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Session VI. High risk patients and antithrombotic strategies

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Venous thromboembolism in the 3rd millennium: challenges to the management of venous thromboembolism in the elderly

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Venous thromboembolism is a major cause of morbidity in the elderly and of growing concern in the medical community. Despite the importance of lowering the risk of venous thromboembolism in the elderly, in most large-scale clinical trials this population has not been represented adequately. The advantages of anticoagulation may be offset by the risk of bleeding in older subjects: these are often on treatment with drugs that influence the pharmacokinetics of antithrombotic drugs and/or have diseases that inhibit/potentiate anticoagulation. Since the risk of bleeding because of oral anticoagulants is time and dose-dependent, measurements of D-dimers may help to identify subjects who require long-lasting treatment. However, age-related increases in D-dimers, hamper the use of this strategy in older individuals. In patients > 65 years of age undergoing general or orthopedic surgery, venous thromboembolism is a potentially fatal complication: without prophylaxis, the risk of any venous thromboembolism ranges between 26-64%. Prophylaxis with heparins reduces the risk by 52-70%. Thus, there is incomplete protection of the risk i.e. there is a significant residual risk of venous thromboembolism. The availability of safer and better antithrombotic drugs thus makes it conceivable to design new paradigms for antithrombotic strategies in the elderly, tailored at the individual risk of bleeding/thrombosis.

Introduction

The risk of venous thromboembolism (VTE) increases with age. After the age of 75, the annual incidence of VTE is 1-3/1,000.[1] VTE is a serious and potentially fatal complication associated with surgical trauma, particularly in elderly patients undergoing joint replacement or hip-fracture surgery. Other major factors [malignancy, abdominal surgery, low physical activity, immobilization, obesity, varicose veins, heart failure, chronic obstructive pulmonary disease, stroke, myocardial infarction, deep vein thrombosis, (DVT), or pulmunary embolism, (PE)] are common in the elderly.[2] Bedridden older patients have a risk of VTE comparable to that of surgical patients at moderate risk.[3] The use of a low-molecular weight heparin (LMWH), enoxaparin, significantly reduces the risk in these subjects. However, in patients ≥ 65 yrs of age, co-morbidities (e. g. renal/liver disease or anemia), and polypharmacy may enhance the risk of bleeding from unfractionated heparin (UFH) as well as by LMWH.[4] Oral anticoagulation (OAC) is the strategy of choice to prevent recurrent events in subjects with VTE. Since the risk of serious bleeding is dose and time-dependent[4,5] strategies to identify subjects at the highest risk of recurrence of VTE i.e. those who require long-lasting OAC, are urgently needed. In addition to compression ultrasonography (CUS), circulating levels of D-dimer (DD) have been suggested to help to identify such subjects. In addition to the risk of bleeding, the incidence of symptomatic VTE during prophylaxis with LMWH and/or warfarin in patients 65 years of age or older who have undergone unilateral total hip arthroplasty, ranges between 1-4%.[6-8] This makes the search for better and safer drugs for the management of VTE mandatory.

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Why are antithrombotic drugs not used more widely in the elderly?

Data from the MEDENOX trial show that bedridden older patients, because of common clinical conditions (chest infections, heart failure, respiratory failure, recent myocardial infarction [MI], recent stroke, malignancy, rheumatic disorders), have a risk of VTE comparable to that of surgical patients at moderate risk.[3] LMWH (enoxaparin 40 mg/d s.c.) reduced the risk by 62% (p<0.001 vs placebo) in this setting.[3] However, the latter information had little impact on the attitude of physicians to limit the use of any antithrombotic prophylaxis in the elderly. As a matter of fact, older patients are often on therapy with drugs that influence the pharmacokinetics of antithrombotic drugs.[4] Renal or liver disease and anemia are associated with an increased risk of bleeding, particularly gastro-intestinal (GI) bleeds, during antithrombotic therapy. Because of delayed excretion, impaired renal function may contribute to the bleeding tendency of older patients on LMWH.[4,9] Accordingly, LMWH should be used with caution in older patients with renal impairment and with hemorrhagic risk factors, and adjustment of the LMWHs dose is recommended in patients with severe renal failure (creatinine clearance <30 mL/min). UFH carries a similar risk. Interaction with other drugs can inhibit/potentiate the anticoagulant activity of warfarin by altering its metabolic clearance or reducing its absorption from the intestine. Interaction with aspirin and other non-steroidal anti-inflammatory drugs can potentiate the hemorrhagic effect of warfarin and increase the risk of bleeding. In support of the attitude

