied PGF2 -III in platelets from 8 male patients with hereditary deficiency of gp91phox, the catalytic subunit of NADPH oxidase, and 8 male controls. Upon stimulation platelets from controls produced PGF2 -III, that was inhibited -8% by aspirin and -63% by a specific inhibitor of gp91phox (gp91phoxds-tat). Platelets from patients with gp91phox hereditary deficiency had normal thromboxane A2 formation but marked PGF2 -III reduction compared to controls. In normal platelets incubated with the gp91phox inhibitor gp91phoxds-tat or with SQ29548, a thromboxaneA2/Isoprostanes receptors inhibitor, platelet recruitment, an in vitro model of thrombus growth, was reduced -50% and -75% respectively; a lower effect (-17%) was seen with aspirin. In gp91phox deficient patients agonist-induced platelet aggregation was within the normal range while platelet recruitment was reduced compared to controls. Incubation of platelets from X-CGD with PGF2 -III dose-dependently (1-100 pmol/L) increased platelet recruitment by mobilizing platelet Ca2+ and activating gpIIb/IIIa; a further increase of platelet recruitment was detected by platelet co-incubation with L-NAME, an inhibitor of NO synthase. This study provides the first evidence that platelet PGF2 -III maximally derives from gp91phox activation and contributes to platelet recruitment via activation of gpIIb/IIIa.

**A HAPLOTYPE WITHIN THE ANNEXIN A5 GENE PREDISPOSE TO GESTATIONAL VASCULAR COMPLICATIONS AND PREGNANCY-RELATED VENOUS THROMBOEMBOLISM**

G.L. Tiscia, D. Colaizzo, L. Fischetti, F. Cappucci, M. Margaglione, E. Grandone

I.R.C.C.S. Casa Sollievo della Sofferenza, S. Giovanni Rotondo, University of Foggia, Italy

Gestational vascular complications (GVC) such as early recurrent fetal loss (ERFL), late fetal loss (LFL), preeclampsia (PE) and fetal growth restriction (FGR) may be a consequence of an utero-placental thrombosis. Pregnancy represents an acquired hypercoagulable state and is itself a risk factor not only for GVC but also, at any gestational age and especially after delivery, for the venous thromboembolism (VTE). Inherited thrombophilias (FV Leiden, Prothrombin mutation, PTm), increase the risk for GVC and VTE. However, many events still remain “unexplained”. Since 1987, the annexin A5 (ANXA5) protein is known to have anti-coagulant properties. Recently, a haplotype (M2) within gene promoter of ANXA5 has been reported as likely responsible of protein level reduction and to be significantly prevalent among 80 German women with a history of ERFL. We investigated the M2 haplotype in two groups of Italian women: - patients with a history of GVC and - patients with pregnancy-related VTE. 398 cases (103 with EFL, 54 LFL, 158 PE, 83 VTE) and 195 controls admitted at our Unit for a thrombophilia screening were investigated. Acquired and genetic thrombophilias was investigated for every individual we have enrolled. Logistic regression (LogR) correcting for age, gravidity, parity and thrombophilias were performed for calculating adjusted OR and 95% CI. The M2 haplotype was found in 30 controls (15%), in 35 women (34%) with ERFL (chi-squared test [2]: p=0.047; OR: 3.1; 95% CI: 1.1-9.5) in 46 (29%) with PE (2: p=0.008; OR: 2.1; 95% CI: 1.2-3.5), in 8 (15%) with LFL (2: p=0.05) and in 27 (32.5%) with pregnancy-related VTE (2: p<0.001; OR: 3.4; 95% CI: 1.7-6.7). In a small sub-group (n= 46 out of 158, 29%) of women with PE, a FGR episode was recorded; among them, 23 (50%) carried the M2 haplotype. The M2 haplotype seems to be a new and relevant susceptibility factor for pregnancy-related VTE as well as for all investigated GVC, except for LFL.
POLYPHOSPHATE: AN ANCIENT MOLECULE AT THE NEXUS OF INFLAMMATION AND BLOOD COAGULATION

