hematology meeting reports

ISSN 1970-7339

9th International Winter Meeting on Coagulation

Basic, Laboratory and Clinical Aspects of Venous and Arterial Thromboembolic Diseases

Bormio (Sondrio), Italy, April 1-4, 2009

hematology meeting reports 2009; vol 3; no. 2
Owned and Published by the Ferrata-Storti Foundation, Pavia, Italy

hematology meeting reports

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Printing
Tipografia PIME Editrice, Pavia, Italy

Subscriptions
volume 3 (6 issues) 2009
Institutional & Personal: €250

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3. Breast Cancer Association Consortium. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. J Natl Cancer Inst 2006;98:1382-96.
4. Reed E. ERCC1 measurements in clinical oncology. N Engl J Med 2006; 355:1054-5.
5. Mouridsen H.T. Letrozole versus tamoxifen as first-line treatment for metastatic breast cancer: a survival analysis. Am J Cancer 2003; 2 supplement 1:7-11.

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6. Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, eds. Clinical oncology. 2nd ed. Churchill Livingstone. 2000.
7. Pizzo PA, Poplack DG. Principles and Practice of Pediatric Oncology. 4th ed. Philadelphia, Lippincott Williams & Wilkins, 2001.
8. Coleman RE, Rubens RD. Bone metastases. In: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, eds. Clinical Oncology. 2nd ed. Churchill Livingstone. 2000. pp 836-871.
9. Lung LKW, Hui AMY, Leung WK, Sung JY, Ng EKW. Gene expressions of human peritoneal mesothelial cells in gastric cancer. Proceedings of the 97th AACR Annual Meeting, April 1-5, 2006, Washington, DC, USA, Proc Amer Assoc Cancer Res 2006;47: [Abstract #122].

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13. Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med In press 1996.
14. Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis [serial online] 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from URL: http://www.cdc.gov/ncidod/EID/ eid.htm

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dabigatran etexilato

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Pradaxa capsule rigide è presente ai due dosaggi da 75 mg e da 110 mg. Poiché il contenuto delle due schede tecniche è identico relativamente alle sezioni 4 e 5, di seguito è riportata solo la Scheda Tecnica del dosaggio da 110 mg.

1. DENOMINAZIONE DEL MEDICINALE
Pradaxa 110 mg capsule rigide

2. COMPOSIZIONE QUALITATIVA E QUANTITATIVA
Ogni capsula rigida contiene 110 mg di dabigatran etexilato (come mesilato)
Eccipienti: Ogni capsula rigida contiene 3 mg di giallo tramonto (E110)
Per l'elenco completo degli eccipienti, vedere paragrafo 6.1.

3. FORMA FARMACEUTICA
Capsula rigida. Capsule con testa color blu chiaro opaco e corpo color crema opaco di misura 1 riempita con pellet di color giallognolo. Sulla testa è stampato il logo di Boehringer Ingelheim, sul corpo "R110".

4. INFORMAZIONI CLINICHE
4.1 Indicazioni terapeutiche. Prevenzione primaria di episodi tromboembolici in pazienti adulti sottoposti a chirurgia sostitutiva elettiva totale dell'anca o del ginocchio. **4.2 Posologia e modo di somministrazione.** Prevenzione di episodi di Tromboembolismo Venoso (TEV) in pazienti sottoposti a chirurgia sostitutiva elettiva del ginocchio. La dose raccomandata di Pradaxa è di 220 mg una volta al giorno, assunta sotto forma di 2 capsule da 110 mg. Il trattamento deve iniziare per via orale entro 1-4 ore dalla conclusione dell'intervento con una capsula e continuare dal giorno successivo con 2 capsule una volta al giorno per un totale di 10 giorni. Prevenzione di episodi di Tromboembolismo Venoso (TEV) in pazienti sottoposti a chirurgia sostitutiva elettiva dell'anca. La dose raccomandata di Pradaxa è di 220 mg una volta al giorno, assunta sotto forma di 2 capsule da 110 mg. Il trattamento deve iniziare per via orale entro 1-4 ore dalla conclusione dell'intervento con una capsula e continuare dal giorno successivo con 2 capsule una volta al giorno per un totale di 28-35 giorni. Per entrambi gli interventi, se l'emostasi non fosse normale, l'inizio del trattamento deve essere rimandato. Se il trattamento non viene iniziato il giorno dell'intervento, si deve cominciare con 2 capsule una volta al giorno. Popolazioni speciali di pazienti: *Insufficienza renale.* Il trattamento con Pradaxa in pazienti con grave insufficienza renale (clearance della creatinina < 30 ml/min) è controindicato (vedere paragrafo 4.3). L'esperienza clinica in pazienti con Insufficienza renale moderata (clearance della creatinina 30-50 ml/min) è limitata. Questi pazienti devono essere trattati con cautela. La dose raccomandata è 150 mg assunta 1 volta al giorno come 2 capsule da 75 mg (vedere paragrafi 4.4 e 5.1). Dopo l'intervento di sostituzione del ginocchio, il trattamento deve iniziare per via orale entro 1-4 ore dalla conclusione dello stesso con una capsula e continuare dal giorno successivo con 2 capsule una volta al giorno per un totale di 10 giorni. Dopo l'intervento di sostituzione dell'anca, il trattamento deve iniziare per via orale entro 1-4 ore dalla conclusione dello stesso con una capsula e continuare dal giorno successivo con 2 capsule una volta al giorno per un totale di 28-35 giorni. *Pazienti anziani:* Nei pazienti anziani (> 75 anni) l'esperienza clinica è limitata. Questi pazienti devono essere trattati con cautela. La dose raccomandata è 150 mg assunta 1 volta al giorno come 2 capsule da 75 mg (vedere paragrafi 4.4 e 5.1). Dopo l'intervento di sostituzione del ginocchio, il trattamento deve iniziare per via orale entro 1-4 ore dalla conclusione dello stesso con una capsula e continuare dal giorno successivo con 2 capsule una volta al giorno per un totale di 10 giorni. Dopo l'intervento di sostituzione dell'anca, il trattamento deve iniziare per via orale entro 1-4 ore dalla conclusione dello stesso con una capsula e continuare dal giorno successivo con 2 capsule una volta al giorno per un totale di 28-35 giorni. *Insufficienza epatica*. I pazienti con enzimi epatici elevati superiori al doppio del limite superiore dei valori normali (ULN), sono stati esclusi dagli studi clinici. Pertanto l'uso di Pradaxa non è raccomandato in questa popolazione (vedere paragrafi 4.4 e 5.2). La misurazione dell'ALT deve essere effettuata come parte degli esami standard pre-operatori (vedere paragrafo 4.4). Peso. L'esperienza clinica al dosaggio raccomandato, in pazienti con peso corporeo < 50 kg o > 110 kg è assai limitata. Sulla base dei dati clinici e di cinetica non è necessario un aggiustamento posologico (vedere paragrafo 5.2) ma si raccomanda uno stretto controllo clinico (vedere paragrafo 4.4). Pazienti post-interveto con aumentato rischio di sanguinamento. I pazienti a rischio di sanguinamento o i pazienti a rischio di sovra esposizione, particolarmente i pazienti con insufficienza renale moderata (clearance della creatinina pari a 30-50 ml/min), devono essere trattati con cautela (vedere paragrafi 4.4 e 5.1). *Bambini e adolescenti.* Non c'è esperienza sull'uso di Pradaxa nei bambini. Pradaxa non è raccomandato nei pazienti al di sotto di 18 anni a causa della mancanza di dati sulla sicurezza e sull'efficacia. *Uso concomitante di Pradaxa e amiodarone*. Il dosaggio di Pradaxa deve essere ridotto a 150 mg al giorno in pazienti che ricevano contemporaneamente dabigatran etexilato e amiodarone (vedere paragrafo 4.5). Passaggio dal trattamento con Pradaxa al trattamento con anticoagulante parenterale. Si raccomanda di attendere 24 ore dall'ultima dose prima di passare da Pradaxa ad un anticoagulante parenterale (vedere paragrafo 4.5). Passaggio dal trattamento con anticoagulante parenterale al trattamento con Pradaxa. Non ci sono dati disponibili, pertanto non è raccomandato iniziare il trattamento con Pradaxa prima della prevista somministrazione della dose successiva di anticoagulante parenterale (vedere paragrafo 4.5). Pradaxa deve essere inghiottito intero con acqua, con o senza cibo. **4.3 Controindicazioni.** • Ipersensibilità al principio attivo o ad uno qualsiasi degli eccipienti. • Pazienti con grave insufficienza renale (ClCr < 30 ml/min). • Sanguinamento attivo clinicamente significativo. • Lesione organica a rischio di sanguinamento. • Alterazione spontanea o farmacologica dell'emostasi. • Insufficienza epatica o malattia epatica che possa avere un qualsiasi impatto sulla sopravvivenza. • Trattamento concomitante con chinidina (vedere paragrafo 4.5). **4.4 Avvertenze speciali e precauzioni di impiego.** Insufficienza epatica. I pazienti con enzimi epatici elevati, superiori al doppio del limite superiore dei valori normali, sono stati esclusi negli studi clinici controllati. Pertanto l'uso di Pradaxa non è raccomandato in questa popolazione. La misurazione dell'ALT deve essere effettuata come parte degli esami standard pre-operatori. Rischio emorragico. Un'attenta osservazione clinica (ricerca di segni di sanguinamento o anemia) è raccomandata durante il trattamento, soprattutto nelle seguenti situazioni che possono aumentare il rischio emorragico: malattie associate ad un aumentato rischio di sanguinamento, come alterazioni della coagulazione congenite o acquisite, trombocitopenia o alterazioni della funzionalità delle piastrine, malattia gastrointestinale ulcerativa attiva, biopsia recente o trauma maggiore, emorragia intracranica recente o cerebrale, chirurgia spinale o oftalmica, endocardite batterica. I pazienti con insufficienza renale moderata sono maggiormente esposti a dabigatran. I dati in pazienti di peso < 50 kg e in pazienti anziani sono limitati (vedere paragrafi 4.2 e 5.2). In queste situazioni Pradaxa deve essere utilizzato con cautela ed è richiesto uno stretto controllo clinico (ricerca di segni di sanguinamento o anemia) per tutta la durata del trattamento (vedere paragrafo 4.2). Quando si verifica un grave sanguinamento il trattamento deve essere sospeso e l'origine del sanguinamento indagata (vedere paragrafo 4.9). Agenti che possono accrescere il rischio di emorragia non devono essere somministrati in concomitanza o devono essere somministrati con cautela con Pradaxa (vedere paragrafo 4.5). Pazienti ad alto rischio di mortalità dovuta a chirurgia e con fattori di rischio intrinseco di eventi tromboembolici. I dati di efficacia e sicurezza disponibili per dabigatran in questi pazienti sono limitati e pertanto devono essere trattati con cautela. Anestesia spinale/anestesia epidurale/iniezione lombare. In pazienti sottoposti a chirurgia ortopedica maggiore, non può essere esclusa l'insorgenza di ematomi epidurali o spinali determinanti paralisi prolungata o permanente con l'uso contemporaneo di dabigatran e anestesia spinale, anestesia epidurale o iniezione lombare. Il rischio di questi rari eventi può essere maggiore con l'uso post-operatorio di catetere permanente epidurale o con la somministrazione concomitante di altri medicinali che alterino l'emostasi. Pertanto l'uso di Pradaxa non è raccomandato in pazienti che debbano essere sottoposti ad anestesia che preveda l'utilizzo di cateteri permanenti epidurali post-operatori. La somministrazione della prima dose di Pradaxa deve avvenire almeno due ore dopo la rimozione del catetere. Questi pazienti richiedono un'osservazione frequente di segni e sintomi neurologici. Chirurgia per frattura dell'anca. Non sono disponibili dati sull'uso di Pradaxa in pazienti sottoposti a intervento per frattura dell'anca. Pertanto il trattamento non è raccomandato. Coloranti. Pradaxa capsule rigide contiene il colorante giallo tramonto (E110) che può causare reazioni allergiche. **4.5 Interazioni con altri medicinali ed altre forme di interazione.** Sono stati effettuati studi di interazione solo negli adulti. Anticoagulanti e trattamenti che agiscono sull'aggregazione piastrinica. I seguenti trattamenti non sono raccomandati in concomitanza a Pradaxa: eparine non frazionate e derivati dell'eparina, eparine a basso peso molecolare (EBPM), fondaparinux, desirudina, agenti trombolitici, antagonisti del recettore della GPIIb/IIIa, clopidogrel, ticlopidina, destrano, sulfinpirazone e antagonisti della vitamina K. Si deve notare che l'eparina non frazionata può essere somministrata alle dosi necessarie per mantenere pervio un catetere centrale venoso o arterioso (vedere paragrafi 4.2 e 4.4). Interazioni legate al profilo metabolico di dabigatran etexilato e dabigatran. Dabigatran etexilato e dabigatran non sono metabolizzati dal sistema del citocromo P450 e non hanno effetti *in vitro* sugli enzimi umani del citocromo P450. Pertanto non sono attese interazioni correlate al medicinale con dabigatran. FANS: Quando Pradaxa è stato co-somministrato con diclofenac, l'esposizione plasmatica di entrambi i medicinali è rimasta inalterata dimostrando un'assenza di interazione farmacocinetica fra dabigatran etexilato e diclofenac. Tuttavia, a causa del rischio di emorragia, soprattutto con FANS con emivita di eliminazione > 12 ore, si raccomanda la stretta osservazione di segni di sanguinamento (vedere paragrafo 4.4). Interazioni del trasportatore. Amiodarone: Amiodarone è un inibitore del trasportatore dell'efflusso P-glicoproteina e dabigatran etexilato è un substrato di questo trasportatore. Quando Pradaxa è stato co-somministrato con amiodarone, la quantità e la velocità di assorbimento di amiodarone e del suo metabolita attivo DEA sono rimaste essenzialmente immodificate. L'AUC e la C_{max} di dabigatran sono aumentate di circa il 60 % e il 50 % rispettivamente. Il meccanismo dell'interazione non è stato completamente chiarito. Considerando la lunga emivita di amiodarone, la potenziale interazione con il farmaco può perdurare per settimane dopo la sospensione di amiodarone. Il dosaggio deve essere ridotto a 150 mg di Pradaxa al giorno in pazienti che siano trattati contemporaneamente con dabigatran etexilato e amiodarone. Inibitori della P-glicoproteina. Forti inibitori della P-glicoproteina quali verapamil, claritromicina ed altri devono essere utilizzati con cautela. La chinidina, inibitore della P-glicoproteina, è controindicata (vedere paragrafo 4.3). Induttori della P-glicoproteina. Potenti induttori della P-glicoproteina quali rifampicina o Erba di San Giovanni (*Hypericum perforatum*), possono ridurre l'esposizione sistemica a dabigatran. Si raccomanda cautela quando questi medicinali sono co-somministrati. Digossina: In uno studio condotto su 24 pazienti sani, quando Pradaxa è stato somministrato in associazione a digossina non sono state osservate né modifiche sulla digossina né alterazioni cliniche significative dell'esposizione a dabigatran. pH gastrico. Pantoprazolo: Quando Pradaxa è stato somministrato in associazione a pantoprazolo, è stata osservata una riduzione di circa il 30% dell'area sotto la curva concentrazione plasmatica-tempo di dabigatran. Pantoprazolo ed altri inibitori della pompa protonica sono stati co-somminsitrati con Pradaxa negli studi clinici e non sono stati osservati effetti sul sanguinamento o sull'efficacia. Ranitidina: La somministrazione di ranitidina con Pradaxa non ha effetti clinicamente rilevanti sull'assorbimento di dabigatran. **4.6 Gravidanza e allattamento.** Gravidanza. Non vi sono dati adeguati riguardanti l'uso di Pradaxa in donne in gravidanza. Gli studi condotti su animali hanno evidenziato una tossicità riproduttiva (vedere paragrafo 5.3). Il rischio potenziale per gli esseri umani non è noto. Le donne in età fertile devono evitare la gravidanza durante il trattamento con debigatran etexilato. Pradaxa non deve essere utilizzato durante la gravidanza se non quando chiaramente necessario. Allattamento. Non vi sono dati clinici riguardanti gli effetti di dabigatran sui lattanti durante l'allattamento. L'allattamento deve essere sospeso durante il trattamento con Pradaxa. **4.7 Effetti sulla capacità di guidare veicoli e sull'uso di macchinari.** Non sono stati effettuati studi sulla capacità di guidare veicoli e sull'uso di macchinari. **4.8 Effetti indesiderati.** Un totale di 10.084 pazienti sono stati trattati in 4 studi di prevenzione di TEV attivamente controllati con almeno un dosaggio del medicinale in studio. Di questi pazienti 5.419 sono stati trattati con 150 o 220 mg di Pradaxa al giorno, mentre 389 hanno ricevuto dosi inferiori a 150 mg al giorno e 1.168 dosi superiori a 220 mg al giorno. Le reazioni avverse più comunemente riportate sono i sanguinamenti che si sono verificati in un totale di circa il 14% dei pazienti; la frequenza di sanguinamenti maggiori (inclusi i sanguinamenti della ferita) è inferiore al 2%. La tabella 1 riporta il numero (%) di pazienti in cui si sono verificati eventi di sanguinamento durante il periodo di trattamento di prevenzione di TEV nei due studi clinici principali, in accordo alla dose.