to limit the use of antithrombotic drugs in the elderly, the following information is relevant:

- with few exceptions[10,11] the majority of studies suggest that older age is a risk factor for bleeding complications.[12-15] In the Italian Study on Complications of Oral Anticoagulant Therapy (ISCOAT), the rate of hemorrhagic complications was 10.5%/year in subjects ≥70 years of age and 6.0%/year in those aged <70%.[13] A review of the safety of anticoagulation in the elderly has revealed a 2-fold increase in bleeding with warfarin in this setting.[16] Landefeld *et al.* found a relative risk of bleeding of 3.2 in patients aged 65 years or older.[17,18] In patients >75 years followed in Italian Coagulation Clinics, the efficacy of warfarin was offset by the increase in major bleedings (p=0.032, relative risk of 6.6);[19]
- there is a direct relationship between the intensity of warfarin treatment and the risk of bleeding in patients with VTE, in those with tissue heart valves and mechanical heart valves, and in patients with atrial fibrillation (AF).[4] Loeliger reported that the incidence of bleeding was 1.6% in the absence of warfarin therapy, 5.0% in the presence of an INR of 2.5 and 50% with an INR of 4.0.[20] In the study by Van Der Meer *et al.*, the risk of bleeding rose significantly with the intensity of the anticoagulation, with age and with the type of coumarin derivative used.[21] As the intensity of anticoagulation is an important predictor of bleeding, starting doses should be lower in older patients, and careful monitoring of the response to dose changes is mandatory to minimize the risk associated with long-term OAC.[22,23]
- In view of the exclusion criteria employed in some large clinical studies on OAC in AF, only a selected groups of *rather young, healthy* subjects have been evaluated. Moreover, because of bleeding and other contraindications, withdrawal of treatment was common in such studies.[24] Thus, despite the impressive results, at least 25% of the AF population do not receive any antithrombotic prophylaxis.[25-28] A nationwide survey of randomly selected office-based practitioners, showed that few doctors were likely to use warfarin in patients >75 years of age with non-valvular AF;[29,30]
- because of the inherent bleeding risk and other contraindications, alternative forms of treatment have been developed to replace warfarin to prevent recurrence in patients with a history of VTE. In 187 patients (aged >65 years) enoxaparin (4,000 U s.c./24 h for 3 months) was as effective as warfarin, and was associated with a lower risk of bleeding (p=0.04).[31] Similar results have been obtained in more recent studies.[32-36]