J.H. Morrissey
Biochemistry Department, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Polyphosphates are highly anionic polymers of inorganic phosphates linked by phosphoanhydride bonds. Polyphosphate is widely distributed in biology and is known to accumulate in large quantities in many infectious microorganisms. Furthermore, polyphosphate is abundant in dense granules of human platelets and is secreted following platelet activation. We recently showed that polyphosphate is a potent modulator of blood clotting, acting at three points in the clotting cascade: 1) it triggers the contact pathway of blood clotting and may be the long-sought (patho)physiologic activator of this pathway; 2) it accelerates factor V activation and may help explain the enhanced activity of platelet factor V; and 3) it is incorporated into fibrin clots, resulting in thicker fibrin fibrils with increased resistance to fibrinolysis. We have also recently shown that polyphosphate is a potent pro-thrombotic and pro-inflammatory mediator in vivo, using mouse models. And finally, we have recently shown that polyphosphate polymer length differentially influences its ability to modulate blood clotting at the three steps outlined above; in particular, efficient triggering of the contact pathway of blood clotting requires very long polyphosphate polymers – of the size that accumulates in microorganisms. Taken together, these studies show that polyphosphate derived from human platelets or infectious microorganisms can be strongly pro-hemostatic, pro-thrombotic and pro-inflammatory, depending on the polyphosphate source and route of administration.

PERIOPERATIVE ANTITHROMBOTIC MANAGEMENT AND PULMONARY EMBOLISM

PERIOPERATIVE HEPARIN BRIDGING IN PATIENTS RECEIVING ORAL ANTICOAGULATION: META-ANALYSIS OF BLEEDING AND THROMBOEMBOLIC RATES

D. Siegel, J. Yudin, J.D. Douketis, A.C. Spyropoulos
McMaster University, Hamilton, ON, Canada

Background. Periprocedural bridging using unfractionated heparin (UFH) or low molecular weight heparin (LMWH) in patients receiving chronic oral anticoagulation (OAC) is often utilized with the view to reduce the risk of thromboembolic (TE) events. Optimal perioperative anticoagulant methods have not been established.


Results. A search of MEDLINE, EMBASE and Cochrane Collaboration databases yielded 32 studies on 6760 bridged patients. Studies were reviewed by 2 independent data collectors (k=0.869). Study quality was generally poor with risk of bias. Thirty-one studies were observational with 1 randomized controlled trial. Low TE risk and/or non-OAC patient groups were used for comparison in 12 observational studies. Major (22/32, 68.8%) and non-major (27/32, 84.4%) procedures were represented. TE events occurred in 67 of 6760 bridged (0.87%; 95% CI 0.40%-1.35%) and 29 of 4897 non-bridged (0.77%; 95% CI 0.24%-1.30%) patients. Using a random effects model, there was no difference in the risk of TE events in bridged versus non-bridged patients (OR 1.02, 95% CI 0.53-1.95). Bridged patients had a significantly increased risk of overall bleeding (OR 5.47, 95% CI 3.89-7.70) and major bleeding (OR 3.43, 95% CI 1.13-10.4) compared to non-bridged patients. There was no difference in TE events (OR 2.44, 95% CI 0.34-17.4) or overall bleeding (OR 2.40 95% CI 0.72-8.05) in patients receiving full versus intermediate/low dose LMWH.