Tabella 1: Eventi di sanguinamento maggiore e qualsiasi sanguinamento nello studio principale dell'anca e del ginocchio

	Dabigatran etexilato 150 mg N (%)	Dabigatran etexilato 220 mg N (%)	Enoxaparina N (%)
Trattati	1866 (100,0)	1825 (100,0)	1848 (100,0)
Sanguinamento Maggiore	24 (1,3)	33 (1,8)	27 (1,5)
Qualsiasi sanguinamento	258 (13,8)	251 (13,8)	247 (13,4)

La tabella 2 mostra le reazioni avverse ordinate per SOC e frequenza utilizzando la seguente convenzione: molto comune (≥ 1/10); comune (≥ 1/100, <1/10); non comune (≥ 1/1.000, <1/100); raro (≥ 1/10.000, <1/1.000); molto raro (≥ 1/10.000).

SOC/Termini Preferiti	Dabigatran etexilato 150 mg N (%)	Dabigatran etexilato 220 mg N (%)	Enoxaparina N (%)
Numero di pazienti trattati	2737 (100)	2682 (100)	3108 (100)
Patologie del sistema emolinfopoietico			
Comune			
Anemia	110 (4,0)	117 (4,4)	141 (4,5)
Non comune			
Trombocitopenia	5 (0,2)	2 (0,1)	5 (0,2)
Patologie vascolari			
Comune			
Ematoma	38 (1,4)	37 (1,4)	55 (1,8)
Ematoma traumatico	37 (1,4)	41 (1,5)	51 (1,6)
Emorragie dalle ferite	35 (1,3)	28 (1,0)	31 (1,0)
Non comune			
Emorragia	5 (0,2)	18 (0,7)	21 (0,7)
Patologie respiratorie, toraciche e mediastiniche			
Non comune			
Epistassi	19 (0,7)	15 (0,6)	13 (0,4)
Patologie gastrointestinali			
Comune			
Emorragia gastrointestinale	33 (1,2)	17 (0,6)	20 (0,6)
Non comune			
Emorragia rettale	12 (0,4)	15 (0,6	5 (0,2)
Emorragia emorroidale	4 (0,2)	8 (0,3)	2 (0,1)
Patologie epatobiliari			
Non comune			
Aumento dell'alanina aminotransferasi	18 (0,7)	7 (0,3)	28 (0,9)
Aumento dell'aspartato aminotransferasi	9 (0,3)	5 (0,2)	15 (0,5)
Funzionalità epatica alterata/ Test di funzionalità epatica alterati	6 (0,2)	10 (0,4)	7 (0,2)
Aumento degli enzimi epatici	4 (0,2)	5 (0,2)	11 (0,4)
Iperbilirubinemia	4 (0,1)	3 (0,1)	4 (0,1)
Aumento delle transaminasi	0 (0,0)	2 (0,1)	1 (0,0)
Patologie della cute e del tessuto sottocutaneo			
Comune			
Emorragia cutanea	45 (1,6)	57 (2,1)	61 (2,0)
Patologie del sistema muscoloscheletrico e del tessuto connettivo			
Non comune			
Emartrosi	9 (0,3)	7 (0,3)	17 (0,6)
Patologie renali e urinarie			
Comune			
Ematuria	38 (1,4)	33 (1,4)	25 (0,8)
Patologie sistemiche e condizioni relative alla sede di somministrazione			
Non comune			
Emorragia al sito di iniezione	21 (0,8)	19 (0,7)	27 (0,9)
Sanguinamento	2 (0,1)	6 (0,2)	6 (0,2)
Emorragia al sito di inserzione del catetere	2 (0,1)	1 (0,0)	7 (0,2)
Esami diagnostici			
Comune			
Calo dell'emoglobina	45 (1,6)	35 (1,3)	74 (2,4)
Non comune			
Calo dell'ematocrito	0 (0,0)	6 (0,2)	4 (0,1)
Traumatismo, avvelenamento e complicazioni da procedura			
Comune			
Secrezione dalle ferite	130 (4,8)	130 (4,9)	93 (3,0)
Anemia post-operatoria	99 (3,6)	87 (3,2)	120 (3,7)
Ematoma post-procedurale	66 (2,4)	45 (1,7)	78 (2,5)
Emorragia post-procedurale	37 (1,4)	54 (2,0)	56 (1,8)
Suppurazione post-procedurale	31 (1,1)	34 (1,3)	31 (1,0)
Procedure mediche e chirurgiche			
Non comune			
Drenaggio post-procedurale	11 (0,4)	13 (0,5)	16 (0,5)
Drenaggio della ferita	1 (0,0)	4 (0,2)	2 (0,1)

Oltre ai risultati relativi alle ALT riportati sono stati misurati i seguenti risultati di analisi di laboratorio negli studi di fase 3, indicati nella tabella 3.

Tabella 3: valori delle ALT da analisi di laboratorio

	Dabigatran etexilato 150 mg N (%)	Dabigatran etexilato 220 mg N (%)	Enoxaparina N (%)
Incidenza totale di Alaninina aminotransferasi mentata di 3 x ULN	68 (2,5)	58 (2,2)	95 (3,5) au-

4.9 Sovradosaggio. Non esiste antidoto per dabigatran. Dosi di dabigatran etexilato superiori a quelle raccomandate espongono il paziente ad un aumentato rischio di sanguinamento. Nell'eventualità di complicazioni emorragiche il trattamento deve essere sospeso e la causa del sanguinamento indagata. Poiché dabigatran è escreto soprattutto per via renale, deve essere mantenuta un'adeguata diuresi. Deve essere preso in considerazione un trattamento appropriato ad es. l'emostasi chirurgica o la trasfusione di plasma fresco congelato. Dabigatran può essere dializzato; non c'è esperienza clinica che dimostri l'utilità di questo approccio negli studi clinici.

5. PROPRIETÀ FARMACOLOGICHE

5.1 Proprietà farmacodinamiche. Categoria farmacoterapeutica: inibitori diretti della trombina, codice ATC: B01AE07. Dabigatran etexilato è un profarmaco di piccole dimensioni molecolari che non esercita alcuna attività farmacologica. Dopo somministrazione orale, dabigatran etexilato è rapidamente assorbito e convertito in dabigatran mediante idrolisi catalizzata da esterasi nel plasma e nel fegato. Dabigatran è un potente inibitore diretto, competitivo, reversibile della trombina ed è il principio attivo principale che si ritrova nel plasma. Poiché la trombina (proteasi della serina) consente la conversione del fibrinogeno in fibrina nella cascata della coagulazione, la sua inibizione previene la formazione di trombi. Dabigatran inibisce la trombina libera, la trombina legata a fibrina e l'aggregazione delle piastrine indotta dalla trombina. Studi effettuati sugli animali *in-vivo* e *ex-vivo* hanno dimostrato l'efficacia antitrombotica e l'attività anticoagulante di dabigatran dopo somministrazione endovenosa e di dabigatran etexilato dopo somministrazione orale in vari modelli animali di trombosi. Esiste una chiara correlazione tra la concentrazione plasmatica di dabigatran e l'entità dell'effetto anticoagulante, sulla base dei dati degli studi di fase II. Allo steady-state (dopo 3 giorni) la concentrazione plasmatica al picco di dabigatran, misurata 2-4 ore dopo la somministrazione di 220 mg di dabigatran etexilato, è attesa essere circa 270 ng/ml, con un intervallo pari a 80-460 ng/ml. La concentrazione a valle di dabigatran, misurata alla fine del periodo di somministrazione (24 ore dopo l'ultima dose di dabigatran da 220 mg), è attesa essere circa 40 ng/ml, con un intervallo pari a 10-90 ng/ml. Origini etniche. Più del 99% dei dati di efficacia e sicurezza provengono da soggetti caucasici. Studi clinici nella profilassi del Tromboembolismo Venoso (TEV) a seguito di chirurgia maggiore di sostituzione di articolazione. In 2 ampi studi, randomizzati, a gruppi paralleli, in doppio cieco, di conferma della dose, i pazienti destinati a chirurgia ortopedica maggiore (uno per intervento di sostituzione del ginocchio ed uno per intervento di sostituzione dell'anca) sono stati trattati con Pradaxa 75 mg o 110 mg entro 1-4 ore dall'intervento e quindi con 150 o 220 mg al giorno, essendo stata valutata normale l'emostasi o con 40 mg di enoxaparina il giorno prima dell'intervento e quindi giornalmente. Nello studio RE-MODEL (sostituzione del ginocchio) la durata del trattamento è stata di 6-10 giorni e nello studio RE-NOVATE (sostituzione dell'anca) di 28-35 giorni. Sono stati trattati rispettivamente un totale di 2076 (sostituzione del ginocchio) e 3494 (sostituzione dell'anca) pazienti. L'insieme di tutti gli episodi di TEV (che comprendeva EP, TVP prossimale e distale, sia sintomatica che asintomatica rilevata con venografia di routine) e la mortalità per tutte le cause costituivano l'endpoint primario di entrambi gli studi. L'insieme di tutti gli episodi maggiori di TEV (che comprendeva EP, TVP prossimale e distale, sia sintomatica che asintomatica rilevata con venografia di routine) e la mortalità correlata a TEV costituivano un endpoint secondario considerato di maggior rilevanza clinica. I risultati di entrambi gli studi hanno dimostrato che l'effetto antitrombotico di Pradaxa 220 mg e 150 mg era statisticamente non inferiore a quello di enoaparina sui TEV totali e sulla mortalità per tutte le cause. La stima dell'incidenza di episodi maggiori di TEV e di mortalità correlata a TEV per la dose da 150 mg era lievemente peggiore che per l'enoxaparina (tabella 4). Risultati migliori sono stati osservati con la dose da 220 mg dove la stima dell'incidenza di episodi maggiori di TEV era lievemente migliore che con l'enoxaparina (tabella 4). Gli studi clinici sono stati condotti in una popolazione di pazienti con età media > 65 anni. Negli studi clinici di fase 3 non sono state riscontrate differenze in termini di efficacia e sicurezza fra uomini e donne. Della popolazione di pazienti che ha partecipato agli studi RE-MODEL e RE-NOVATE (5.539 pazienti trattati), il 51% soffriva di ipertensione concomitante, il 9% di diabete concomitante, il 9% di coronaropatia e il 20% aveva una storia di insufficienza venosa. Nessuna di queste patologie ha mostrato di interferire sugli effetti di dabigatran sulla prevenzione di TEV o sulla frequenza di sanguinamento. Dati relativi all'endpoint TEV maggiore e mortalità correlata a TEV erano omogenei rispetto all'endpoint primario di efficacia e sono indicati nella tabella 4. I dati dell'endpoint per la TEV totale e la mortalità per tutte le cause sono riportati nella tabella 5. I dati degli endpoint di sanguinamenti giudicati maggiori sono elencati nella tabella 6 sotto riportata.

Tabella 4: Analisi di TEV maggiore e mortalità correlata a TEV durante il periodo di trattamento negli studi di chirurgia ortopedica RE-MODEL e RE-NOVATE

Studio	Dabigatran etexilato 220 mg	Dabigatran etexilato 150 mg	Enoxaparina 40 mg
RE-NOVATE (anca)			
N	909	888	917
Incidenza (%)	28 (3,1)	38 (4,3)	36 (3,9)
Rischio relativo rispetto enoxaparina	0,78	1,09	
95% IC	0,48 - 1,27	0,70 - 1,70	

RE-MODEL (ginocchio) N	506	527	511
Incidenza (%)	13 (2,6)	20 (3,8)	18 (3,5)
Rischio relativo rispetto enoxaparina	0,73	1,08	
95% IC	0,36 - 1,47	0,58 - 2,01	

Tabella 5: Analisi di TEV totale e mortalità per tutte le cause durante il periodo di trattamento degli studi di chirurgia ortopedica RE-NOVATE e RE-MODEL

Studio	**Dabigatran etexilato 220 mg**	**Dabigatran etexilato 150 mg**	**Enoxaparina 40 mg**
RE-NOVATE (anca) N	880	874	897
Incidenza (%)	53 (6,0)	75 (8,6)	60 (6,7)
Rischio relativo rispetto enoxaparina	0,9	1,28	
95% IC	(0,63 - 1,29)	(0,93 - 1,78)	
RE-MODEL (ginocchio) N	503	526	512
Incidenza (%)	183 (36,4)	213 (40,5)	193 (37,7)
Rischio relativo rispetto enoxaparina	0,97	1,07	
95% IC	(0,82 - 1,13)	(0,92 - 1,25)	

Tabella 6: Episodi di sanguinamento maggiore (ESM) a seguito del trattamento nei singoli studi RE-MODEL e RE-NOVATE

Studio	**Dabigatran etexilato 220 mg**	**Dabigatran etexilato 150 mg**	**Enoxaparina 40 mg**
RE-NOVATE (anca) Pazienti trattati N	1146	1163	1154
Numero di ESM N(%)	23 (2,0)	15 (1,3)	18 (1,6)
RE-MODEL (ginocchio) Pazienti trattati N	679	703	694
Numero di ESM N(%)	10 (1,5)	9 (1,3)	9 (1,3)