Optimal duration of anticoagulation

The direct relationship between the intensity and the duration of anticoagulation and the risk of bleeding (1-4%/year)[4,5] has fostered the need for proper stratification of patients at risk of recurrence of VTE. As to personal history, prospective cohort studies[37] have shown a lower risk of recurrence after withdrawing anticoagulant treatment in individuals with initial thromboembolic episodes triggered by time-limited factors (surgery, trauma, immobilization, estrogens) than in those with persistent predisposing factors (inherited thrombophilia, lupus-like anticoagulant). As to ultrasonography, in 313 patients with proximal DVT followed-up for 6 years after a 3-6 months period of anticoagulation, those with persistent venous obstruction were at the highest risk of recurrence (hazard ratio, 2.4, 95% CI 1.3-4.4, p=0.004 after adjustment for thrombophilia and spontaneous clinical presentation).[38] A similar prognostic value was observed in 179 patients with symptomatic first episode of DVT and in 104 patients with DVT occurring after hip replacement surgery, serially monitored by ultrasonography over a period of 12 months.[38] Recently, persistent vein obstruction, documented by repeated ultrasonography after proximal DVT, has been confirmed as an independent risk factor for recurrent VTE.[39] To improve the identification of high-risk individuals i.e. to refine the therapeutic guidance in the individual patient, there have been attempts to develop laboratory methods to provide a global measure of the degree of thrombophilia. In this respect, promising results have been obtained employing methods to measure DD during[40] or following[41] withdrawal of OAC. However, in a study examining the effect of age, race and functional status on plasma DD levels in community-dwelling elderly people, each decade of age was associated with a 25.9% elevation in DD or a 1.8 increased odds of having a DD >600 μg/L.[42] Baseline DD levels have been reported to be higher in patients >70 years undergoing abdominal surgery than in those <60 years.[43] Thus, *ad hoc* studies are needed to define age-related normal control values of DD in order to exploit this measurement in very old individuals, i.e. in subjects at the highest risk of bleeding/VTE. In addition to DD, quantification of the effect of activated protein C on thrombin formation has been suggested to help identify subjects at the highest risk of recurrences.[44,45] Besides raised median levels of fibrinogen, factors VII, VIII and IX, in the Third Glasgow MONICA survey, prothrombin fragment F1+2 (F1+2), and thrombin-antithrombin complexes (TAT) also increased significantly with age (46). In healthy subjects, the mean plasma levels of F 1+2, TAT and DD are 2- to 3- fold higher in individuals ≥60 years of age.[46-49]

Residual risk of VTE

A decline in the incidence of VTE among hospitalized and post-operative patients is presently perceived by the medical community. Although this is true in general, the incidence of PE is still a significant cause of death, and older subjects are particularly vulnerable to PE.[50,51] By the year 2030, 17% of the population in the United States will be >65 years of age.[52] Between 300,000 and 600,000 patients are hospitalized in the US each year for DVT[53] and nearly 30,000 die each year of PE.[54] Without prophylaxis, the risk of any DVT, diagnosed by routine venography, in hip or knee replacement or hip-fracture surgery ranges from 48-64.3%.[55] Under these conditions, the mortality following total hip replacement is 6-12%, PE being the most common cause of death (range 0.19-3.4%).[53] A 40-70% relative risk reduction is currently achieved by antithrombotic prophylaxis. Randomized studies show similar safety and higher efficacy (higher relative risk reduction) of LMWH as compared to low-dose UFH. In spite of this, a significant subset of subjects receiving LMWH for prophylaxis in orthopedic surgery is at risk of developing VTE. In other words, despite LMWH having improved efficacy with respect to UFH, it provides incomplete protection from the risk of VTE. An incomplete protection from VTE has also been documented in general surgery trials. In both instances, LMWH were as safe as and better at preventing VTE than low-dose UFH.[55] The possibility of replacing subcutaneous LMWH with adjusted-dose warfarin, hirudin or danaparoid to prevent VTE in patients at high risk of VTE undergoing total joint replacement or hip-fracture surgery is well documented: a residual risk of VTE is present with these drugs too.[55]