Summary. Patients receiving heparin bridging during OAC interruption appear to be at increased risk of bleeding and similar risk of TE events compared to non-bridged patients. Studies of high methodologic quality are needed to develop an optimal anticoagulation strategy and inform clinical decision-making.
THERAPEUTIC-DOSE ‘NORTH AMERICAN’ HEPARIN BRIDGING REGIMEN: RATIONALE AND IMPLICATIONS FOR FUTURE RESEARCH

J.D. Douketis

Department of Medicine, McMaster University, Hamilton, ON, Canada

There is no standardized heparin bridging regimen for patients who require temporary interruption of warfarin around the time of an elective surgical or other invasive procedure. In North America, the dominant bridging regimen consists of therapeutic-dose low-molecular-weight heparin, at a dose comparable to that used for the treatment of acute venous thromboembolism or an acute coronary syndrome. With this regimen, which is typically applied to patients considered at moderate-to-high risk for thromboembolism, the perioperative incidence of thromboembolism is approximately 1% and the incidence of major bleeding is 2-4%. Whether this higher-intensity regimen is more effective than a less intense heparin bridging regimen is not known. This paper will review the rationale for a therapeutic-intensity heparin bridging regimen and the justification for its incorporation into ongoing clinical trials assessing the efficacy and safety of perioperative heparin bridging.

VITAMIN K ANTAGONISTS AND LMWH

G.T. Gerotziafas

Department of Thrombosis, Service d’Hématologie Biologique, Hôpital Tenon, Paris, France

Thrombin generation assay performed in the presence of low tissue factor (TF) concentration is sensitive to the anticoagulant effect of LMWHs and VKA. Studies performed by our group in patients receiving monotherapy with LMWH or VKA or combination of both showed that treatment with LMWH adjuvant to VKA enhances the anticoagulant effect induced by VKA but it is not sensitive to the anticoagulant activity of LMWHs. Activated partial thromboplastin time (aPTT), explores the intrinsic clotting pathway, is sensitive to UFH, but it is hardly influenced by VKA treatment and by LMWHs at therapeutic doses. The measurement of anti-Xa activity in plasma of patients treated with LMWH is hardly correlated to the clinical outcome. Thrombin generation assay performed in the presence of low tissue factor (TF) concentration is sensitive to the anticoagulant effect of LMWHs and VKA. Studies performed by our group in patients receiving monotherapy with LMWH or VKA or combination of both showed that treatment with LMWH adjuvant to VKA enhances the inhibition of thrombin generation compared to VKA monotherapy. In VKA treated patients having an INR within the therapeutic range (2 to 3) thrombin generation is inhibited by 50%. The hypo-coagulation in patients treated with LMWH and VKA cannot be predicted by the INR values and the correlation with the anti-Xa activity is weak. In patients receiving VKA and LMWH with INR values lower than 2 and anti-Xa activity about 0.5 anti-Xa IU/ml thrombin generation is reduced by about 50. The anticoagulation induced by therapeutic doses of LMWH at the instauration of VKA treatment is equivalent in terms of thrombin generation inhibition, to that obtained by full VKA anticoagulation with an INR within the therapeutic range. This might be a field where thrombin generation assessment, should be prospectively evaluated as a tool for the estimation of the global anticoagulation produced by LMWH and VKA treatment.