5.2 Proprietà farmacocinetiche. Dopo somministrazione orale, dabigatran etexilato è rapidamente e completamente convertito in dabigatran, che è la forma attiva nel plasma. La scissione del profarmaco dabigatran etexilato per idrolisi catalizzata da esterasi al principio attivo dabigatran è la reazione metabolica predominante. La biodisponibilità assoluta di dabigatran dopo somministrazione orale di Pradaxa è pari a circa il 6,5%. Dopo somministrazione orale di Pradaxa a volontari sani, il profilo farmacocinetico di dabigatran nel plasma è caratterizzato da un rapido aumento delle concentrazioni plasmatiche con C_{max} raggiunta in 0,5-2,0 ore dopo l'assunzione. Assorbimento. Uno studio che valutava l'assorbimento post-operatorio di dabigatran etexilato, 1-3 ore dopo l'intervento, ha dimostrato un assorbimento relativamente lento rispetto a quello riscontrato nei volontari sani, dimostrando un profilo concentrazione plasmatica-tempo senza elevati picchi di concentrazioni plasmatiche. Le concentrazioni plasmatiche al picco sono raggiunte 6 ore dopo la somministrazione in un periodo post-operatorio a causa di fattori quali anestesia, paresi intestinale ed effetti chirurgici, indipendentemente dalla formulazione orale del medicinale. In un ulteriore studio è stato dimostrato che un assorbimento lento e ritardato si manifesta solitamente solo il giorno dell'intervento. Nei giorni successivi l'assorbimento di dabigatran è rapido con concentrazioni plasmatiche al picco raggiunte 2 ore dopo la somministrazione del medicinale. Il cibo non altera la biodisponibilità di dabigatran etexilato, ma ritarda di 2 ore il tempo per il raggiungimento della concentrazione plasmatica al picco. Distribuzione. È stato osservato un basso legame (34-35%), indipendente dalla concentrazione, di dabigatran alle proteine plasmatiche umane. Il volume di distribuzione di dabigatran pari a 60-70 L supera il volume dei fluidi corporei totali indicando moderata distribuzione tissutale di dabigatran. La C_{max} e l'area sotto la curva concentrazione plasmatica-tempo erano proporzionali alla dose. Le concentrazioni plasmatiche di dabigatran hanno mostrato un calo biesponenziale con un'emivita media terminale di 12-14 ore nei volontari sani e di 14-17 ore nei pazienti sottoposti a chirurgia ortopedica maggiore. L'emivita era indipendente dalla dose. Metabolismo ed eliminazione. Metabolismo ed escrezione di dabigatran sono stati studiati a seguito di somministrazione di una dose singola di dabigatran radioattivo per via endovenosa a soggetti maschi sani. Dopo una dose endovenosa, la radioattività derivata da dabigatran era eliminata principalmente con le urine (85%). L'escrezione fecale era stimata essere il 6% della dose somministrata. Il recupero della radioattività totale era compreso fra 88 e 94 % della dose somministrata entro 168 ore dalla somministrazione. Dabigatran è soggetto a coniugazione con la formazione di acilglucuronidi farmacologicamente attivi. Esistono quattro isomeri posizionali 1-O, 2-O, 3-O, 4-O degli acilglucuronidi ciascuno stimato per meno del 10 % del dabigatran totale nel plasma. Tracce di altri metaboliti sono rilevabili solo con metodi analitici altamente sensibili. Dabigatran è eliminato principalmente in forma immodificata con le urine, ad una velocità di circa 100 ml/min corrispondente alla velocità di filtrazione glomerulare. Popolazioni speciali. *Insufficienza renale*. L'esposizione (AUC) a dabigatran dopo somministrazione orale di Pradaxa è approssimativamente 2,7 volte maggiore nei volontari con insufficienza renale moderata (ClCr compresa tra 30 e 50 ml/min) rispetto a quelli senza insufficienza renale. In un ristretto numero di volontari con grave insufficienza renale (ClCr 10-30 ml/min), l'esposizione (AUC) a dabigatran era approssimativamente 6 volte maggiore e l'emivita circa 2 volte più lunga di quella osservata in una popolazione senza insufficienza renale (vedere paragrafi 4.2, 4.3 e 4.4). *Pazienti anziani*. Studi specifici di farmacocinetica condotti in soggetti anziani mostravano un aumento dal 40 al 60% dell'AUC e di più del 25 % della C_{max} rispetto ai soggetti giovani. Studi di farmacocinetica basati sulla popolazione hanno valutato la farmacocinetica di dabigatran dopo dosi ripetute in pazienti (fino a 88 anni). L'aumento osservato dell'esposizione a dabigatran era correlato alla riduzione della clearance della creatinina dovuta all'età (vedere paragrafi 4.2 e 4.4). *Insufficienza epatica*. Non è stata rilevata alcuna alterazione dell'esposizione a dabigatran in 12 soggetti con insufficienza epatica moderata (Child Pugh B) rispetto a 12 soggetti di controllo (vedere paragrafi 4.2 e 4.4). *Peso corporeo*. Studi di farmacocinetica basati sulla popolazione hanno valutato la farmacocinetica di dabigatran in pazienti di peso corporeo compreso tra 48 e 120 kg. Il peso corporeo aveva un minor effetto sulla clearance plasmatica di dabigatran risultante in una maggiore esposizione nei pazienti con basso peso corporeo (vedere paragrafi 4.2 e 4.4). *Genere*. L'esposizione al principio attivo nelle pazienti di sesso femminile è circa dal 40% al 50% superiore che nei pazienti di sesso maschile e non è raccomandato un aggiustamento posologico. *Origine etnica*. La farmacocinetica di dabigatran è stata investigata in volontari caucasici e giapponesi a seguito di somministrazione di dosi singole e multiple. L'origine etnica non influisce sulla farmacocinetica di dabigatran in modo clinicamente rilevante. Non sono disponibili dati di farmacocinetica in pazienti di razza nera. *Interazioni farmacocinetiche*. Gli studi di interazione in vitro non hanno mostrato alcuna inibizione o induzione dei principali isoenzimi del citocromo P450. Ciò è stato confermato dagli studi in vivo effettuati su volontari sani, in cui non è stata evidenziata alcuna interazione tra questo trattamento ed i seguenti principi attivi: atorvastatina (CYP3A4), digossina (interazione con il trasportatore P-glicoproteina) e diclofenac (CYP2C9). L'esposizione a dabigatran nei soggetti sani era aumentata del 60% in presenza di amiodarone. **5.3 Dati preclinici di sicurezza.** I dati degli studi non-clinici non rivelano rischi particolari per l'uomo sulla base di studi convenzionali di sicurezza farmacologica, tossicità per dose ripetuta e genotossicità. Gli effetti osservati negli studi di tossicità per somministrazione ripetuta erano dovuti all'effetto farmacodinamico amplificato di dabigatran. È stato osservato un effetto sulla fertilità femminile nella forma di diminuzione degli impianti ed aumento della perdita pre-impianto a dosi di 70 mg/kg (5 volte il livello di esposizione plasmatica nei pazienti). A dosi tossiche per la madre (da 5 a 10 volte il livello di esposizione plasmatica nei pazienti), nei ratti e nei conigli è stato osservato un calo del peso corporeo del feto e della vitalità con un aumento delle variazioni fetali. In uno studio pre- e post-natale, è stato osservato un aumento della mortalità fetale a dosi tossiche per la madre (dose corrispondente a un livello di esposizione plasmatica 4 volte superiore a quello osservato nei pazienti). Gli studi di carcinogenicità con dabigatran non sono ancora stati completati.

6. INFORMAZIONI FARMACEUTICHE

6.1 Elenco degli eccipienti. Contenuto della capsula. • Acido tartarico. • Gomma arabica. • Ipromellosa. • Dimeticone 350. • Talco. • Idrossipropilcellulosa. Capsula. • Carragenina. • Potassio Cloruro. • Titanio Diossido. • Indigo Carminio (E132). • Giallo tramonto (E110). • Ipromellosa. • Acqua depurata. Inchiostro nero per stampa. • Gommalacca shellac. • Alcool N-butilico . • Alcool isopropilico. • Etanolo denaturato industriale. • Ferro ossido nero (E172). • Acqua depurata. • Glicole propilenico. **6.2 Incompatibilità.** Non pertinente. **6.3 Periodo di validità.** Blister e flacone: 3 anni. Una volta aperto il flacone, il prodotto deve essere utilizzato entro 30 giorni. **6.4 Precauzioni particolari per la conservazione.** Blister. Conservare nella confezione originale per tenerlo al riparo dall'umidità. Flacone. Conservare nella confezione originale per tenerlo al riparo dall'umidità. Tenere il flacone ben chiuso. **6.5 Natura e contenuto del contenitore.** Confezioni contenenti 1, 3 o 6 blister strip di alluminio, divisibili per dose singola (10 x 1, 30 x 1, 60 x 1). Il blister consiste in uno strato superiore di alluminio rivestito da copolimeri acrilati di polivinilcloruro-polivinilacetato (PVCAC acrilati) a contatto con il prodotto e in uno strato inferiore di alluminio rivestito da polivinilcloruro (PVC) a contatto con il prodotto. Flacone di polipropilene con tappo a vite contenente 60 capsule rigide. È possibile che non tutte le confezioni siano commercializzate. **6.6 Precauzioni particolari per lo smaltimento e la manipolazione.** Quando si utilizza Pradaxa confezionato in blister, devono essere osservate le seguenti istruzioni: • La capsula rigida deve essere estratta dal blister sollevando il foglio di alluminio posto sulla parte posteriore. • La capsula rigida non deve essere spinta attraverso il blister. • Il foglio di alluminio del blister deve essere sollevato solo quando occorre una capsula rigida. Quando si utilizzano le capsule confezionate in flacone, devono essere osservare le seguenti istruzioni: • Il flacone si apre premendo e ruotando il tappo. Il medicinale non utilizzato ed i rifiuti derivati da tale medicinale devono essere smaltiti in conformità alla normativa locale vigente.

7. TITOLARE DELL'AUTORIZZAZIONE ALL'IMMISSIONE IN COMMERCIO
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EU/1/08/442/005. EU/1/08/442/006. EU/1/08/442/007. EU/1/08/442/008

9. DATA DELLA PRIMA AUTORIZZAZIONE/RINNOVO DELL'AUTORIZZAZIONE
18 marzo 2008

10. DATA DI REVISIONE DEL TESTO
10 giugno 2008

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Boehringer Ingelheim

hematology meeting reports 2009
volume 3
number 2

table of contents

9th International Winter Meeting on Coagulation

Basic, Laboratory and Clinical Aspects of Venous and Arterial Thromboembolic Diseases

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PRIMARY HEMOSTASIS I

COOPERATIVE CALCIUM SIGNALING AND PLATELET RECEPTORS UNDER FLOW

M.R. Cozzi, L. De Marco

National Cancer Institute, CRO, Aviano, Italy

Collagen plays a central role in hemostasis and thrombosis; it activates platelets through collagen receptor glycoprotein (GP) VI and integrin α2β1 and induces platelet plug formation and occlusion at sites of vessel damage under physiological shear condition by recruting platelets to exposed collagen. At arterial shear rates von Willebrand Factor (VWF), which binds to collagen via A3 domain, plays a critical role in the initiation of thrombus formation by tethering platelets to the VWF/collagen complex through GPIb-IX-V binding to the VWF-A1 domain. Subsequent firm platelet adhesion is supported by the two collagen receptors GPVI and α2β1. Increase in intracellular calcium is one of the most common occurring transduction mechanism in response to different platelet agonists. Thus, we have investigated the calcium signaling relationship between GPIb-IX-V, GPVI and α2β1 in regulating adhesion of platelets perfused over immobilized collagen in the presence of dimeric WVFA1A2A3. We analyzed concurrently platelet adhesion and calcium transients in single FLUO3-AM labeled platelets interacting with fibrillar type I collagen using a videoimaging apparatus.

By analyzing individual $[Ca^{++}]_i$ traces in platelet perfused over collagen/VWFA1A2A3 at 3000 s^{-1} (arterial shear rate), we can identify four types of signals: Full, Reduced, Partial and Transient, each starting with rapid raise in intracellular calcium but differing in form and intensity. Activated control platelets exhibited a Full or Reduced response. Both types of response were essentially abolished by treating the platelets with anti α2β1 or anti GPVI antibody and only Partial or Transient calcium oscillation were observed. Experiments performed in normals with either low or hight α2β1 densities revealed a decreased surface coverage by almost 50% in the former, accompanied by significantly decreased Full types of signals and an increase of the Reduced ones. Our results demonstrate that α2β1 plays a pivotal role in platelet arrest following platelet tethering through A1 domain-GPIb-IX-V interaction and that amplification and temporal modification of calcium signals are obtained by the concerted action of the two receptors, reinforced by the activation through GPVI, leading to full platelet activation.

A threshold of calcium signals generated by GPIb-VWFA1 interaction and by collagen receptor activated machinery must be reached to produce global and high magnitude calcium signals. Sustained calcium elevation signals are therefore necessary to induce an effective and withstanding platelet adhesion.

INHERITED PLATELET FUNCTION DISORDERS

M. Cattaneo

Unità di Ematologia e Trombosi, Ospedale San Paolo, DMCO, Università di Milano, Italy

Congenitalplatelet disorders can alter circulating platelet numbers, function or both. These conditions are typically manifested by symptoms of excessive mucocutaneous bleeding and rapid onset, excessive bleeding following invasive surgical and dental procedures or trauma. Disorders of platelet function include defects of: 1) platelet receptors for adhesive proteins (e.g., Bernard-Soulier syndrome, Glanzmann thrombasthenia), 2) platelet receptors for soluble agonists (e.g., defects of $P2Y_{12}$), 3) platelet granules (e.g., storage pool deficiency); 4) signal transduction pathways (abnormalities of the arachidonate/thromboxane A_2 pathway, of the stimulatory G-protein alpha-subunit), 4) procoagulant phospholipids (Scott syndrome). The diagnostic laboratory assessment for evaluation of a suspected platelet function defect should include an assessment of blood counts, a careful evaluation of the blood smear, and an evaluation of platelet size (mean platelet volume). Assessments of platelet function, by assays of aggregation and secretion, are commonly used for the diagnostic evaluation of platelet disorders. More specialized tests are helpful to confirm conditions that have been suspected based on the results of the initial screening tests. Therapy is not warranted for bruising. Platelet transfusions should be reserved for individuals with serious bleeding unresponsive to medical therapies. Recombinant Factor VIIa is useful in the treatment of bleeding episodes of patients with alloimmunization from platelet transfusions. Desmo-pressin and fibrinolytic inhibitors are useful for treatment of less severe bleedings. Treatment of menorrhagia needs to be individualized, and should take into consideration the individual's wish for pregnancies or contraception.

INHERITED HUMAN $gp91^{phox}$ DEFICIENCY IS ASSOCIATED WITH IMPAIRED ISOPROSTANE FORMATION AND REDUCED PLATELET RECRUITMENT

P. Pignatelli,[1] R. Carnevale,[1] S. Di Santo,[1] S. Bartimoccia,[1] V. Sanguigni,[2] A. Plebani,[3] S. Basili,[1] F. Violi[1]

[1]Department of Experimental Medicine, Division of Internal Medicine H, University of Rome, La Sapienza; [2]Department of Internal Medicine, University of Rome Tor Vergata, Rome; [3]Department of Pediatrics, University of Brescia, Italy

Platelet isoprostane PGF2α-III, a pro-aggregating molecule, is believed to derive from non enzymatic oxidation of arachidonic acid. We assessed the hypothesis that PGF2α-III derives also from $gp91^{phox}$ activation and

contributes to the phenomenon of platelet recruitment. We studied PGF2α-III in platelets from 8 male patients with hereditary deficiency of $gp91^{phox}$ (Nox2) the catalytic subunit of NADPH oxidase, and 8 male controls. $Gp91^{phox}$ deficency is a very rare human disease (prevalence 1:1000.000) characterized by life-threatening infections. Patients were a subgroup from a multicentre study in collaboration with the Italian Primary Immunodeficiency Network (IPINET). Upon stimulation platelets from healthy subjects produced PGF2α-III, that was inhibited 8% by aspirin and 43% by an inhibitor of $gp91^{phox}$. Also, in platelets incubated with a $gp91^{phox}$ inhibitor or with SQ29548, a thromboxane A2 receptors inhibitor, platelet recruitment, an *in vitro* model of thrombus growth, was reduced by 52% and 84% respectively; a lower effect (-13%) was seen with aspirin. Incubation of normal platelets with PGF2·-III dose-dependently (1-100 pM) increased platelet recruitment by mobilizing platelet Ca^{2+} and activating gpIIb/IIIa. Platelets from patients with $gp91^{phox}$ hereditary deficiency had normal thromboxane A2 formation and 75% PGF2α-III reduction compared to controls. In $gp91^{phox}$ deficient patients agonist-induced platelet aggregation was within the normal range while platelet recruitment was reduced compared to controls. Incubation of $gp91^{phox}$-deficient platelets with PGF2α-III (1-100 pM) partially restored platelet recruitment. In conclusion this study provides the first evidence that platelet PGF2α-III maximally derives from Nox2 activation and contributes to platelet recruitment via activation of gpIIb/IIIa.

VARIABILITY OF ASPIRIN RESPONSE: FUNCTIONAL VS THROMBOXANE-DERIVED TESTS

G. Davì

Chieti, Italy

In clinical practice, treatment failure is a common phenomenon, well known by physicians, occurring with ant cardiovascular drug used to prevent atherothrombosis. Therefore, the clinical diagnosis of an atherothrombotic event in a patient while on a therapeutic dose of aspirin should be more appropriately classified as "treatment failure", rather than "clinical resistance" to therapy. The vast majority of studies reporting the occurrence of aspirin "resistance" in different clinical settings have relied upon single or repeated measurements of platelet function *ex vivo* using one or more of the following techniques: i) light transmittance aggregometry in platelet-rich plasma; ii) the platelet function analyzer (PFA)-100 developed as a bedside, rapid whole blood assay; iii) the VerifyNow Rapid Platelet Function Assay (RPFA), also a bedside, whole blood assay. Each of these techniques challenges the capacity of blood platelets to respond to an aggregating stimulus(i) (eg, arachidonic acid) added at variable concentration in a largely artificial environment. Platelet aggregation, as measured by conventional methods ex vivo, has less than ideal intrasubject and intersubject variability, and displays limited sensitivity to the effect of aspirin, which is often considered to be a "weak" antiplatelet agent based on such measurements. Moreover, the relevance of changes in this index of functional capacity to the actual occurrence of platelet activation and inhibition *in vivo* is largely unknown. The clinical studies relying on these functional assays are biased by several other major limitations, including lack of biochemical or witnessed verification of compliance, use of arbitrary criteria to define the normal versus the aspirin resistance range and variable assay conditions. These biases, together with the fact that non of these techniques directly reflect the mechanism of action of aspirin, contribute to a highly variable detection rate of "aspirin-nonresponsive" patients. Serum TXB_2 and urinary 11-deydro-TXB2 provide reliable information on the maximal biosynthetic capacity of circulating platelets *ex vivo* and on the actual rate of TXA_2 biosynthesis *in vivo*, respectively. These measurements have been used extensively to characterize the clinical pharmacology of aspirin as an antiplatelet drug. Because the maximal biosynthetic capacity of human platelets is several thousand times as high as the basal rate of TXA_2 biosynthesis in healthy subjects, the relationship between the inhibition of platelet COX-1 activity and TXA_2 biosynthesis *in vivo* is strikingly nonlinear. The inhibition of platelet COX-1 attains functional relevance when the maximal biosynthetic capacity to generate TXA_2 is reduced by at least 95%. Assessing the adequacy of platelet COX-1 inhibition by low-dose aspirin would require the availability of a pre-dosing measurement of serum TXB_2 or reference to a standardized threshold level of this analyte. Urinary 11-dehydro-TXB2 excretion provides a non-invasive, time-integrated index of whole body TXA2 production. Because platelets are not the only source of TXA2 biosynthesis, the urinary excretion of 11-dehydro-TXB2 is only reduced incompletely by 60 to 80% following aspirin administration. Thus, persistent metabolite excretion in aspirin-treated patients may provide a useful index of aspirin-insensitive mechanisms of TXA2 biosynthesis. The clinical relevance of aspirin-insensitive TXA2 biosynthesis has been explored by Eikelboom et al, who reported, in a nested case-control study, that aspirin-treated, high-risk patients in the upper quartile for baseline urinary 11-dehydro-TXB2 excretion had a 1.8 times higher risk of major vascular events than those in the lower quartile. Because of the highly variable rate of 11-dehydro-TXB_2 excretion and the variability in the platelet *v s.* extra-platelet origin of its formation, defining a threshold level of metabolite excretion for assessing the adequacy of platelet COX-1 inhibition by aspirin is simply not feasible. Recently, the effect of aspirin has been reported to be variably and randomly detected by functional assays in healthy volunteers, given the documented fluctuating nature of this apparent "non-responder" phenotype, most likely reflecting the relatively poor intra-subject reproducibility of functional measurements. The variability of the thromboxane-independent component of the different aggregation signals and the instability of the "resistant" phenotype are likely to be amplified, rather than diminished, by cardiovascular diseases.