Newer antithrombotic strategies

Newer agents, including direct thrombin inhibitors, inhibitors of the factor VIIa/tissue factor pathway, and factor Xa inhibitors are under development and may allow higher selectivity and better control of anticoagulation than that currently achieved with warfarin or LMWH. Ongoing studies are evaluating new thrombin inhibitors in the prophylaxis of VTE in orthopedic surgery and in the treatment of acute VTE. Melagatran is a potent, synthetic, low molecular weight (430 Da) inhibitor that binds rapidly, competitively and reversibly to the active site of thrombin. Animal studies have demonstrated that melagatran may be given orally.[56] When administered s.c. melagatran is well tolerated in healthy subjects and in patients undergoing orthopedic surgery.[57] A novel, oral direct thrombin inhibitor, ximelagatran, has recently been developed. It is administered orally and is absorbed rapidly and transformed into its active form, melagatran. In the METHRO I study, a randomized, parallel group, controlled trial, 103 patients (mean aged 69 years) scheduled for elective total hip or total knee replacement, received s.c. melagatran (1, 2 or 4 mg *bid*) for 2 days commencing immediately before surgery. This was followed by oral ximelagatran (6, 12 or 24 mg *bid*) for 6-9 days. Anther 33 patients (mean aged 69 years) received dalteparin 5,000 IU s.c once daily for 8-11 days starting the evening before surgery. Venographically, DVT was found in 20.5% of patients who had received s.c. melagatran plus ximelagatran and in 18.5% of patients on dalteparin. No difference was found between the three doses of melagatran and ximelagatran. Nor was a difference found between melagatran, ximelagatran and dalteparin with regards to bleedings.[58] Promising results with melagatran have been achieved in 48 patients (age range: 58.8-64.8 years) with phlebographically documented acute DVT.[59] The initiation of coagulation is triggered by the activation of factor IX and factor X by factor VIIa/tissue factor complex (VIIa/TF). Strategies to block this pathway include inhibitors of TF, of factor VIIa and of the VIIa/TF complex.[56] A soluble TF variant exhibits a marked antithrombotic activity in a rabbit model of arterial thrombosis.[60] *In vitro*, peptide analogs of TF inhibit the co-factor activity of TF by competing with TF for binding to factor VIIa.[56] An active-site-blocked factor VIIa that competes with factor VIIa for TF binding, has antithrombotic activity in primate and rabbit models of thrombosis.[56] Agents that inhibit the factor VIIa/TF complex include the natural anticoagulant TFPI (tissue factor pathway inhibitor), and the nematode anticoagulant peptide c2 (NAPc2). Recombinant TFPI and NAPc2 are in advanced stages of development. Because TFPI is rapidly cleaved into non-functional truncated forms by unknown protease(s) when administered intravenously, it has a short half-life. In pigs, TFPI attenuates injury-induced neointimal hyperplasia, and *in vitro* inhibits smooth muscle cell migration.[56] TFPI attenuates coagulopathy and improves survival in sepsis models in baboons and rabbits.[56] Accordingly, TFPI is now undergoing phase III evaluation in patients with sepsis. Unlike some small peptides isolated from *Ancylostoma caninum*, which contain *Ascaris*-type protease motifs that directly inhibit FXa, NAPc2 binds to a non-catalytic site on factor X or factor Xa and inhibits factor VIIa within the factor VIIa/TF complex. After s.c. injection, NAPc2 has a half-life of almost 50 hours. This is related to its ability to bind Factor X as well as Xa. Similar to TFPI, NAPc2 attenuates sepsis-induced coagulopathy in laboratory animals.[56] NAPc2 is currently undergoing phase II testing for prevention of VTE in patients undergoing elective knee arthroplasty.[56] Direct factor Xa inhibitors are either in preclinical or relatively early stages of development.[56] Fondaparinux sodium is the first in a new class of selective, indirect factor Xa