LMWH. Nevertheless, in real-life clinical practice the duration of co-administration of LMWH and VKA is often longer and the bleeding risk during the initiation of VKA treatment is somewhat higher compared to that reported in clinical trials. Early hospital discharge of patients is widely applied and it is expected that in real-life clinical practice a non negligible percentage of outpatients are treated with LMWH and VKA association. In patients, treated at home or in non-hospital institutions the duration of dual treatment with LMWH and VKA is significantly longer compared to hospitalized ones and the bleeding risk seems to be higher. Probably in these situations a more careful management of dual anticoagulant treatment is needed. The monitoring of the anticoagulant effect induced by co-administration of VKA and LMWH could be useful in some patients who are in high bleeding or thrombotic risk such as elderly or unstable patients, pregnant women or patients hospitalized in intensive care units. The lack of an appropriate assay is a significant drawback for optimization of the combined anticoagulant treatment with VKA and LMWH. The international normalized ratio (INR), based on prothrombin time (PT), explores in vitro the extrinsic clotting pathway and adequately reflects the anticoagulation induced by VKA but it is not sensitive to the anticoagulant activity of LMWHs. Activated partial thromboplastin time (aPTT), explores the intrinsic clotting pathway, is sensitive to UFH, but it is hardly influenced by VKA treatment and by LMWHs at therapeutic doses. The measurement of anti-Xa activity in plasma of patients treated with LMWH is hardly correlated to the clinical outcome. Thrombin generation assay performed in the presence of low tissue factor (TF) concentration is sensitive to the anticoagulant effect of LMWHs and VKA. Studies performed by our group in patients receiving monotherapy with LMWH or VKA or combination of both showed that treatment with LMWH adjuvant to VKA enhances the inhibition of thrombin generation compared to VKA monotherapy. In VKA treated patients having an INR within the therapeutic range (2 to 3) thrombin generation is inhibited by 50%. The hypo-coagulation in patients treated with LMWH and VKA cannot be predicted by the INR values and the correlation with the anti-Xa activity is weak. In patients receiving VKA and LMWH with INR values lower than 2 and anti-Xa activity about 0.5 anti-Xa IU/ml thrombin generation is reduced by about 50. The anticoagulation induced by therapeutic doses of LMWH at the instauration of VKA treatment is equivalent in terms of thrombin generation inhibition, to that obtained by full VKA anticoagulation with an INR within the therapeutic range. This might be a field where thrombin generation assessment, should be prospectively evaluated as a tool for the estimation of the global anticoagulation produced by LMWH and VKA treatment.
Venous thromboembolism (VTE) may complicate the course of acute medical diseases in hospitalized patients. A number of studies have identified medical patient groups at increased risk for VTE, including patients with congestive heart failure, respiratory insufficiency, infectious or inflammatory diseases, or neurologic disorders. In the absence of thromboprophylaxis, the overall incidence of VTE in these patients may be as high as about 20%. At least 3 large, randomized controlled trials comparing low molecular weight heparins and fondaparinux with placebo have shown a significant reduction in the incidence of VTE when pharmacologic prophylaxis was administered. A subsequent meta-analysis of these and smaller trials has demonstrated that thromboprophylaxis in medical patients significantly reduces the incidence of symptomatic events and of pulmonary embolism-related mortality. Based on these findings, clinical guidelines recommend the use of thromboprophylaxis in medical patients at increased risk of VTE. Observational studies have shown that about 40% of patients admitted to medical departments are eligible for this treatment. The optimal duration of prophylaxis is less established: guidelines currently recommend a maximum of 14 days, but some patients with persistent immobilization may benefit from longer treatment. The EXCLAIM study was the first study to compare extended-duration, out-of-hospital VTE prophylaxis with low molecular weight heparin with the currently recommended standard in acutely ill medical patients. Extended prophylaxis significantly reduced the incidence of VTE, there including symptomatic VTE, but at the cost of a significant increase in the incidence of major bleeding. The results of the EXCLAIM study have stressed the need for a more accurate stratification of medical patients, both for the risk of VTE and bleeding. In medical patients, the risk of VTE is determined by the concomitant presence of immobilization, acute illness leading to hospitalization, and patient specific risk factors. The definition of immobilization remains particularly controversial and ranges from total bed rest to limited mobility (e.g. bathroom privilege). Up to two thirds of patients have concomitant risk factors, each possibly playing a role in determining the individual risk of VTE. On the other hand, up to half of patients admitted to medical wards have moderate to severe renal insufficiency, and the majority receive multiple concomitant therapies, therefore increasing the risk of bleeding. The problem of extended prophylaxis remains open, since observational studies have reported a non negligible incidence of symptomatic VTE in rehabilitation facilities of 2.4%. Clinical trials evaluating new oral anticoagulant drugs for the prevention of VTE in medical patients may further contribute to this issue. In particular, a clinical trial evaluating rivaroxaban for the prevention of VTE in high risk medical patients, the MAGELLAN study, has been recently completed and the results are expected to be presented at the next American College of Cardiology meeting. In MAGELLAN, a double blind, double dummy study, patients were randomized to receive rivaroxaban administered at the dose of 10 mg od for 35 ± 4 days or enoxaparin 40 mg od for 10 ± days.