PRIMARY HEMOSTASIS II

PATHOPHYSIOLOGY OF ACUTE CORONARY THROMBOSIS: THE ROLE OF TISSUE FACTOR

V. Toschi

Department of Hematology and Blood Transfusion, San Carlo Borromeo Hospital, Milan, Italy

Experimental evidences have demonstrated that acute coronary thrombosis occurs upon rupture of the thin fibrous cap of a vulnerable atherosclerotic plaque. Tissue factor (TF) becomes exposed to flowing blood and activation of the coagulation takes place by binding of TF to factor VII/VIIa [1]. It has been shown that TF is particularly abundant into the lipid rich, necrotic core of the plaque which is therefore the most thrombogenic component of the lesion [2]. Binding of TF to factor VIIa causes thrombin generation which in turn induces platelet activation and fibrin formation which leads to partial or total thrombotic occlusion of the coronary artery and to the acute coronary syndromes (ACS): myocardial infarction and unstable angina [3]. We have shown that the activation of coagulation within the coronary vessels at sites of atherosclerotic plaque rupture is largely TF-dependent, since a significant reduction in both platelet and fibrin deposition was obtained by local pretreatment of atherosclerotic lesions with the recombinant analogue of the physiologic tissue factor pathway inhibitor (rTFPI) or with a specific anti-TF antibody [4]. It has been shown that, besides local factors, systemic pro-thrombotic mediators may contribute to coronary thrombosis. Specifically, circulating plasma soluble TF (sTF) might be an important clinical biomarker in patients with ACS and my also contribute, as additional causative factor, to coronary obstruction [5]. An increase in sTF is independently associated with cardiovascular mortality in patient with ACS thus suggesting that plasma sTF levels may have a prognostic significance in ACS patients. Studies by us and others showed that circulating TF may be important to sustain thrombosis in addition to locally active TF present into the plaque. Blood-borne TF, which is mainly released by leukocytes, is essential for propagation of thrombus after its initial formation on the vascular surface [6]. Further studies suggested that platelets might be also a potential source of circulating TF. Immunoreactive and functionally active TF has been demonstrated on platelet membrane and its expression may be increased after stimulation of these cells by agonists such as ADP and thrombin [7]. It is therefore conceivable that platelet associated TF may be delivered by these cells at the site of thrombus formation and may thus contribute to its growth and to coronary obstruction.

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PLATELET ACTIVATION AFTER CORONARY STENT IMPLANTATION

M. Camera

Dept. of Pharmacological Sciences, University of Milan and Centro Cardiologico Monzino IRCCS Milan, Italy

Coronary stenting has become a mainstay of interventional cardiology and drug-eluting stent (DES) are, nowadays, the treatment of choice for patients with symptomatic coronary artery disease undergoing percutaneous coronary intervention (PCI). Platelet activation is an inherent part of the vascular response to PCI with stent implantation and may affect both short- and long term outcomes in patients treated with DES. In fact, PCI induces injury to the vessel, disrupting the atherosclerotic plaque, thereby triggering the production of thrombin, the stimulation of platelets, and ultimately, mural thrombosis at the site of vascular injury. Additionally, patients requiring PCI often present with existing platelet hyper-reactivity; therefore, PCI induced platelet activation may occur in an already prothrombotic environment. The clinical implications of thrombotic events following PCI include stent thrombosis, myocardial infarction, and consequently, potentially increased mortality.

For these reasons, antiplatelet therapy is the cornerstone of peri- and post-procedural care for patients undergoing PCI. In particular, dual antiplatelet therapy with aspirin and clopidogrel is associated with improvement in long-term clinical outcomes in such patients and is presently the antiplatelet therapy of choice for secondary prevention of atherothrombotic ischemic events in patients who undergo PCI.

Notwithstanding, a significant number of patients experience recurrent events while on such therapy. This is not surprising given that even dual antiplatelet therapy with aspirin and clopidogrel does not inhibit all pathways of platelet activation. Insights into the pathophysiology of late DES thrombosis indicate that DES cause substantial impairment in arterial healing characterized by lack of complete re-endothelialization and persistence of fibrin. This delayed healing is the primary substrate underlying all cases of late DES thrombosis at autopsy. To prevent late and very late ST following DES implantation, careful patient selection, meticulous implantation technique and antithrombotic drug administration for at least 12 months and in high-risk patients even longer is recommended.

DIFFERENT CLINICAL EFFICACY OF ANTIPLATELET TREATMENTS IN PATIENTS WITH CLAUDICATION

F. Violi

Prima clinica medica, Centro Aterotrombosi, La Sapienza, Università di Roma, Italy

Peripheral artery disease (PAD) is a common atherosclerotic disorder that involves not only the arteries of the leg but also the coronary and cerebral arteries. In 2,000, there were an average of 5 million adults aged more than 40 years who were classified as having PAD on the basis of an ankle-brachial index (ABI) <0.90. If risk factors remain stable, more than 8 million US residents, who are older than 40 years and over, will have PAD by the year 2020, according to census population projections. PAD has a tremendous social impact since its natural history could be complicated by myocardial infarction (MI) and stroke; accordingly, the risk of MI and stroke is much higher than in PAD free patients. Platelets play an important role in the process of atherothrombosis via release of molecules that are injurious for vascular walls precipitating thrombosis at the site of plaque injury. There is evidence indicating that platelets are activated in patients with PAD. In the last meta-analysis of Antithrombotic Trialists' Collaboration (ATC), on the clinical efficacy of antiplatelet treatment in PAD patients, it appeared evident that this drug class could favourably influence cardiovascular outcomes. Thus, antiplatelet treatment was associated with a 23% risk reduction of vascular events including MI, stroke and vascular death in overall population with PAD. Taking into account that patients with claudication represent the vast majority of patients affected by PAD, it could be relevant to be certain that this clinical setting is actually favorably influenced by antiplatelet treatment. Our new meta-analysis shows that thienopyridines reduce the risk of cardiovascular events by 22% (p=0.018) while a non significant reduction was observed with aspirin (-13%, p=0.273) or picotamide (-21%, p=0.302). The different impact of drugs affecting several intraplatelet activation signalling, may provide new insight on the role of platelets in the pathophysiology of atherosclerotic progression in patients with generalized atherosclerosis such as those with claudication.

MECHANISMS IN HEMOSTASIS AND THROMBOSIS

MOLECULAR BASES OF THE MODULATION OF COAGULATION FACTOR LEVELS

F. Bernardi, B. Lunghi, G. Marchetti

Department of Biochemistry and Molecular Biology, University of Ferrara, Italy

The control of clotting factor levels is crucial for the hemostatic balance. High levels of coagulation factor VIII (FVIII), factor IX (FIX), and factor XI (FXI) have been consistently associated with an increased risk of venous thrombosis. Moreover, factor VII (FVII) levels in patients with ongoing myocardial infarction were independent predictors of mortality and reinfarction. Although the heritability of plasma levels of several coagulation factors is high (around 0.40 for FVIII and FIX, and higher than 0.50 for FVII) the molecular bases of raised levels are poorly known for several factors. Whereas several polymorphisms contribute to predict FVII levels in the population, only one intragenic variation (*D1241E*), and particularly the E1241 allele, has been found to be associated with minor differences (10% reduction) in circulating FVIII level. At present, the genetic bases of raised FIX and FXI levels remain to be explained. Genes involved in the biosynthesis, processing and survival in plasma of coagulation factors might play a part in determining factor levels, and thus in the modulation of coagulation through pleiotropic effects. Oligosaccharide structures, like determinants of ABO blood groups, might be implicated in structure-function relationships of coagulation factors and in their catabolism/clearance. ABH antigenic determinants are carried on N-linked oligosaccharide chain of both von Willebrand factor (vWF) and FVIII and the ABO locus strongly influences FVIII and vWF levels. Specifically, non-0 group is associated with increased levels of FVIII and vWF. Relation between ABO groups and other coagulation factors has been poorly investigated. Receptors could participate in binding and sequestration of coagulation factor from plasma. The low density lipoprotein receptor-related protein (LRP) is a multifunctional member of the LDL receptor superfamily, abundantly expressed on a wide range of different cell types, including hepatocytes, monocytes and smooth muscle cells. It recognizes a broad range of ligands, belonging to several families of proteins and including serine proteases and protease/inhibitor complexes. Several evidences have suggested that LRP might play a role in hemostasis. LRP has been demonstrated, in cellular and animal models, to participate in the regulation of “clearance” of FVIII, FIXa and FXa, by binding and directing them to the intracellular degradation pathways. The carriership of alleles predicting differential LRP expression has been associated with with lower FVIII and FXI levels. The interaction of genetic components involved in transcription, post-translational modification and in clearance of circulating proteins could

produce ample differences in circulating levels of coagulation factors. Combined ABO/LRP genotypes have been associated with gradient in FVIII levels and up to 50% variations in mean FVIII activity levels in plasma. Taken together data provide strong evidence for genetic "regulators" of coagulation factor levels within coagulation factor genes, and in genes involved in protein expression and survival. Genome wide studies have also provided strong genetic evidence for additional unknown "regulators" of coagulation factor levels. A number of genetic loci (quantitative trait loci, QTLs) have been identified, and among those QTLs on chromosome 18, 5 and 11 have been suggested to predict FVIII levels. The contribution of genotype combinations to thrombosis liability needs to be investigated in large epidemiological studies.

TOWARD A BETTER CLASSIFICATION OF ANTIPHOSPHOLIPID SYNDROMES

V. Pengo

Clinical Cardiology, Thrombosis Center, University of Padova, Italy

The main problem with the diagnosis of APS is that we are not dealing with a rare combination of signs and symptoms but with very common disorders (thrombosis and pregnancy loss) potentially sustained by the presence of circulating 'antiphospholipid antibodies'. It is obvious that if laboratory criteria for diagnosis include any single positive test result out of all those determining the presence of aPL antibodies, the number of diagnosed patients will be very large. A limitation was made by the new Sydney statement according to which the *medium high titer of aCL* was defined as >40 GPL or 40 MPL or (better) >99th percentiles of normal controls. Likewise, the newly introduced a β2GPI criterion was considered positive when titers exceeded the 99th percentiles. The number of (false positive) diagnoses of APS will thus be reduced and dangerous, long-term anticoagulant treatment will be avoided. Nevertheless, the criterion of single test positivity was maintained and patients will continue to be classified in categories according to the sole positivity for LA (IIa), for aCL (IIb) or for aβ2GPI (IIc). It is possible that laboratories in which the diagnosis of APS is based on the determination of a single test may formulate incorrect diagnoses because of misinterpretation and/or lack of standardization.

PLACENTAL HEMOSTASIS – ROLE OF MICROPARTICLES

A. Aharon, B. Brenner

Thrombosis and Hemostasis Unit, Rambam, Health Care Campus, Haifa, Israel

Pregnancy is an acquired hypercoagulable state, with gestational vascular complications (GVC) including preeclampsia, intrauterine growth restriction (IUGR), pregnancy loss and placental abruption being a major cause of maternal morbidity and fetal mortality. Placental thrombotic findings inwomen with GVC are associated with inherited and acquired thrombophilia, yet the mechanisms leadin toward thrombosis inwomen with GVC and the underlying placental-maternal crosstalk providing for its development are not clearly understood.[1] Micro-particles play a dominant role in coagulation initiation and thrombus formation.[2] Microparticles bearing tissue factor (TF), the central coagulation cascade initiator, and adhesion molecules such as P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1), accumulate in developing clots in areas of cell injury, and promote thrombus formation.[3] The high levels of negatively charged phospholipids, such as phosphatidylserine (PS), on microparticle membranes may enable the transition of TF from its dimeric, nonactive encrypted form to the active, procoagulant TF-VIIa complex.[4] Moreover, the PS leaflets promote membrane fusion and transfer of both proteins and lipids to cells, including activated platelets endothelial cells and leukocytes.[5] Normal pregnancy is characterized by increased levels of platelet and endothelial-derived MPs.[6] Studies on the prevalence of MPs in pregnancy found increased levels of procoagulant MPs in the pregnancy loss group compared to healthy pregnant women.[7] Preeclamptic women also displayed increased levels of circulating leukocyte, platelet, T-cell and granulocyte-derived MPs[8] when compared to healthy pregnant women. Syncytiotrophoblast microvilli are shed into the maternal circulation and their levels have been monitored to rise significantly in preeclamptic women.[9] Excess shedding of syncytiotrophoblast derived MPs is a feature of early- onset preeclampsia, but not of normotensive intrauterine growth restriction. The increased shedding of syncytiotrophoblast-derived MPs, seen during pre-eclampsia may be induced by placental ischemia and oxidative stress. In a recent study we evaluated MPs TFPI expression and its relation to normal pregnancies and GVC hypercoagulability. Using two different methods of flow cytometry, a significant reduction in the number of TFPI-positive MPs in healthy pregnant, GVC and LMWH groups compared to non-pregnant group was observed.[10] These observations suggest that the altered hemostatic balance measured during pregnancy may result from decreased TFPI levels and shifts in levels of circulating TF-positive MPs. The MPs TF\TFPI ratio has the potential to contribute to detection of hypercoagulability in human pregnancy.

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CANCER AND VENOUS THROMBOEMBOLISM

HEMOSTASIS AND THROMBOSIS CENTER

D. Imberti

Piacenza Hospital, 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 cancer patients; for example, the optimum duration of pharmacological treatment, the management of thrombosis related to the use of central venous catheters or of VTE recurrences, the possible role of vena cava filters or of the new antithrombotic drugs. Finally, VTE treatment is particularly problematic in oncohaematological patients, especially on account of the high risk of haemorrhage caused primarily by prolonged and often severe thrombocytopenia. Unfortunately, the efficacy and safety of the various treatment methods in this category of patients are not supported by adequate evidence in different contexts, and there are considerable differences in approach in clinical practice.

LIMITATIONS OF EXISTING EVIDENCE TO PREVENT VENOUS THROMBOEMBOLISM IN THE CANCER PATIENT AND FUTURE PERSPECTIVES

A. Falanga

Hemostasis and Thrombosis Center, Department of Hematology-Oncology, Ospedali Riuniti di Bergamo, Italy

Cancer represents a recognized acquired thrombophilic condition. Thrombotic complications commonly occur in patients with cancer. In particular, the estimated risk of VTE is 0.5%/year or 0.04%/month and is 6.5-fold increased by chemotherapy. Preventing VTE complications is important in cancer patients because VTE significantly affects morbidity and mortality. Furthermore, the diagnosis of VTE can be often more difficult, and the treatment of VTE can be less effective and more toxic. Finally, cancer-associated VTE can cause interruption of chemotherapy and can impact on the use of health care resources. However the effective and safe prevention of VTE in this population remains a challenge because of variable evidence from clinical trials and meta-analyses. The available guidelines offer recommendations for pharmacological or non-pharmacological prophylaxis of VTE in patients with cancer in several situations (i.e., surgery, hospitalization, cancer chemotherapy on ambulatory basis, central venous catheters, recent prior VTE), and for use of anticoagulation as a possible adjunct to cancer therapy. Nevertheless, the existing data addressing these issues are limited and there is a need for further research by means of large randomised controlled clinical trials. Limitations of the existing guidelines issued from the major International Scientific Societies to prevent VTE in cancer patients include issues in: 1. the surgical setting, 2. the medical setting, and 3. the setting of treatment of established VTE. Also the benefits and harms of extended VTE prophylaxis in patients at high risk, such as the elderly or those with CNS malignancies, require further evaluation. In addition, the role of new anticoagulant agents, including the indirect anti-factor Xa inhibitor fondaparinux, the direct anti-Xa inhibitors, and the direct thrombin inhibitors, has to be tested. Finally the anti-cancer effect of anticoagulant drugs in various experimental models has been suggested by several studies, particularly addressing the role of LMWH on mortality in these patients. However, given the limitations of the available data, the use of anticoagulants in patients with cancer without VTE to improve survival cannot be currently recommended. Taken together, all these data may be encouraging and provide information for the design of future studies to evaluate anticoagulation in the cure of cancer. No studies have tested the effect of new anticoagulants on survival.