inhibitors and the farthest along in clinical development, having recently completed phase III trials for prevention of VTE in orthopedic surgery. Fondaparinux sodium is an antithrombin (AT; formerly referred to as antithrombin III)-mediated factor Xa inhibitor that is devoid of any anti-factor IIa (thrombin) activity. Unlike heparins, it selectively inhibits factor Xa without significant effects on aPTT, PT, and on platelet aggregation and adhesion. Four phase III trials[61-64] have compared the efficacy and safety of fondaparinux with that of 40 mg enoxaparin for VTE prophylaxis in patients undergoing hip fracture surgery (PENTIFRA study, 1,250 patients, mean age >75 years), elective hip replacement (EPHESUS, 1,817 patients mean age 66-67 years and PENTATHLON 2000, 1,584 patients, mean age 67 years), and elective knee replacement (PENTAMAKS study, 724 patients, mean age > 65 years). Analysis of the efficacy data for 5,385 evaluable patients (fondaparinux 2,682; enoxaparin 2,703) show that the administration of fondaparinux is associated with a significant (>50% vs enoxaparin, $p<0.001$) reduction in the relative risk of VTE without any increased risk of death or of clinically relevant bleeding,[65,66] the superiority being consistent across all patient sub-populations examined (age, gender, obesity, and surgical considerations). In subjects >75 years of age, fondaparinux had a safety profile comparable to that in the entire population when the drug was administered >6 hours post-operatively. Accordingly, the recommended dosing regimen in subjects >75 years is 6-8 hours postoperatively. The Rembrandt trial compared the efficacy and safety of three different doses of fondaparinux (5.0, 7.5, and 10.0 mg) with that of the LMWH, dalteparin, for treatment of acute symptomatic DVT.[67] Fondaparinux decreased thrombus size in 46%, 48%, and 42% of patients for the 5.0, 7.5, and 10.0 mg doses, respectively, and dalteparin decreased thrombus site in 49% of patients (p=NS, fondaparinux combined vs dalteparin). There were 8 recurrent thromboembolic complications (2.4%) in the 334 patients treated with fondaparinux and 6 (5.0%) in the 119 dalteparin recipients, with a relative risk reduction of 50% in favor of fondaparinux. All groups had similar low incidences of major bleeding, and no deaths occurred during the initial treatment period.

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Economy-class syndrome: media hype or real risk?

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Venous thromboembolism (VTE) is a multifactorial disease resulting from the interaction between genetic and environmental risk factors. The former include abnormalities causing inherited thrombophilia, such as deficiencies of the naturally occurring anticoagulant proteins antithrombin, protein C, protein S, and the gain-of-function mutations in genes encoding coagulation factor V (factor V Leiden) and prothrombin. The environmental, transient risk factors associated with an increased risk of VTE are cancer, recent surgery, pregnancy and puerperium, use of oral contraceptives and prolonged immobilization. For many decades, flights have been considered a risk factor for VTE. Recently, the interest in this topic increased both in the lay and medical press because of the death from pulmonary embolism of a 27-year old woman at the arrival hall in Heathrow airport (London) after a 20-hour flight from Australia.[1]

In 1946, Homans first referred to flights as a possible risk factor for VTE reporting an episode of venous thrombosis in a doctor after a 14-hour flight.[2] The most important pathogenic mechanism for VTE during air travel is stasis in the lower limbs. During the London Blitz in the Second World War it was observed that the incidence of fatal pulmonary embolism was increased 6-fold. The main reason for this was that the mechanical impairment of venous circulation due to squatting for a prolonged period in air raid shelters, promoted formation of venous thrombosis in the lower limbs and therefore pulmonary embolism.[3] In 1977, Symington and Stack used, for the first time, the term *economy-class syndrome*, underlying the pathogenic role of stasis during long flights in restricted seats, such as those of the economy class.[4] In 1986, Sarvesvaran observed that 18% of 61 cases of sudden death occurring in the arrival hall were attributable to pulmonary embolism, compared to 3.5% of 28 cases of sudden death occurred at the departure hall of Heathrow airport in London during a three-year period.[5] Although various case reports[6] and some retrospective observations[7,8] became available following Homans' observation of a patient with deep-vein thrombosis after an airflight, we had to wait until 1999 for studies estimating the risk of VTE related to air travel. Three case-control studies on this topic appeared in the literature, giving conflicting results. Two French studies found a positive association between VTE and long-haul flights.[9,10] In particular, Ferrari *et al.*,[9] in 160 consecutive patients with deep-vein thrombosis and 160 healthy controls, estimated a relative risk of 4 for any travel (car, train or flight). In contrast a Dutch study[11] failed to confirm such findings; only 17 out of 788 (2%) individuals with VTE had taken a flight before the event. However, these studies had some limitations that need to be discussed. The two French, positive studies[9,10] analyzed the different types of transport together without dividing air travel from travel by car or train. Moreover, both of them suffered from referral bias and in one study[9] the control group was inappropriately selected. In contrast, the referral bias was limited in the Dutch study,[11] since cases and controls were individuals consecutively referred to a Hospital for a suspected deep-vein thrombosis and in whom objective techniques had confirmed (cases) or did not confirm (controls) the presence of the disease. Despite the large sample size, the study did not show an association between air travel and deep-vein thrombosis. Unfortunately, one problem of this study was that only 4 cases (2%) and 13 controls (2%) had been exposed to the factor of interest (air travel) in the month preceding symptoms.