References

UNFRACTIONATED HEPARIN FOR VENOUS THROMBOEMBOLISM PREVENTION IN THE HOSPITALIZED MEDICAL PATIENT: SHOULD DOSING BE ONCE, TWICE, OR THRICE DAILY?
C.E. Mahan, M. Pini, A.C. Spyropoulos
1University of New Mexico Health Sciences Center & Clot Prevention Intervention Management Programs, LLC, Albuquerque, New Mexico, USA. 2Medicina Interna II, Ospedale di Fidenza, Parma, Italy; 3Department of Medicine, McMaster University, Hamilton General Hospital, Thrombosis Unit, Hamilton, ON, Canada
No head-to-head trials have compared the safety and efficacy of unfractionated heparin (UFH) 5,000 U subcutaneously thrice (i.e. q8 h or TID) daily versus twice daily (q12 h or BID) for venous thromboembolism (VTE) prevention in the hospitalized medical patient. Currently, the American College of Chest Physicians (ACCP) and the Italian Association of Medical Oncology (AIOM) guide-

THROMBOPROPHYLAXIS IN THE ACUTELY-ILL MEDICAL PATIENT
W. Ageno
Department of Clinical Medicine, University of Insubria, Varese, Italy

SYSTEMATIC REVIEW OF THROMBOPROPHYLAXIS IN ACUTELY ILL MEDICAL PATIENTS

Hematology Reports 2011; 3 (s1)
lines do not specify a frequency for UFH while the International Union of Angiology (IUA), the Italian Society for Studies on Haemostasis and Thrombosis (SISET), the National Comprehensive Cancer Network (NCCN), and the American Society of Clinical Oncology (ASCO) recommend a frequency of TID UFH. Well-designed primary studies comparing low molecular weight heparin (LMWH) and UFH, and UFH and placebo were reviewed. Meta-analyses evaluating safety and efficacy of LMWH versus UFH, or BID UFH versus TID UFH were also evaluated. Although BID UFH showed some efficacy in one primary study, in another study it was no more beneficial than no prophylaxis. LMWH is more efficacious than BID UFH, but comparable in safety and efficacy to TID UFH. Meta-analytic data demonstrate that BID UFH may have some reduction in deep vein thrombosis, but also suggest that TID UFH is more efficacious than BID UFH at the cost of more major bleeding. Lastly, case-fatality rates from pulmonary embolism (PE) are additive (i.e. recurrent PE) and appear to be higher than with major bleeding. Therefore, risk-benefit favors TID UFH over BID UFH due to increased efficacy even when taking into consideration the increased rate of major bleeds. The medical patient with VTE risk factors appears to be at moderate to high risk. Newly published risk-assessment models, such as the IMPACT-ILL, may be beneficial in determining which patients would best benefit from BID UFH (i.e. moderate risk) or TID UFH (i.e. moderate to high risk). Because it appears that UFH is best administered in a TID frequency, LMWH or emerging oral anticoagulants should likely be preferred agents since drugs with more frequent dosing are omitted more often. International guidelines for VTE prevention should incorporate a frequency for UFH to guide use and likely favor LMWH over UFH for this reason.

OPTIMAL DURATION OF THROMBOPHYLAXIS IN ACUTELY-ILL MEDICAL PATIENTS

J.D. Douketis
Department of Medicine, McMaster University, Hamilton, ON, Canada

Hospitalized medical patients are at increased risk for venous thromboembolism (VTE) and there is strong evidence that 7-10 days of thromboprophylaxis with a heparin reduces the risk that patients will develop clinically-important VTE by 40-60%. Extended-duration heparin thromboprophylaxis, typically for an additional 3-4 weeks, has been shown in high-risk surgical patients to further reduce the incidence of VTE. However, the use of extended-duration prophylaxis in medical patients is controversial. Furthermore, unlike surgical patients, medical patients are more heterogeneous in regard to their VTE risk profile and have more variable hospital lengths of stay and convalescent periods. This paper will review the rationale for both short-duration (7-10 days) and extended-duration (26-35 days) heparin prophylaxis in medical patients who required hospitalization.