JAK2V617F MUTATION FOR THE EARLY DIAGNOSIS OF PH- MYELOPROLIFERATIVE NEOPLASMS IN PATIENTS WITH VENOUS AND ARTERIAL THROMBOEMBOLISM. A META ANALYSIS

F. Dentali

Department of Clinical Medicine, Insubria University, Varese, Italy

Patients with Philadelphia-negative myeloproliferative disease (MPD) have an high risk to develop venous and arterial thrombosis. Recent studies suggested that JAK2V617F mutation is frequent in patients with splanchnic vein thrombosis (SVT) but not in patients with other venous thromboembolic events (VTE). However, whether screening for the JAK2V617F mutation in VTE patients is justified remains unclear. Furthermore, limited data are available regarding the prevalence of the JAK2V617F mutation in patients with arterial thrombosis without distinct features of MPD. The aim of our systematic review was to assess the prevalence of the JAK2V617F mutation in VTE patients and in patients with arterial thrombosis. Further, we sought to examine the role of this mutation in the diagnosis of non overt Philadelphia negative myeloproliferative disease (MPD). Two reviewers, independently, selected studies evaluating JAK2 mutation in adult patients using the MEDLINE and EMBASE electronic databases. Study characteristics were extracted in duplicate. Pooled odds ratios (ORs) of case-control studies and weighted mean proportion of the prevalence of JAK2V617F mutation of uncontrolled series were calculated. Twenty four studies involving 3123 patients with VTE were included in our meta-analysis. Mean prevalence of JAK2 mutation was 32.7% (95%CI 25.5, 35.9%) in patients SVT. JAK2 mutation was associated with increased risk of SVT (OR, 53.98; 95% CI, 13.10, 222.45). MPD was diagnosed in 59.5% of SVT patients with JAK2V617F mutation (95%CI 51.3, 67.5%). Mean prevalence of JAK2 mutation in other VTE patients was low (range 0.88-2.57%). Six studies for a total of 535 patients with arterial thrombosis were included. The JAK2V617F mutation was found in five patients with arterial thrombosis, with a mean prevalence of 1.1% (95% CI 0.40, 2.29%). JAK2V617F mutation does not appear to be associated with a statistically significant increased risk of arterial thombosis in case control studies (OR 2.18; 95% CI 0.15, 30.64). In conclusion, the JAK2V617F mutation is strongly associated with the SVT risk. Presence of mutation in these patients is associated with a subsequent diagnosis of MPD in many patients. Therefore, routine screening of JAK2 mutation appears to be indicated in these patients. In contrast, this suggestion does not apply to patients presenting with VTE in other sites and to patients with arterial thrombosis, since the observed prevalence of this mutation was low and appeared to be similar to the prevalence reported in the general population. Thus, a routine screening of JAK2 mutation does not seem to be indicated in these patients.

UPDATES IN HEMOSTASIS AND THROMBOSIS

A NEW TOOL FOR THE AUTOMATED MEASUREMENT OF PROCOAGULANT PHOSPHOLIDS IN PLASMA: IS IT WORTH DOING AND DO THE RESULTS HAVE ANY CLINICAL RELEVANCE?

B. Woodhams, A. Rousseau, P. Van Dreden

R&D, Diagnostica Stago, Genevilliers, France

To date no simple methodology is available to routinely monitor procoagulant phospholipids (PPL) in plasma. The most common methodology involves use of flowcytometry which is slow, complicated, needs a high level of skill and the smaller microparticles may not be seen as they fall below the level of sensitivity of the instrument. These types of assay tend to quantitate the number and the type of microparticles and do not measure their activity. Working with T Exner (Australia) we have developed a very simple automated assay for PPL activity. This assay measures the total PPL activity in the plasma and does not differentiate between the types of microparticles or the source of the PPL. Diluted citrated plasma is mixed with phospholipid depleted substrate plasma and a FX calcium mixture and the clotting time measured: the shorter the clotting time the higher the PPL concentration. The assay is totally dependent on PPL and is not affected by Tissue Factor or activation of the intrinsic or extrinsic pathway. Like all assays of this type sample collection is most important as any cellular contamination of the sample could influence results especially if samples are stored frozen before testing. Double centrifugation should be used for all sample preparation. Essentially samples need to be prepared in a similar way to those used to prepare samples for Lupus Anticoagulant activity assays. Users need to be aware that as the assay is measuring PPL it will be sensitive to higher titre antiphospholipid antibodies. The APS status of the patients needs to be known as some APS antibodies will correct the PPL assay results, resulting in a higher clotting time (possibly a false negative result). This assay is now under evaluation in a number of different clinical situations. Examples of the types of clinical results being obtained are shown in the Table 1.

Table 1.

	PPL (sec)
Controls	57.6 (50.9-74.9)
Thyroid cancer – no metastasis	47.7
Thyroid cancer – with metastasis	28.1
Type I diabetes	33.8
Type II diabetes	48.3
Non-pregnant women	56.2
Normal pregnancy	34.6
Pre-eclampsia	31.1

The results obtained to date are very encouraging and a clear increase in PPL activity is seen in a number of hypercoagulable states. Further clinical studies are ongoing to validate and show the clinical utility of the test. The assay will be discussed with the problems and pitfalls known to date along with the clinical data that has been obtained to date. The data to date suggests that this new assay capable of measuring the contribution of the PPL in plasma may be a useful new assay both for clinical research and in the routine laboratory as an aid to diagnosis and monitoring patient therapy.

CURRENT RECOMMENDATIONS FOR THE MANAGEMENT OF ACQUIRED HEMOPHILIA

F. Baudo

International Panel on Acquired Hemophilia (IPAcHe), Milano, Italy

Acquired hemophilia A (AHA) is a rare bleeding disorder caused by an autoantibody to FVIII and characterized by sudden bleeding in patients without personal or family history. Bleeding is variable, ranging from acute, life threatening hemorrhage, with 9-22% mortality, to mild bleeding that requires no treatment. AHA usually presents to clinicians without prior experience with this syndrome, therefore diagnosis is frequently delayed and bleeds undertreated. Data on epidemiology and clinical picture are consistent but there is concern on the optimal treatment modalities to control the bleeding and to eradicate the inhibitor. Although a number of comprehensive reviews on AHA have been published, an international consensus for clinical practice is lacking. The aim of IPAcHe group was to compile a set of practice guidelines based on our collective clinical experience in treating a large number of patients with AHA. Structured literature searches were used to support expert opinion in the development of recommendations for the management of patients with AHA. Recommendations were formulated according to the method of Guyatt *et al.* where "we recommend" represents a strong (Grade 1) recommendation and "we suggest" a weak (Grade 2) recommendation. In contrast to congenital haemophilia, no high-level evidence exists to support treatment recommendations for patients with AHA. Data generated in congenital haemophilia patients with or without inhibitors may occasionally be used to support treatment decisions in patients with AHA. However treatment recommendations must generally rely on the expertise and clinical experience of physicians who have treated patients with this disorder.

Immediate consultation with a hemophilia centre experienced in the management of inhibitors is essential to ensure accurate diagnosis and appropriate treatment. Control of acute bleeding is the first priority and we recommend first-line therapy with bypassing agents such as recombinant activated FVII or activated prothrombin complex concentrate. Treatment to eradicate the inhibitor should be started as soon as the diagnosis has been achieved. Corticosteroids or combination of corticosteroids and cyclophosphamide are

suggested as first-line therapy. Rituximab is suggested as second-line therapy if first-line therapy fails or is contraindicated. These recommendations are intended to increase the awareness of this rare but often fatal syndrome among health care professionals to whom patients with AHA usually present and to provide a set of practice guidelines to improve the management of these patients.

RECOGNIZING ANTIPHOSPHOLIPID ANTIBODY SYNDROME (APLS)

B.L. Davidson

Weill Cornell Medical College-Qatar, Doha, Qatar

There is consistent agreement over many years and across international boundaries that patients with thrombosis and demonstrable antiphospholipid antibody levels warrant indefinite therapeutic anticoagulation unless contraindicated. This is due to the devastating types of thrombotic complications and the frequency of their occurrence. In fact, this might be the sole internationally agreed-upon indication for indefinite therapeutic anticoagulation, since there is controversy regarding both duration, intensity, and need for anticoagulation with other types of thrombophilic states. With international consensus on the pragmatic clinical need for therapeutic anticoagulation in APLS, it is perplexing that there is such controversy, perhaps even hair-splitting, regarding the technical aspects of making the diagnosis. A patient case will be presented, along with a proposal for increasing general and specialty physician awareness of the syndrome, improving the ease of diagnosis, and hence changing the paradigm of APLS from a highly specialized topic to one of general recognition, a diagnosis not to be missed.

TREATMENT OF RETINAL VEIN OCCLUSIONS

D. Prisco, E. Grifoni

Dipartimento di Area Critica Medico Chirurgica, Università degli Studi di Firenze; Centro Trombosi, DAI del Cuore e dei Vasi, Azienda Ospedaliero-Universitaria Careggi, Firenze, Italy

Retinal vein occlusion (RVO) is the second most common retinal vascular disease after diabetic retinopathy and an important cause of visual morbidity and blindness. It occurs with an estimated incidence of 0.53-1.6 per 1000 person/years and affects mainly patients over 50 years. RVO is frequently associated with systemic diseases and/or cardiovascular risk factors, such as hypertension, diabetes mellitus, and dyslipidaemia. In recent years, the possible role of thrombophilic factors in the pathogenesis of RVO has also been evaluated. RVO are classically divided into central or branch RVO based on the site of vascular occlusion. The pathogenesis, natural history, risk factors, prognosis and treatment vary significantly between the two entities. There is no current standard treatment for RVO in the acute setting. No medical or ocular intervention can reverse the retinal occlusion once it has occurred. Current management is therefore divided into identifying and managing modifiable risk factors to prevent a recurrence, and preventing and treating sight-threatening complications. Although various medical and surgical treatments have been attempted with varied success, laser therapy remains the mainstay intervention in RVO for preventing and/or treating complications such as macular oedema, retinal neovascularization, vitreous haemorrhage and tractional retinal detachment. The effect of anti-VEGFs intravitreal injection appears to be promising in the treatment of macular oedema, but its role in restoring and maintaining vascular homeostasis remains to be demonstrated. Other approaches such as the administration of intravitreal steroids or hemodilution are not supported by current evidence. The use of antithrombotic and thrombolytic agents in patients with RVO has been described in several reports, but there are currently no adequate clinical trials that have evaluated their efficacy and safety in this setting. However, there is a rationale for the use of both antiplatelet agents and anticoagulant drugs in patients with RVO: the former because RVO is frequently associated with cardiovascular risk factors and the latter because the thrombotic event occurs in the venous system. Recently, trials comparing the effect of low molecular weight heparin and aspirin for the treatment of RVO have been performed. Two randomized clinical trials were conducted by Faravash et al. to compare the effect of dalteparin and aspirin in patients with recent-onset central and branch RVO, respectively. Patients in the dalteparin group received subcutaneous dalteparin 100 IU/kg twice daily for 10 days, then 100 IU/kg once daily for another 10 days, whereas patients in the aspirin group were given aspirin 100 mg daily throughout the study. Dalteparin was found to be superior to aspirin in terms of improving visual acuity and preventing iris neovascularization in patients with central RVO, whereas no significant difference was found in the final visual acuity between patients treated by dalteparin or aspirin for recent-onset branch RVO. In a multicenter, randomized, double-blind, controlled trial by Ageno et al. patients with recent-onset RVO were randomized to receive a fixed daily dose of parnaparin, 12,800 IU for 7 days followed by 6,400 IU for a total of 3 months, or aspirin 100 mg/day for 3 months. Parnaparin was found to be more effective than aspirin alone in the prevention of functional worsening after RVO, significantly reducing vascular damage and improving visual acuity without any increase of either intraocular or systemic bleeding complications. Thus, these results suggest that the use of an initial course of anticoagulant treatment could be useful for the management of acute RVO; however, further studies on a larger number of patients are needed.

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MECHANISMS IN HEMOSTASIS AND THROMBOSIS I

TURNING THROMBOMODULIN ON PHARMACOLOGICALLY CAN TURN DISEASE PROGRESSION OFF

C. Esmon,[1,2] L. Thompson,[1] D. Qu,[1] M. Delvaeye,[3] E. Conway[3]

[1]Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; [2]Howard Hughes Medical Institute, Oklahoma City, OK, USA; [3]KU Leuven, Belgium

Thrombomodulin (TM) is a well established endothelial cell surface glycosaminoglycan that accelerates thrombin dependent activation of protein C, an anticoagulant enzyme with anti-inflammatory and cytoprotective activity, and the procarboxypeptidase, thrombin activatable fibrinolysis inhibitor (TAFI). Although TAFIa can inhibit fibrinolysis, its primary biological function is likely to inhibit vasoactive peptides like bradykinin and C5a. Thrombin binding to thrombomodulin blocks thrombin's ability to clot fibrinogen and activate platelets. Altering the rates and extents of biological processes is not limited to the reactions above. The lectin like domain of thrombomodulin has been shown to bind to and inhibit HMGB 1, a reported late stage mediator of sepsis. In addition, it can impair leukocyte attachment to the activated endothelium. Soluble TM has long been recognized as an effective therapeutic in animal studies of acute inflammatory disease. Recently, clinical studies have found it to be much safer than heparin with similar, if not superior, clinical outcomes in patients with infection induced DIC. Since soluble TM is only a weak protein C activator and a modest direct inhibitor of thrombin clotting activity, other activities associated with TM were likely to contribute in part to these protective effects. Recently, TM was shown to accelerate factor I inhibition of C3b and C4b, thereby contributing to the down regulation of complement. The latter activity is likely to contribute significantly to the protective effects of soluble TM in acute inflammatory disease. Down regulation of cellular TM is commonly observed in disease. Recent studies from Berend Isermann's laboratory has revealed down regulation of TM in experimental diabetes. This down regulation appears to contribute to disease progression since increasing activated protein C levels ameliorates the disease progression. To approach preventing TM down regulation in inflammation, we have recently begun to examine the role of specific adenosine receptors in preventing this process. A subgroup of these receptors is known to elevate cellular cyclic AMP levels and previous studies have shown in cell culture that this elevation of cyclic AMP can impair TM down regulation by inflammatory cytokines. We have now shown in murine disease models that this pathway is indeed important in preventing down regulation of TM. Thus we have gained a new understanding of the multiple important activities of thrombomodulin and a potentially efficacious approach to preventing down regulation with its important pathophysiological consequences.

INTERACTIONS BETWEEN MICROBES AND THE CLOTTING SYSTEM

J.H. Morrissey, T.H. Yun, S.H. Choi, S.A. Smith

Departments of Biochemistry and Internal Medicine, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Disseminated intravascular coagulation (DIC) can be a life-threatening complication of sepsis, in which the blood clotting system is activated extensively throughout the vasculature. The coagulopathies associated with sepsis are thought to be driven, in large part, by upregulation of tissue factor on the surface of monocytes/macro-phages. On the other hand, a number of direct interactions between infectious microorganisms and the blood clotting system have been reported, which may contribute both to normal host responses to pathogens as well as sepsis-induced coagulopathies. Here we examine two novel microbial activators/enhancers of blood clotting: polyphosphate and omptins. Polyphosphates are inorganic linear polymers of phosphate that are secreted from dense granules of human platelets; they also accumulate in a wide variety of infectious microorganisms. Polypho-sphate enhances blood clotting by acting at three points in the clotting cascade: (1) it triggers the contact pathway of blood clotting (and may be a long-sought pathophysiologic activator of this limb of the clotting cascade); (2) it accelerates the conversion of factor V to Va; and (3) it enhances fibrin clot formation, resulting in fibrin fibrils that are thicker and more resistant to fibrinolysis. Omptins are a family of outer membrane proteases found on the surface of certain gram-negative bacteria. We recently showed that omptins can accelerate tissue factor-induced blood clotting by proteolytically inactivating tissue factor pathway inhibitor (TFPI), the main plasma inhibitor of the initiation phase of blood clotting. The ability of omptins to inactivate TFPI requires the cells to express lipopolysaccharide with short O-antigens (rough LPS). Interestingly, *Yersinia pestis*, the etiologic agent of plague, naturally expresses rough LPS. The *Y. pestis* omptin (termed Pla) is required for virulence and is well known to convert plasminogen to plasmin via limited proteolysis. We have found, however, that TFPI is a far better substrate for Pla than is plasminogen. We hypothesize that Pla has a dual function in supporting the bubonic form of plague caused by *Y. pestis*. *This work was supported by grants from the National Institutes of Health and the Roy J. Carver Charitable Trust.*

PATIENTS WITH SEVERE SEPSIS AND MULTIPLE ORGAN FAILURE AFTER ADULT CARDIAC SURGERY. THE ROLE OF HUMAN PROTEIN C ZYMOGEN CONCENTRATE

G. Landoni, V. Testa, C. Gerli C.

Ospedale San Paolo, Milano, Italy

Background: In this observational study we report on our initial experience of PCc administration to consecutive patients who developed sepsis-related double organ failure shortly after cardiac surgery over a one-year period.
Objectives: To describe outcome and changes in clotting and inflammatory parameters in a case series of consecutive adult patients with severe sepsis at high risk for bleeding who received protein C concentrate after cardiac surgery.
Patients/Methods: Nine consecutive critically ill adult patients with severe sepsis or septic shock and two organ failure after cardiac surgery during the period from January 2007 to January 2008.
Interventions: All patients received protein C concentrate (Ceprotin® – Baxter) with a 50 IU/Kg bolus followed by a 3 IU/Kg/h continuous infusion for 72 hours.
Results: Normalization of protein C levels was accompanied by an early drop in interleukins followed by a marked improvement in prothrombin time, and antithrombin levels. No patient experienced drug related side effects. 30-day mortality was 11% (1 patient) compared to the expected mortality of 68%.
Conclusions: In this pilot study of nine patients with double organ failure induced by sepsis following cardiac surgery we observed a normalization of inflammatory and clotting parameters, no bleeding complications and a low 30-day mortality.