Recently, an observational study, carried out over a ten-year period at the arrival hall of the Charles de Gaulle airport in Paris, showed a strong association between flights, in particular long-haul flights of more than 8 hours, and symptomatic, non-fatal pulmonary embolism.[12] Following this observation, several companies advised people to follow simple measures during flight, such as to drink water, avoid alcohol consumption, sedative drugs and crossing legs, use comfortable clothes, and move leg muscles by walking the aisle or doing mild exercise. In fact, besides stasis, hemoconcentration due to a low intake of water, to the diuretic effect of alcohol, and perspiration in the dry atmosphere in the cabin, and lower limb muscle areflexia due to sedative drugs are possible pathogenic factors for VTE. It has been recently observed that the relative hypoxia and low pressure in a flight cabin can also contribute to the occurrence of VTE through activation of the coagulation cascade.[13]

To date, there few and inconclusive data are available on the interaction between air travel and thrombophilia. A small, uncontrolled retrospective study[14] on 20 patients with VTE after air travel showed the presence of thrombophilia in 6 of them (30%), previous thrombotic events in 4 (20%), other risk factors such as oral con-

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traceptives, cancer or plaster casts on a leg in 10 (50%), whereas in the remaining 5 (25%) no risk factors were identified. Another study,[15] on 16 patients with a deep- or superficial-vein thrombosis after long-haul flights, showed the presence of factor V Leiden or the prothrombin mutation in 3 cases. However, all the patients with deep-vein thrombosis had thrombosis in the veins of the calf diagnosed with ultrasonography, which is an inaccurate technique for diagnosing distal thrombosis.[16] Therefore, the possibility of misdiagnosis in this study cannot be ruled out.

In order to establish whether or not air travel is a risk factor for VTE and to investigate the interaction between air travel and thrombophilia or the use of oral contraceptives, we carried out a case-control study on 210 patients with proximal, objectively documented deep-vein thrombosis of the lower limbs with or without pulmonary embolism, and 210 healthy controls.[17] Thirty-one patients (15%) and 16 controls (8%) had flown in the month preceding the event (cases) or the visit (controls), for an odds ratio of 2.1 (95% CI 1.1-4.0). Three-quarters of the cases and controls had made short flights (less than 8 hours) and in economy class. The odds ratio for VTE was slightly higher (3-fold) for long-haul flights (more than 8 hours). Thrombophilia was found in 49% of patients and 12% of the controls, and oral contraceptives were used by 61% of women in fertile age in patients and in 27% of the corresponding controls. After stratification for the presence of air travel and thrombophilia, considering as the referral group individuals who did not fly and did not have thrombophilia, the risk for VTE was 6-times higher in the presence of thrombophilia, 2-times higher in the presence of air travel, and 16-times higher in the presence of both risk factors. Similarly, when stratification was done according to the presence of air travel and oral contraceptive use, considering as the referral group women who did not fly and were not taking oral contraceptives, the risk for VTE was 4-times higher in oral contraceptive users, 2-times higher in those who flew and 14-times higher in the presence of both risk factors. This study demonstrates that air travel is a minor risk factor for VTE, being associated with a 2-fold increase thrombotic risk. The risk for VTE is greatly increased in the presence of thrombophilia or oral contraceptive use, indicating a multiplicative interaction between these risk factors and air travel.