THROMBOPROPHYLAXIS IN HOSPITALIZED CANCER PATIENTS

Oncology Institute of Vojvodina, Sremska Kamenica, Serbia

The precise mechanism of hypercoagulability in cancer patients is unclear, although there are many theories related to this phenomenon (liberation of tumor-associated thromboplastins, the effects of chemotherapy and hormonal drugs, presence of foreign materials such as indwelling intravenous lines, obesity, infections, radiotherapy, and surgical procedures). Primary hematological causes may also include defects of coagulation proteins, both inherited and acquired. It should be recognized that some cancer patients are at high risk for VTE. The appearance of venous thromboembolic (VTE) disease is diagnosed in 11% to 20% of all cancer patients. Fifty percent of cancer patient deaths are associated with venous thromboembolic disease. Patients with VTE have poor prognosis. Thromboprophylaxis in hospitalized cancer patients is an important part of supportive care. According to our guidelines for thromboprophylaxis it is mandatory in patients undergoing surgical or gynecological intervention and with one of risk factors for thrombosis (recurrences, obesity, etc.). These patients have heparin prophylaxis one day before operation and 10 days after. All patients with glioblastoma during irradiation received thromboprophylaxis with LMWH heparin. Patients with myeloma multiplex during the treatment with thalidomide have thromboprophylaxis with heparin or warfarin. Breast cancer patients who receive hormonal therapy and have one of risk factors for thrombosis are also on thromboprophylactic treatment. When possible, therapy should be changed for a drug with less thrombogenic elements. The patients receiving high-dose therapy and autologous transplantation of peripheral stem cell should also be under thromboprophylaxis measure. Our guidelines in such patients suggest continual therapy with UF heparin. These criteria for thromboprophylaxis expose our patients to minimum risk of thromboembolic events.

USE OF RISK-ASSESSMENT MODELS

A.C. Spyropoulos
Department of Medicine, McMaster University, Hamilton, ON, Canada

Risk-assessment models (RAMs) for hospitalized medical patients at risk for venous thromboembolism (VTE) have attempted to identify at-risk patients using a point system or binary approach of exposing risk factors (acute medical illness) or predisposing risk factors (genetic or clinical characteristic) for VTE. These RAMs were derived from data predominately from patient subgroups within randomized-controlled trials and were cumbersome, not subject to rigorous validation, and were based on limited evidence of how these risk factors interacted in a quantitative manner. Recently simplified RAMs have
been proposed comprised of various point systems and a
threshold which would identify at-risk patient groups that
would benefit from thromboprophylaxis. The RAMs and
some of the point systems have been validated in large
patient cohorts either prospectively or retrospectively and
have shown good sensitivity. The presence of malignan-
cy, prior VTE, hypercoagulability, advanced age and
immobility all conferred increased risk of VTE during
hospitalization or in the post-hospital discharge period in
the various models.

IMPROVING PROPHYLAXIS AND DECREASING
VENOUS TROMBOEMBOLIC EVENTS IN THE
HOSPITALIZED PATIENT

C.E. Mahan,1 M.A. Hussein,2 A.N. Amin,3
A.C. Spyropoulos4
1University of New Mexico Health Sciences Center &
Clot Prevention Intervention Management Programs,
LLC, Albuquerque, NM, USA; 2Health Economics and
Outcomes Research, IMS, Falls Church, Virginia, USA;
3Department of Medicine Executive Director, Hospitalist
Program, University of California - Irvine, Irvine, Ca,
USA; 4Department of Medicine, McMaster University,
Hamilton General Hospital, Hamilton, On, Canada