MECHANISMS IN HEMOSTASIS AND THROMBOSIS II

MECHANISMS OF CLOTTING FACTOR-MEMBRANE INTERACTION

J.H. Morrissey, V. Pureza, R.L. Davis-Harrison, M.A. McLean, S.G. Sligar, C.M. Rienstra, J. Boettcher, Y.Z. Ohkubo, E. Tajkhorshid

Departments of Biochemistry, Chemistry and Pharmaco-logy, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

The blood clotting cascade involves the assembly of serine proteases with their cognate protein cofactors on membrane surfaces, provided the membranes contain exposed anionic phospholipids. Despite their critical importance, the molecular details of protein-membrane interactions in blood clotting are not well understood. Calcium ions have been shown to induce anionic phospholipids to coalesce into membrane microdomains, and we propose that the proteins in blood clotting bind preferentially to these anionic lipid-rich microdomains, representing membrane "hot spots" upon which blood clotting reactions preferentially take place. Unfortunately, the experimenter typically cannot control the formation of membrane microdomains or nanodomains, nor can the experimenter control the partitioning of clotting proteins into or out of specific membrane subdomains; in essence, blood clotting proteins are free to find the membrane nanodomain for which they have the greatest affinity. Because of these limitations, we typically understand only the average contributions of phospholipids to blood clotting. The development of nanoscale membrane bilayers (Nanodiscs) now allows us to understand, with nanometer resolution, how the local phospholipid composition regulates the activity of protease-cofactor complexes in blood clotting. Furthermore, exciting progress in solid-state NMR and large-scale molecular dynamics simulations are allowing us to obtain new, highly detailed structural insights into the interactions between proteins and membrane surfaces. *This work was supported by grants from the National Institutes of Health and the Roy J. Carver Charitable Trust.*

D-DIMER WHAT FOR? MESSAGES FROM THE MOLI-SANI STUDY

M.B. Donati, A. De Curtis, L. Iacoviello, on behalf of the Moli-Sani Investigators

Research Laboratories, "John Paul II" Centre for High Technology Research and Education in Biomedical Sciences, Catholic University, Campobasso, Italy

Fibrin degradation in vivo leads to different products among which D-Dimer has been extensively studied as a marker of venous thrombosis/pulmonary embolism. Only recently interest has also been addressed to the plasma levels of D-Dimer as a parameter of the risk for cardiovascular (CV) ischemic disorders and a marker of hypercoagulability predicting cancer development. We

are presently evaluating the possible role of D-Dimer levels as a predictor of both cardiovascular and cancer events ("common soil hypothesis") in the general population. The Moli-Sani Study is an on-going epidemiological cohort study, on 25,000 men and women, aged ?35 years, randomly recruited from the general population of a Southern Italian region. D-dimer levels were measured on fresh citrated plasma by an automated latex enhanced immunoassay (HemosIL, IL, Milan, Italy). Until March 2008, 15,339 subjects were recruited. After exclusion of subjects with cardiovascular and malignant disease and incomplete questionnaires or missing D-Dimer values, 11,247 subjects, 5,909 women and 5,338 men, aged 54 ±12 (mean±SD) years, (34-98 range) were available for the analysis. Women had higher D-Dimer levels than men (211,5 ng/mL (206,6-216,5) vs. 198,9 (194,3-203,6), p<0.0001, respectively). In women, after multivariate ANOVA adjusted for age, physical activity, social status, diabetes, menopausal status, white blood cell count, CRP levels, smoking habits, D-dimer levels were positively associated with age and CRP, and negatively with physical activity and social status; moreover non-smoker women had D-dimer levels higher than former and current smokers. In men, multivariate ANOVA, adjusted for age, social status, diabetes, hypertension and CRP levels, showed a positive association of D-dimer levels with age and CRP levels). The association of D-Dimer levels with age was only apparent after the age of 66 in women and 56 in men.Thus in this preliminary analysis of the Moli-Sani study population, D-Dimer levels were independently associated with risk factors for cardiovascular disease both in men and in women. Such an association might help better characterizing the significance of D-Dimer as a bio-marker of the risk of cardiovascular disease, while the follow-up phase of the study (to be started in the second half of 2009) will allow us to answer the question whether D-Dimer levels may represent a predictive marker of cardiovascular or cancer events.

Supported by an unrestricted grant from Instrumentation Laboratory (Milan, Italy).

PLATELETS AND FIBRINOLYSIS: A COMPLEX RELATIONSHIP

M. Colucci, N. Semeraro, T.C. Ammollo, F. Semeraro

Department of Biomedical Sciences, Section of General and Experimental Pathology, University of Bari, Bari, Italy

Platelets play a pivotal role in the fibrinolysis resistance of arterial thrombi. Available data, mainly derived from *in vitro* studies, indicate that activated platelets may influence the fibrinolytic process through several mechanisms which may either up- or down-regulate the plasminogen-mediated degradation of fibrin. Earlier studies on this subject showed that platelets facilitate clot lysis, an effect largely dependent on clot retraction, and it was hypothesized that the contraction of the clot hastened fibrinolysis by increasing the local concentration of fibrinolytic enzymes. Platelets were also shown to bind plasminogen and promote plasmin formation, providing an additional mechanism of fibrinolysis stimulation. One limitation of these studies is that the experimental conditions were rather "unphysiological" (e.g. purified systems, extremely diluted plasma) and probably inadequate to unmask the true effect of platelets on clot lysis. In later studies, several *in vitro* models of fibrinolysis/thrombolysis, that better mimic the physiological conditions, have been developed to investigate the role of platelets in fibrin digestion. These studies consistently reported that the presence of platelets made the clot resistant to lysis through two main mechanisms: release of intraplatelet PAI-1 and, ironically, clot retraction, which was demonstrated to hamper fibrinolysis by reducing the diffusion of the fibrinolytic enzymes within the clot, particularly in areas containing platelet aggregates. Platelet dense granules contain polyphosphate, a linear polymer of phosphate units shown to alter the structure of fibrin fibers, yielding clots with increased resistance to fibrinolysis. It is unclear, however, whether platelet-derived polyphosphate inhibits fibrinolysis in a physiological environment.Another potential mechanism of fibrinolysis resistance induced by platelets involves TAFI (thrombin activatable fibrinolysis inhibitor). Platelet alpha granules contain detectable amounts of TAFI that are released upon cell activation and might increase the local concentration of this fibrinolysis inhibitor within a thrombus. Activated platelets, on the other hand, may promote TAFI activation by increasing the rate and time frame of thrombin generation. To date, the studies on this issue are scant and inconsistent. We investigated the role of TAFI in platelet-mediated fibrinolysis resistance by thromboelastography using whole blood made platelet-poor (PP-WB, <40,000/uL) or platelet-rich (PR-WB, >400,000/uL) by removing or adding autologous platelets. The susceptibility to t-PA-induced fibrinolysis decreased in parallel with the increase in platelet concentration (4-fold longer lysis time in PR-WB as compared to PP-WB). The platelet-dependent antifibrinolytic effect was reduced by more than 50% by a specific inhibitor of TAFIa or by a monoclonal antibody that prevents thrombin-mediated TAFI activation. A strong inhibition of fibrinolysis was also observed when PP-WB was enriched with platelet membranes, which are still capable of promoting TAFI activation but unable to induce clot retraction. Interestingly, the inhibition of fibrinolysis by platelets was less pronounced in the presence of a neutralizing anti-factor XI antibody, suggesting a role of the thrombin-mediated feedback activation of factor XI. Moreover, the efficacy of anticoagulants in stimulating fibrinolysis (by a TAFI-mediated mechanism) was variably influenced by platelets. Clinically relevant concentrations of unfractionated and low molecular weight heparins were strongly profibrinolytic in PP-WB but virtually inactive in PR-WB. On the contrary, the direct thrombin inhibitor dabigatran maintained most of its profibrinolytic activity also in the presence of a high platelet concentration. These new mechanistic insights may open new avenues in the management of thrombotic diseases.

NEUTROPHIL ACTIVATION AND THROMBOSIS: FRIENDS OR FOES?

N. Maugeri, A. Manfredi

University Vita-Salute San Raffaele, Milan, Italy

Epidemiologic, clinical, animal and experimental studies indicate a link between neutrophil function and thromboembolic events.[1-9] This observation is supported both from the effects of purely mechanical stimuli, including rheological changes and from biochemical findings, including the effect of neutrophil-derived eicosanoids or proteases, the expression of tissue factor induced by platelet P-selectin, TNF, C5a or antiphospholipid antibodies or by the effect of neutrophil products on coagulation cascade.[9-14] The attention of our group is now focused on the possible negative regulation exerted by neutrophils on thromboembolic events, which could justify both the association between neutrophil activation and "high risk" of thrombosis[11-14] and the transient cellular activation observed in patients with acute coronary syndromes.[1-3] We will discuss in particular two novel mechanisms, the cardioprotective function of pentraxin 3[15-16] and the clearance of activated platelets by neutrophils in circulation.[17] All together, clinical and experimental evidence suggest that the neutrophils can act as modulators of thromboembolic events. A better characterization of these mechanisms possibly represents a suitable target for molecular intervention.

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DIAGNOSIS AND TREATMENT OF THROMBOEMBOLISM

NEW THROMBOTIC RISK FACTORS IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

M. Ruggeri

Hematology Department, San Bortolo Hospital, Vicenza, Italy

Polycythemia vera (PV) and essential thrombocythemia (ET) are two chronic myeloproliferative diseases (cMPD)[1] with a clinical course characterized by a long median survival and with a low rate of transformation into acute myeloid leukemia and myelofibrosis,[2-4] but complicated by venous and arterial thrombotic episodes and, less frequently, by bleeding events.[5,6] At initial presentation, the incidence of thrombosis in PV and ET varied from 12-39% and 11-25%, respectively; during follow-up the incidence of thrombotic complications remain substantial. At diagnosis, the frequency of hemorrhages varies from 1.7-20% and during the follow-up the incidence is around 1.5-2% in both diseases. (Table 1-4).

Age (patients older than 60) and an history of previous thrombotic events are the most important predictors of thrombosis in patients with PV and ET.[5-9] The presence of either of these define an high- risk category of patients, candidate to cytoreductive treatment, with the aim to reduce/avoid the vascular episodes. The role of conventional cardiovascular risk factors, such as smoking, hypertension, diabetes, dyslipidemia is more contentious, and they are not considered in risk classification. Several studies have explored the contribution of hereditary and acquired thrombophilic states, without to reach a firm conclusion about a clear association with thrombosis.[13-15] Patients with PV and ET may have a higher risk of development of hemostatic complication after surgical procedures. A retrospective, multicenters survey was recently carried out in 716 PV and 1462 ET patient within GIMEMA framework16. 255 patients (105 PV and 150 ET) underwent to at least one surgery, for a total of 311 interventions. In this cohort, 23/311 major thrombotic events and 23/311 (7.3%) major hemorrhage episodes were recorded, with need of transfusion treatment, a prolonged hospital admission and new interventions in some cases. These rates appear higher than that observed in clinical trials with cancer surgical patients treated with heparin prophylaxis. There was no correlation between thrombotic or bleeding episodes and type of diagnosis, use of antithrombotic prophylaxis and type of surgery (major or minor intervention, urgent or elective procedure). Recently, somatic mutations within the Janus Kinase 2 (JAK2) gene have been shown to occur in more than 95% of PV and 50% of ET and PMF.[17-20] Most often the mutation occurs in the pseudo-kinase domain JH2 (JAK2V617F) but other mutations have been identified within exon 12 of the JAK2 gene which may also lead to a constitutive activation of the tyrosine kinase activity and its signaling.[21] Indeed, the JAK2V617F somatic mutation induces a deregulation and a gain of function of the JAK2 kinase, which leads to an abnormal cytokine response of hematopoietic progenitors, a subsequent increased production of blood cells and a constitutive functional activation of granulocytes and platelets. On the basis of these data mutations of the JAK2 gene are now considered not only as a key pathogenetic event for this group of diseases but also a possible, new risk factor for thrombosis. PV patients harboring greater than 75% JAK2V617F allele were found at higher risk to develop cardiovascular events compared to those with less than 25% mutant allele in a prospective evaluation22, but this finding was not confirmed in another study.[23] An association of JAK2 mutation and thrombosis was observed by some investigators,[24-25] but conflicting results were reported by other authors.[26-27] Finally, leukocytosis was recently found to be a risk factor for thrombosis in PV and ET patients. In a large prospective study on aspirin prophylaxis in PV (ECLAP, European Collaboration on Low-dose Aspirin in Polycythemia),[28] patients with a leukocyte count greater than 15×10^9/L had an increased risk of thrombosis compared with patients with white blood count lower than 10×10^9/L (HR 1.71; CI 1.1-2.6); this finding was confirmed by Mayo's researchers.[29] Similar results were shown in retrospective studies in ET cohorts.[30-31] Anyway, these two new risk factors should be validated in large, prospective studies before their incorporation in the classification of thrombotic risk

Table 1. Thrombosis and haemorrhage at diagnosis in polycythemia vera.

Authors	*Patients N*	*Asymptomatic %*	*Major thrombosis (AT plus VTE)*	*Major bleeding %*
Berk *et al.* 1981	431	NR	13	NR
Anger *et al.* 1989	141	NR	18	9
Najean *et al.* 1987	58	21	12	1.7
GISP 1995	1213	NR	20	NR
Petti et al. 1998	199	NR	13	NR
Passamonti *et al.* 2002	163	37	34	3
ECLAP 2005	1638	NR	38	8

AT, arterial thrombosis; VTE, venous thrombosis; NR, not reported.

Table 2. Thrombosis and haemorrhage at diagnosis in essential thrombocythemia.

Authors	Patients N	Asymptomatic %	Major thrombosis (AT plus VTE)	Major bleeding %
Belluci *et al.* 1985	94	67	22	3.2
Fenaux *et al.* 1990	147	36	18	4
Cortelazzo *et al.* 1990	100	34	11	9
Colombi *et al.* 1991	103	73	23	1.9
Van Genderen *et al.* 1997	68	14	7	1
Besses *at al.* 1999	148	57	25	NR
Jensen *et al.* 2000	96	52	14	5.2
Wolanskyj *et al.* 2007	322	63	26.3	10.6
Carobbio *et al.* 2007	439	NR	26[§]	NR

AT, arterial thrombosis; VTE, venous thrombosis; [§]at diagnosis or in the previous history; NR, not reported.

Table 3. Thrombosis and haemorrhage in polycythemia vera during follow-up.

Authors	Patients N	Asymptomatic %	Major thrombosis (AT plus VTE)	Major bleeding %
Berk *et al.* 1981	431	NR	25	NR
Anger *et al.* 1989	141	NR	40	NR
Najean *et al.* 1987	58	NR	10	1.7
GISP 1995	1213	NR	19	NR
Petti *et al.* 1998	199	NR	18.4	NR
Passamonti *et al.* 2002	163	NR	18.4	1.8
ECLAP 2005	1638	-	11.5	NR

AT, arterial thrombosis; VTE, venous thrombosis; NR, not reported.

Table 4. Thrombosis and haemorrhage in essential thrombocythemia during follow-up.