Venous thromboembolism (VTE) prevention remains a
key healthcare initiative internationally with nearly one
million deaths occurring in the US and EU from fatal PE
annually. In US hospitals, there has been a recent and
continued focus on national quality initiatives to prevent
hospital-acquired VTE. Two recent concepts have emer-
ged in the medical literature including “appropriate”
prophylaxis (AP) and “preventable” VTE (PVTE). AP is
defined as appropriate type, dose and duration. PVTE is
defined as VTE in which AP was NOT utilized prior to
objective verification of the event. Various strategies
exist to prevent VTE by increasing prophylaxis rates.
The most effective current interventions are: multiface-
ted; active including reminders to the provider; hospital
staff, patient and provider education; and regular audit
and feedback to medical staff. Successful active inter-
vention programs have included both electronic alerts,
with or without computerized clinical decision support
software and, more recently, human alerts. Several
recent human alert studies have utilized in-hospital cli-
nical pharmacists embedded within the medical team.
Passive strategies, such as guideline dissemination or
posters, should not be used as a lone method. Audit and
feedback should be perpetual and should closely moni-
tor physicians that are ignoring reminders for at-risk
patients with no contraindications to thromboprophyla-
xis. Inappropriate duration remains a leading reason as
to why at-risk patients do not receive AP within the
hospital. Few intervention studies address duration
compared with hospital length of stay or drug labeling.
The Joint Commission has a new core measure set for
VTE prevention, treatment, and an outcome measure for
preventable VTE. However to date, PVTE and AP are
only measured in two and one intervention studies,
respectively. In addition, only 60 out of 5000 US acute
care hospitals are currently reporting on the VTE core
measure set. Mandating reporting of the VTE core mea-
asure set would improve preventative measures within
the US and reduce morbidity, mortality, and costs asso-
ciated with VTE. Future studies should focus on: head-
to-head multifaceted intervention trials assessed over
time; AP; overall and PVTE; and rates of unnecessary
prophylaxis for patients not at risk.
Index of Authors

Ageno W. 18
Aharon A. 6
Amin A.N. 20
Ammollo C.T. 11
Balduini A. 2
Bartimoccia S. 15
Bauersachs R. 13
Betti S. 6
Bjelobrk-Kolarov I. 19
Brambilla M. 3
Brenner B. 6
Camera M. 3
Canzano P. 3
Cappucci F. 15
Carnevale R. 15
CATCH investigators 13
Ciminello A. 6
Cohen M. 5,11
Colaizzo D. 15
Colombo G. 3
Colucci M. 14
D’Angelo A. 2
Dahlbäck B. 11
Dazzi D. 10
De Marco L. 1
De Stefano V. 6
Degl’Innocenti G. 4
Di Santo S. 15
Douketis J.D. 16, 17, 19
Esmon C. 5,11
Esmon N.L. 11
Finocchi A. 15
Fischetti L. 15
Gerotziafas G.T. 17
Girolami A. 1
Grandone E. 15
Hussein M.A. 20
Imberti D. 13
Jovanovic Da. 19
Jovanovic Du. 19
Lenti L. 15
Lupu F. 11
Mahan C.E. 18,20
Manfredi A.A. 2
Marcucci R. 4
Marenzi G. 3
Margaglione M. 15
Maugeri N. 2
Moia M. 10
Morrisey J.H. 12,16
Nadir Y. 6
Pattacini C. 10
Petrovic D. 19
Pignatelli P. 15
Pini M. 10, 18
Plebani A. 15
Popovic L. 19
Prandoni P. 9
Prisco D. 4
Rezaie A. 5,12
Rossi E. 6
Sanguigni V. 15
Semeraro F. 11
Siegel D. 16
Soresina A.R. 15
Spyropoulos A.C. 16, 18, 19, 20
Tirloni E. 3
Tiscia G.L. 15
Toschi V. 4
Tremoli E. 3
Tsung A. 11
Violi F. 15
Xu J. 5,11
Yudin J. 16
Za T. 6