Authors	Patients N	Asymptomatic %	Major thrombosis (AT plus VTE)	Major bleeding %
Belluci *et al.* 1985	94	NR	17	3.2
Fenaux *et al.* 1990	147	NR	13.6	0.7
Cortelazzo *et al.* 1990	100	NR	20	1
Colombi *et al.* 1991	103	NR	10.6	5.8
Van Genderen *et al.* 1997	68	NR	28	21
Besses *et al.* 1999	148	NR	22.3	4.1
Jensen *et al.* 2000	96	NR	16.6	7.3
Wolanskyj *et al.* 2007	322	NR	31[1]	11.9[1]
Carobbio *et al.* 2007	439	NR	15.5[2]	NR

AT, arterial thrombosis; VTE, venous thrombosis; NR: not reported; [1]cumulative, at 5 years follow-up; [2]6.2 years follow-up.

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D-DIMER AND CLINICAL DECISION RULES REVISITED FOR THE DIAGNOSIS OF DEEP VEIN THROMBOSIS

M.H. Prins, A. Ten Cate-Hoek, M. Joore, on behalf of the AMUSE study group

Department of Epidemiology, Care and Public Health Research Institutes, University of Maastricht, the Netherlands

Background. Up to 90% of referred patients with suspected deep venous thrombosis of the leg do not have this disease. It would be ideal to safely exclude deep venous thrombosis at initial presentation in a large proportion of these patients. We conducted a management study in primary care to evaluate the safety and efficiency of excluding deep venous thrombosis using a clinical decision rule combined with a point-of-care D-dimer assay. In addition we analysed whether the clinical decision rule – in combination with the used D-dimer, needed an update and we analysed the cost-effectiveness of the new strategy. *Methods and results of the clinical study.* We included in a prospective cohort in primary care (approximately 300 practitioners) consecutive patients with clinically suspected deep venous thrombosis. Patients were managed based on the result of the clinical decision rule combined with a D-dimer result. Patients with a score ≤3 were not referred for ultrasound and received no anticoagulant treatment; patients with a score ≥4 were referred for ultrasound and received care as usual. The primary outcome was symptomatic, objectively confirmed, venous thromboembolism during 3 months of follow-up. The mean age of the 1028 study patients was 58 years and 37% were males. A valid score was obtained in 1002 patients (98%). In 500 patients (49%) the score was ≤3, of whom 7 developed venous thromboembolism within 3 months (1.4%; 95% CI 0.6-2.9%). In 502 patients (49%) the score was ≥4 and ultrasound was not performed in 3. Ultrasound showed deep venous thrombosis in 125 (25%). Of the 374 patients in whom the ultrasound was normal, 4 developed venous thromboembolism within 3 months (1.1%, 95% CI 0.3-2.7%). *Methods and results of the rule calibration* We applied three updating methods to the data of the above mentioned management study, to determine whether adjusting the weights of the included predictors or adding new diagnostic predictors could further improve the accuracy of the rule. The weights of the eight individual predictors did not need to be adjusted, but inclusion of 'history of DVT' and 'prolonged travelling' significantly added predictive value (p<0.05 using the likelihood ratio test). Although these new diagnostic variables were independent predictors of the presence of DVT, adding these to the rule did not improve the safety and efficiency. In fact, at equal safety (1.4% missed diagnoses among the non-referred patients), the efficiency was lower (43.5%) when using the updated rule compared to the original rule (49.4%). *Methods and results cost-effectiveness analysis.* A model based cost-effectiveness analysis was conducted in conjunction with a recent multi-centre prospective diagnostic study (AMUSE, N=1002). A

Markov model with a five year time horizon was used to compare the AMUSE strategy to hospital based strategies, with or without a rule. Probabilities were derived from AMUSE and the literature. Societal costs and health state utilities were used. One way and probabilistic sensitivity analyses were conducted. Cost-effectiveness acceptability curves were constructed. The AMUSE strategy had both slightly lower costs and less QALYs than both care as usual strategies. We compared the AMUSE strategy to the best hospital strategy. This resulted in a saving of € 138, and a QALY loss of 0,002. The iCER is € 56.436. The cost-effectiveness acceptability curves show that the AMUSE strategy has the highest probability of being cost-effective, even exceeding ceiling ratios over € 80.000. *Conclusions.* A diagnostic management strategy for suspected deep venous thrombosis in primary care using a simple clinical decision rule and a point-of-care D-dimer assay is feasible in clinical practice, reduces the need for referral by almost 50% and is associated with a low risk of subsequent venous thromboembolic events.
The clinical rule that was used in AMUSE does not need to be updated. In addition the AMUSE strategy to exclude DVT in primary care is cost-effective as compared

AN ORAL FXa INHIBITOR IN ORTHOPEDIC SURGERY

F. Piovella, M. Barone, C. Beltrametti, C. Picchi

U.O. Angiologia, Malattie Tromboemboliche, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Rivaroxaban is a novel, oral, direct Factor Xa inhibitor in advanced clinical development for the prevention and treatment of thromboembolic disorders. Four multinational, randomized, double-blind, double-dummy phase III studies (RECORD1, 2, 3 and 4) investigated rivaroxaban safety and efficacy for the prevention of venous thromboembolism (VTE) in patients undergoing major orthopaedic surgery. A total of 12,729 patients were randomized to receive oral rivaroxaban 10 mg once daily (od) starting 6-8 hours after surgery, or subcutaneous enoxaparin 40 mg od starting the evening before surgery (RECORD1–3), or 30 mg bid starting 12–24 hours after wound closure or adequate hemostasis (RECORD4). In these phase III trials it was effective in reducing deep vein thrombosis (DVT) and pulmonary embolism (PE) after orthopedic surgery and it is under investigation for the treatment of DVT and PE and for anticoagulation in atrial fibrillation. The RECORD2 study was designed to compare short-term thromboprophylaxis with a low molecular weight heparin – enoxaparin – with extended thromboprophylaxis for up to 5 weeks with a novel, oral, direct Factor Xa inhibitor – rivaroxaban – after total hip arthroplasty (THA). Patients received subcutaneous enoxaparin 40 mg once daily (od), beginning the evening before surgery, continuing for 10-14 days (short-term prophylaxis), and followed by placebo until day 35±4, or oral rivaroxaban 10 mg od beginning 6-8 hours after surgery and continuing for 35±4 days (extended prophylaxis). Mandatory, bilateral venography was conducted at the end of the extended treatment period. The primary efficacy endpoint was the composite of any DVT, non-fatal PE, and all-cause mortality. The main secondary efficacy endpoint was major VTE; the composite of proximal DVT, non-fatal PE, and VTE-related death. Major and non-major bleeding during double-blind treatment were the primary and secondary safety endpoints, respectively. A total of 2509 patients were randomized; 2457 were included in the safety population and 1733 in the modified intention-to-treat (mITT) population. Extended thromboprophylaxis with rivaroxaban was associated with a significant reduction in the incidence of the primary efficacy endpoint and major VTE, compared with short-term thromboprophylaxis with enoxaparin. The incidences of major and non-major bleeding were similar in both groups. In conclusion, extended duration rivaroxaban was significantly more effective than short term enoxaparin for the prevention of VTE, including major VTE, in patients undergoing THA. Furthermore, this large trial demonstrated that extended thromboprophylaxis provides substantial benefits for patients undergoing THA, and that the oral, direct Factor Xa inhibitor rivaroxaban provides a safe and effective option for such a strategy. Rivaroxaban was also compared with subcutaneous enoxaparin both given as extended thromboprophylaxis after total hip arthroplasty in the RECORD1 Trial. This was a phase III, multinational, randomized, double-blind, double-dummy trial, conducted to determine the efficacy and safety of oral rivaroxaban, compared with subcutaneous enoxaparin, for 5 weeks of thromboprophylaxis in patients undergoing THA. Patients received rivaroxaban 10 mg beginning 6-8 hours after surgery and once daily (od) thereafter, or enoxaparin 40 mg od, beginning the evening before surgery (restarting 6-8 hours after surgery). Therapy continued for 35±4 days and mandatory, bilateral venography was conducted the next day. The primary efficacy endpoint was the composite of any DVT, non-fatal PE, and all-cause mortality. The primary efficacy analysis was a test for non-inferiority in the per-protocol (PP) population, followed by a test for superiority in the mITT population. The main secondary efficacy endpoint was major VTE: the composite of proximal DVT, non-fatal PE and VTE-related death. Major and non-major bleeding during the active treatment period were the primary and secondary safety endpoints, respectively. A total of 4541 patients were randomized; 4433 were eligible for the safety population, 3153 for the mITT population, and 3029 for the PP population. The criteria for non-inferiority were met and testing for superiority was performed. Rivaroxaban significantly reduced the incidence of the primary efficacy endpoint ($p<0.001$) and major VTE ($p<0.001$), compared with enoxaparin, in the mITT population. The incidence of major and non-major bleeding events was similar in both groups. Rivaroxaban was here also significantly more effective than enoxaparin for extended prophylaxis after THA, with a similar safety profile. In RECORD3, a phase III trial, the efficacy and safety of once-daily (od) rivaroxaban was compared with od enoxaparin for the prevention of VTE in patients undergoing total knee arthroplasty (TKA). In this multicenter, double-blind, double-dummy trial, patients undergoing TKA were randomized to receive either oral riva-

roxaban 10 mg od or subcutaneous enoxaparin 40 mg od. Enoxaparin was initiated 12 hours before surgery, and rivaroxaban 6-8 hours after surgery; both were continued for 10-14 days. The primary outcome was the composite of DVT, PE, and all-cause mortality. Secondary efficacy outcomes included major VTE (the composite of proximal DVT, PE and VTE-related death) and symptomatic VTE. The main safety outcome was major bleeding, and secondary outcomes included non-major bleeding and adverse events. A total of 2531 patients were randomized; 2459 were eligible for inclusion in the safety population and 1702 for the modified intention-to-treat population. The primary efficacy outcome occurred in 9.6% of patients receiving rivaroxaban compared with 18.9% of those receiving enoxaparin (relative risk reduction 49%; $p<0.001$). Rivaroxaban was also significantly more effective than enoxaparin at reducing major VTE, with a relative risk reduction of 62%. Symptomatic VTE was also lower with rivaroxaban than with enoxaparin. The incidences of major and non-major bleeding were similar in both the rivaroxaban and enoxaparin groups, as was the incidence of other adverse events. During the treatment period, there were no deaths or PEs in the rivaroxaban group, and two deaths and four PEs occurred in the enoxaparin group. In conclusion, Rivaroxaban was superior to enoxaparin for the prevention of VTE after TKA in this study, with a similar, low rate of bleeding. RECORD4 was designed to determine the efficacy and safety of rivaroxaban compared with enoxaparin 30 mg administered twice daily after TKR. Patients (N=3,148) were randomized to receive either oral rivaroxaban 10 mg od (initiated 6–8 hours after surgery) or s.c. enoxaparin 30 mg every 12 hours (initiated 12 to 24 hours after surgery) for 10 to 14 days. Patients underwent mandatory, bilateral venography between day 11 and day 15. The primary efficacy endpoint was the composite of any DVT, non-fatal PE, and all-cause mortality up to day 17. The primary efficacy analysis was a test for non-inferiority in the per-protocol population (n=1,702), followed by a test for superiority in the modified intention-to-treat population (n=1,924) (if non-inferiority was established in the PP population). The main secondary efficacy endpoint was major VTE: the composite of proximal DVT, non-fatal PE, and VTE-related death. Treatment-emergent major bleeding observed no later than two days after the last intake of study was the main safety endpoint. The results are shown in the table. Rivaroxaban significantly reduced the incidence of the primary efficacy outcome compared with enoxaparin (6.9% *vs*. 10.1%, respectively; $p=0.012$; relative risk reduction 31%). Rivaroxaban was non-inferior to enoxaparin for the prevention of major VTE in the PP population ($p<0.001$). The observed incidences of major VTE and symptomatic VTE in those receiving rivaroxaban or enoxaparin were 1.2% vs 2.0% ($p=0.124$), and 0.7% *vs*. 1.2% ($p=0.187$), respectively, and the rates of major bleeding were 0.7% *vs*. 0.3% ($p=0.110$) respectively, and major and clinically relevant non-major bleeding 3.0% *vs*. 2.3% ($p=0.179$) in the rivaroxaban and enoxaparin treated groups, respectively. The data demonstrate that rivaroxaban has superior efficacy to enoxaparin 30 mg administered every 12 hours for the prevention of VTE after TKR, without significantly increasing the risk of bleeding. All outcomes, including symptomatic outcomes, were adjudicated by the same independent, blinded committees for all four studies. A pooled analysis of the four studies has subsequently been performed. In each of the studies, the rivaroxaban regimens tested significantly reduced the incidence of the primary efficacy outcome (total VTE; the composite of any DVT, non-fatal PE, and all-cause mortality) compared with enoxaparin regimens tested, with similar rates of bleeding in both groups. The rivaroxaban regimens also consistently reduced the incidence of major VTE (the composite of proximal DVT, non-fatal PE, and VTE-related death) in all four trials compared with the enoxaparin regimens tested. This pre-specified pooled analysis was performed on all randomized patients who received at least one dose of double-blind study medication to evaluate the effect of rivaroxaban on the composite of symptomatic VTE (comprising DVT or PE) and death, and bleeding. These primary outcomes were analyzed at day 12±2 in the active treatment pool (i.e. during the enoxaparin-controlled period common to all studies, to allow for unbiased comparison with enoxaparin), and for the total study duration pool (planned treatment period and 30-35 days follow-up). The results are shown in the table. Rivaroxaban significantly reduced the incidence of symptomatic VTE and death compared with enoxaparin regimens at day 12±2 (0.47% *vs*. 0.97%; $p=0.001$) and for the total study duration (0.81% *vs*. 1.63%; $p<0.001$). Rivaroxaban was not associated with a statistically significant increased risk of major bleeding. These data demonstrate that in the regimens tested, rivaroxaban reduced the composite of major clinical outcomes compared with enoxaparin regimens, with no significant increase in the risk of major bleeding in patients undergoing major orthopaedic surgery.

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TOO MUCH FIBRIN DEPOSITION

THE EXTRACELLULAR MATRIX REMODELING IN PERIPHERAL NERVE REGENERATION: A KEY ROLE FOR FIBRIN

S.C. Previtali

INSPE, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy

Our knowledge of the regulatory mechanisms and signaling cascade underlying the axonal regeneration program in human peripheral nervous system is still very limited. Furthermore the majority of the molecular players involved in nerve regeneration await identification. The extracellular matrix (ECM) in the endoneurium plays a crucial role in nerve regeneration. We selected nerve biopsies from patients with peripheral axonal neuropathy that were assigned in two distinct groups by the presence of efficient axonal regeneration or the absence of axonal regeneration, independently from their etiology. We investigated differences in the expression of several ECM components, including laminins, collagen, fibrin(ogen), fibronectin and vitronectin. Our results identified different ECM composition in the two groups. Patients with efficient axonal regeneration showed high expression of fibronectin while vitronectin and fibrin(ogen) were almost absent. Instead, patients with absence of axonal regeneration presented low expression of fibronectin and high expression of vitronectin and fibrin(ogen). Fibrin and vitronectin would constitute a primordial ECM necessary for immediate repair after damage. The subsequent transformation of the primordial ECM into mature ECM mainly composed by fibronectin is necessary for adequate and efficient nerve regeneration. Persistence of fibrin and vitronectin reflects defective/inappropriate ECM degradation that may cause defective nerve regeneration. Our result would suggest that different ECM composition might influence the outcome of axonal peripheral neuropathies.

THE EFFECTS OF PANCREAS AND ISLET TRANSPLANT ON THE COURSE OF SECONDARY DIABETIC COMPLICATIONS

A. Secchi

San Raffaele Scientific Institute, Internal Medicine, Università Vità e Salute, Milano, Italy

Pancreas/kidney-pancreas and islet transplant (tx) are a procedures still affected by major risk, particularly the first. Positive effects of these procedures on diabetic complications, were shown. Here we will discuss the positive effects of combined kidney-pancreas (KP) and kidney-islet (KI) tx on glucose metabolism, long term diabetic complications and survival. A reduction of diastolic dysfunction was observed 4 years after tx in KP (pre-tx: 73%, post-tx: 26%) but not in kidney-alone group (pre-tx: 88%, post-tx: 77%). Even after a successful islet transplantation a benefit on cardiovascular outcomes are evident. A normal endothelial function, lower intima media thickness were evident in KP and KI groups, and only 51% of KP remain hypertensive at 1 year after tx, versus 81% of kidney-alone group. Actuarial survival at 7 years improved both in KP and KI transplant. In conclusion, KP, and KI when successful, showed a better survival and reduced complications than kidney-alone group.

RISK ASSESSMENT AND MANAGEMENT

CURRENT CONCEPTS AND DATA FOR VENOUS ULTRASOUND IN CLINICAL TRIALS

S. Schellong

Dresden, Germany

In symptomatic patients with suspicion of DVT, venous ultrasound has to be considered as the standard procedure. Further evidence has been provided that the approach of a single examination of the entire venous system is feasible and safe. It awaits full recognition and implementation into clinical practice. In symptomatic patients with suspicion of PE, venous ultrasound should gain more attention as several studies, including one large outcome study, indicate that identification of patients with VTE by venous ultrasound reduces the number of CT scans, thereby contributing to radiation protection and resource saving. Several recent attempts have been made to validate venous ultrasound in asymptomatic patients against venography. Even if no direct comparison has been made, the results seem to be more promising in medically ill patients than in those early after major orthopaedic surgery. However, there is still insufficient data about the accuracy of centrally read ultrasound. This fact directly points to the unmet need of a consensus of standardization of venous ultrasound as an endpoint measure in clinical trials regarding the examination procedure itself, its documentation, and the adjudication process. Already finished as well as currently ongoing large scale trials in medically ill patients will hopefully contribute to this discussion. Suggestions of regulatory authorities seem to be made without full backup by trial results.

VENOUS THROMBOEMBOLISM PREVENTION INTERVENTION MANAGEMENT PROGRAMS

C.E. Mahan

Albuquerque, NM, USA

Introduction. Extensive gaps in the International Union of Angiology (IUA) and American College of Chest Physicians (ACCP) VTE prevention guidelines and current practice within international hospitals currently exist. "Appropriate Prophylaxis (AP)" has recently emerged in the medical literature as a new concept and is defined as appropriate type, dose and duration of thromboprophylaxis. Thus far in the US hospitals, published data for AP rates demonstrate rates of only 13-30%. Unpublished data from the VTE Study to Assess Rates of Thrombo-prophylaxis (VTE-START) confirm these rates. In 2006 and 2007, the National Quality Forum (NQF) and The Joint Commission (TJC) piloted (i.e. beta-tested) eight measures for the prevention of VTE in hospitalized patients in 55 United States (US) hospitals. In May of 2008, six of these measures were endorsed by the NQF for future implementation by both TJC and the Center for Medicaid Medicare Services (CMS) within US hospitals. "Preventable" VTE is the only outcome measure recently endorsed by the NQF and is defined as a non-upper extremity (UE) VTE for which AP was NOT used. Several programs have demonstrated that prophylaxis rate increases and/or VTE outcome reductions can be obtained by computerized alerts or flags. In addition, several recent pharmacy programs have demonstrated either an increase in prophylaxis or a decrease in VTE outcomes. To date, no published studies have shown reduced rates of "Preventable VTE" or the impact of a pharmacy program on the more stringent definition of AP. *Methods.* In conjunction with the NQF/TJC pilot, a physician-championed, pharmacy-led program was implemented at Lovelace Medical Center (LMC) in January of 2007 to prevent VTE in hospitalized patients. Utilizing the 7th ACCP guidelines, pharmacists used an individualized risk-assessment form to determine patient contraindications to pharmacologic prophylaxis as well as VTE risk factors for newly admitted patients and for patients transferred to a critical care setting. Physicians were contacted by pharmacists for at-risk patients not currently prescribed AP. For patients at-risk in whom pharmacologic prophylaxis was contraindicated, mechanical methods were recommended. At LMC, a prospective case-cohort study utilizing 2006 data as a control group and 2007 patients as the intervention group was conducted. AP and VTE events within 90 days of hospital discharge were measured by patient group. Patients' charts were reviewed to determine if the VTE event was hospital-acquired and preventable or non-preventable. Utilizing the NQF/TJC specifications, VTE events were defined as preventable if AP was not used. UE VTE's were counted as non-preventable. *Results.* In 2006 and 2008 respectively, 1879 and 1646 patients were assessed. The Odds Ratio (OR) for improved AP comparing 2007-2006 were 2.5, 1.6, 2.1, and 1.8 for critical care, medical, surgical patients, and overall patients, respectively. Overall compared to 2006, preventable VTE was also reduced by 74% (18.6/1000 *vs*. 5/1000 patients, p=0.0006) in 2007. *Conclusions.* A pharmacy-led intervention can significantly increase rates of AP and reduce preventable VTE in hospitalized patients. Moreover, with highly-trained staff, an individualized risk-assessment model is a validated method to improve VTE outcomes. Future studies are needed to determine if this program can be replicated at other hospitals to bridge the gap between recommended guidelines for VTE prophylaxis and actual practice. *Disclosures.* Kurt Mahan is an employee of Cardinal Health, speaks and consults for Sanofi-Aventis and Eisai Pharmaceuticals, and has received investigator-initiated research grants from Sanofi-Aventis.

VENOUS THROMBOEMBOLISM RISK FACTORS IN ACUTELY ILL HOSPITALIZED MEDICAL PATIENTS

A.C. Spyropoulos

University of New Mexico Health Sciences Center, Albuquerque, NM, USA

Introduction. Limited information is available on VTE risk factors present at hospital admission in acutely ill medical patients. There are few VTE risk scores in this patient population. Between July 2002 and September 2006, 15,156 patients in 52 hospitals and 12 countries

were enrolled in IMPROVE.[1] Univariate and multivariate analyses from IMPROVE to determine which patient characteristics at hospital admission were associated with clinical VTE risk for up to 3 months of follow up were performed using Cox regression modeling. Upper extremity DVT was excluded. *Results.* In total, 143 (0.9%) patients developed VTE. PE accounted for 53% of total VTE events. Most patients developed VTE events 8-30 days after hospital admission and nearly half of all events occurred post-hospital discharge. The multivariate analysis is shown in the Figure. A VTE risk score from 0-10 was developed using results from the multivariate analysis (Table) found the 3-month predicted VTE risk similar to the observed VTE rate. For patients with a score of 5-10, 3-month predicted VTE risk was 7.2% and the observed rate was 6.5%.

Table. Risk score points assigned to each independent venous thromboembolism risk factor identified.

VTE risk factor	*Points for the risk score*
Age >60 years	1
ICU/CCU stay	1
Immobilization ≥7 days	1
Current cancer	2
Lower limb paralysis	2
Thrombophilia	2
Previous VTE	3

ICU, intensive care unit; CCU, critical care unit; VTE, venous thromboembolism.

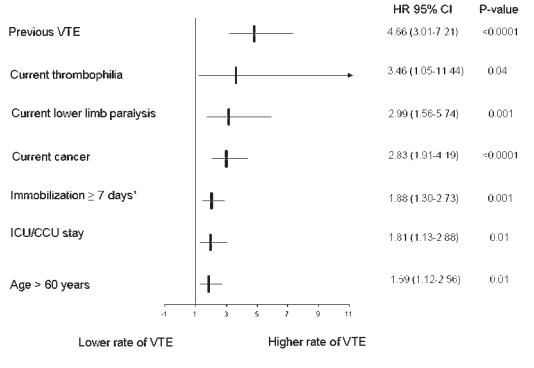

Figure. Multiple Cox regression estimates: factors at-admission associated with 3-month VTE risk. (n=15,125 patients with 143 VTE's).*Days immediately before admission plus days in hospital.

The crude death rates for the 30 patients who developed PE and the 17 patients who developed lower extremities DVT with a score of 0-1, are 20% and 6% respectively, while the crude death rates for the 46 patients who developed PE and the 50 patients who developed lower extremities DVT with a score >1 is 48% and 24%, respectively. *Conclusions.* Previous VTE, current thrombophilia, current lower limb paralysis, current cancer, immobilization ≥7 days, intensive/critical care unit (ICU/CCU) stay and age >60 years are risk factors which when present at hospital admission are associated with 3 month VTE risk. A VTE risk score using risk factors from this analysis was able to predict the observed VTE rate and importantly, death from VTE.

Reference

1. Tapson V et al. Chest 2007;132:936-45.

FROM CLINICAL RESEARCH TO CLINICAL PRACTICE: AN AGREED-UPON, WRITTEN PROTOCOL FOR PROPHYLAXIS OF POST-OPERATIVE VENOUS THROMBOEMBOLISM

M. Pini, C. Pattacini

Medicina II, Ospedale di Fidenza, Italy

Background. Results of sound clinical research should be applied to clinical practice in the best patient interest. Health authorities of our province called for adoption of written protocols for thromboprophylaxis in every Department of Surgery. A policy of active participation of surgeons and nurses in the development of agreed-upon, written protocols was recommended. *Methods.* We explained the program to at least 2 colleagues, including the Chief, of every surgical ward of our hospital and of the Anaesthesiology team. They were asked to compile a complete list of all type of operations performed in their Surgical Unit, and to classify the thromboembolic risk of each intervention as low, moderate, high, or very high. They were also asked to classify the hemorrhagic risk of the operation, as normal or high. We then developed a provisional protocol for every Surgical Units of our hospital, in which 4 prophylaxis modalities were suggested for every type of operations, according to the presence or absence of the main individual risk factors for thrombosis and bleeding. *Results.* The provisional protocol was discussed in a number of meetings with the surgical and anaesthesiology teams of every Unit, and the final protocol – which will be presented – was recently approved by the Directors of the Departments involved. It will be operative since March 16th, 2009, and will be subject to audit every 6 months, both in terms of compliance and of efficacy (rate of symptomatic thromboembolic events and major bleeding). *Conclusions.* An agreed-upon, written protocol for prophylaxis of post-operative venous thromboembolism is needed in every hospital. We present here an example of such a protocol, and the pathway followed to obtain consensus of the colleagues involved in its application.

GIUSEPPE MOSCATI: THE HOLY DOCTOR FROM NAPLES

J. Schellong

Dresden, Germany

Giuseppe Moscati was born July 25, 1880 in Benevent as an offspring of a family of lawyers. He started the study of medicine in 1897 and received his doctorate from the Faculty of Medicine at the University of Naples in 1903. Immediately upon receiving his degree, Moscati joined the staff of the Ospedale Incurabili. He won recognition for his actions in the aftermath of the eruption of Mount Vesuvius on April 8, 1906. In 1908 he became fellow of the institute of physiological chemistry and habilitated in 1911. The same year, he became a member of the Royal Academy of Surgical Medicine. When cholera broke out in Naples in 1911, Moscati was charged by the civic government with performing public health inspections, and with researching both the origins of the disease and the best ways to eradicate it. Besides his work as a researcher and as a doctor, Moscati was responsible for overseeing the directions of the local Institute of Anatomical Pathology. In 1917, he rejected to take over the chair of physiological chemistry at the University in order to continue with his clinical and educational activities. In 1922 Moscati was given a *libera docenza* in clinical medicine, which allowed him to teach at institutes of higher education. Notably, he was the first to use insulin for diabetes treatment in Naples. Moscati was of great piety, having vowed chastity early on. Still during lifetime, he was named the “doctor of the poor” due to his social engagement and charity, often providing free of charge care for underprivileged people. When he unexpectedly died in April 12, 1927, a crowd formed in the streets of Naples saying: “Morto il medico santo!” Moscati's body was initially buried in the cemetery of Poggio Reale, but three years later was exhumed and reinteered in the church of Gesù Nuovo. Based on ongoing adoration and popularity Moscati was beatified by the Roman Catholic Church in 1975 by Paul VI, and canonized in October 25, 1987 by Pope John Paul II. His feast day is November 16. The Major shrine is located at Gesù Nuovo, Naples, his attribute is the white coat.

SELECTED ABSTRACTS

PLASMA AND PLATELET PROTEIN S ANTIGEN LEVELS IN PATIENTS UNDER TREATEMENT WITH WARFARIN

C. Bulato, C.M. Radu, S. Gavasso, L. Spiezia, G. Tognin, D. Bertini, P. Simioni

Department of Cardiac, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Medical School, Padua, Italy

Background. Protein S (PS), a vitamin K-dependent anticoagulant protein, is synthesized by hepatocytes, endothelial cells and by a megakaryocytic cell line. In plasma, 40% of PS circulates in free form and the remaining 60% is complexed to the complement regulatory C4b-binding protein. PS is also contained in platelets but whether this is derived from megakaryocytic synthesis or from uptake of plasma PS is not known. It is hypothesized that platelet PS, released upon thrombin stimulation, becomes available as a cofactor for APC in the degradation of activated factor V on the platelet surface. Warfarin, a vitamin K-antagonist, is the principal anticoagulant agent used to prevent thrombosis in many disorders. *Aim of the study.* To evaluate the effect of warfarin on plasma and platelet PS antigen levels. *Patients and methods.* Thirty eight healthy subjects and thirty four patients under Oral Anticoagulant Therapy (OAT) with warfarin were enrolled. Plasma and platelet PS antigen levels were quantified with enzyme-linked immunoassorbent assay and expressed in percentage of normal plasma and platelet pool (normal range 70-120%). *Results.* PS plasma total levels (mean±SD) in normal and OAT patients were 120.8±17.0% and 83.1±26.7%, respectively. PS plasma free levels (mean±SD) in normal and OAT patients were 109.7±16.4% and 49.5±17.5%, respectively. PS platelet levels (mean±SD) in normal and OAT patients were 97.6±29.8% and 14.9±7.4%, respectively. Plasma and platelet PS concentrations were significantly lower in OAT patients than in controls ($p<0.001$). OAT patients showed a significant reduction of platelet PS compared with plasma total and free PS ($p<0.001$). *Conclusions.* Warfarin seems to have a stronger effect on PS concentration in platelets rather than on PS (free and total) in plasma. Further studies are necessary to clarify the origin of platelet PS and the mechanism of warfarin action on PS platelet levels.

PROCOAGULANT PHOSPHOLIPIDS ACTIVITY IN CANCER PATIENTS WITH VENOUS THROMBO-EMBOLISM

L. Spiezia,[1] C. Radu,[1] E. Campello,[1] S. Gavasso,[1] C. Abalotti,[1] B. Woodhams,[2] P. Simioni[1]

[1]Department of Cardiologic, Thoracic, and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Padua, Italy; [2]R&D, Diagnostica Stago, Gennevilliers, France

Background. Procoagulant phospholipids (PPL) activity depends mainly on the phosphatidylserine externalization and exposure on the membrane lipid bilayer which allow the activation of coagulative cascade and subsequently thrombus formation. The possible role of PPL in the pathogenesis of venous thromboembolism (VTE) in cancer patients is still unclear. *Aim of the study*. To investigate the association between PPL depending clotting time and clinical manifest VTE in cancer patients. *Methods.* PPL mean activity in 20 patients with cancer, who had an objectively proven acute VTE, and in 40 healthy subjects, age and sex matched, were evaluated. PPL test (Diagnostica Stago, Gennevilliers, France) consist of the measurement of clotting time (CT), in second, of a system in which the addition of a phosphilipid depleted substrate plasma makes the test dependent on the PPL of the same being tested. The shorter CT the higher PPL activity. *Results.* PPL depending clotting time (mean±SD) was shorter in patients with cancer plus VTE (48.25±17.92 sec.) than in healthy subjects (55.23±12.55 sec.) the difference was not statistically significant (p 0.085). *Conclusions*. Patients with cancer who presented with manifested VTE had an increase in procoagulants PPL levels than healthy controls. The observed association underscores the possibility that *in vivo* generated PPS initiate coagulation which may lead to thrombus formation. Further much larger studies are needed to validate our results.

HYPOFIBRINOLYSIS DUE TO INCREASE TAFIa PLASMA LEVELS IN CANCER PATIENTS WITH ACUTE VENOUS THROMBOEMBOLISM

L. Spiezia,[1] M. Castelli,[1] V. Rossetto,[1] S. Gavasso,[1] F. Dalla Valle,[1] B. Woodhams,[2] P. Simioni[1]

[1]Department of Cardiac, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Medical School, Padua, Italy; [2]R&D, Diagnostica Stago, Gennevilliers, France

Background. Despite the strong association between malignant disease and thromboembolic disorders, the molecular and cellular basis of this relationship remains unclear. *Aim of the study*. To investigate the association between activation of

fibrinolytic system and clinical manifest venous thromboembolism (VTE) in cancer patients. *Methods*. Thrombin Activatable Fibrinolysis Inhibitor (TAFIa) mean plasma levels in 20 patients with cancer, who had an objectively proven acute VTE, and in 40 healthy subjects, age and sex matched, were evaluated. To detected TAFIa plasma levels we used an enzyme immunoassay for TAFIa/TAFIai according to the instruction of the manufacturer (R&D Diagnostica Stago, Gennevilliers, France). *Results*. TAFIa plasma levels (mean ± standard deviation, SD) were higher in cases (29.76±24.53 ng/mL) than in controls (17.94±8.92 ng/mL). The difference was statistically significant ($p<0.005$). *Conclusions*. Patients with cancer who presented with manifested VTE had an hypofibrinolityc state due to the increase of the TAFIa mean plasma levels. The observed association could induce a thrombosis risk enhancement in cancer patients. The interplay between cancer and blood coagulation merits further experimental and clinical research.

INDEX OF AUTHORS