Whether or not simple measures such as walking in the aisle, drinking water, avoiding alcohol, might be sufficient to prevent VTE, even during short flights, in high risk subgroups of individuals, such as those with known thrombophilia or oral contraceptive users, remains to be established. Only one randomized trial is available on the efficacy of prophylaxis with drugs in high risk individuals. This trial showed that a single dose of low-molecular weight heparin before a flight was more effective than aspirin in preventing venous thrombosis.[18] The use of elastic stockings was also effective in reducing the risk of VTE in high risk individuals.[19] To date, specifically tailored studies are needed to establish which type of prophylaxis (heparin, elastic stockings or other) should be suggested before taking a flight to individuals with thrombophilia or to women who use oral contraceptives.

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Managing acute venous thromboembolism in the emergency department

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Acute venous thromboembolism (VTE) is a potentially fatal disease.[1] Its high prevalence in the general population (1/1000 cases),[2] makes the correct management of this disease essential in all Institutions. However, the situation is clearly different among countries. In Italy for instance, the specific units for VTE are not equally distributed across the country and management of VTE is often demanded in the Emergency Department (ED).[3] Although enormous amounts of data have been produced concerning VTE patients,[1,4] it is still unclear whether this information can be reliably translated to the emergency setting; in fact, patients evaluated at VTE clinics may be different from those seen in the ED.[5,6]

Recently, we prospectively evaluated the prevalence of venous thrombosis and its relation to the clinical characteristics among patients with suspected acute VTE referred to the ED and those referred to VTE clinics.[7] Our data show that ED patients had a higher prevalence of deep vein thrombosis (DVT) and a shorter time-interval between first visit and beginning of symptoms than those evaluated at the clinics.

Although the introduction of simplified approaches for diagnosing DVT and the use of low molecular weight heparins (LMWH) allows home treatment of patients with uncomplicated venous thromboembolism,[8] the situation is different for those with co-morbidity. However, these patients too (especially when they are frequently hospitalized) may ask for home therapy. In these cases, short hospitalization in the ED may furnish reliable information (laboratory and clinical) for assessing the risk of the home-treatment. Little information is available about the safety of treating patients with hemodynamically stable pulmonary embolims (PE) at home.

Another issue poorly investigated concerns what the ED physician can do when immediate testing is not available (e.g. during the night and week-end or in poorly equipped institutions). No validated guidelines are present at the moment and, therefore, while waiting for confirmatory tests, physicians are left with the decision to treat or not and to hospitalize or not patients clinically suspected of having VTE.

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The experience at the Emergeny Department of IRCCS Policlinico S. Matteo, Pavia, Italy

In order to answer some of these questions, since 1999 structured guidelines have been implemented at the ED of Policlinico S. Matteo of Pavia, in patients with clinically suspected acute DVT and/or PE. The topics concern:

1. Diagnostic accuracy of pre-test clinical probability (PCP) and D-dimer (D-d) in a population with acute symptoms and co-morbidity.
2. Diagnostic accuracy of D-d in relation to clinical variables (age of symptoms, location of DVT and concomitant heparin administration).
3. The use of LMWH as *protective anticoagulation* for up to 72 hours when immediate testing for VTE is not available.
4. Home treatment program for DVT and hemodynamically stable PE.

Diagnostic accuracy of PCP and D-dimer in a population with acute symptoms and co-morbidity

The use of standardized clinical probability (SCP), either alone or in combination with other tests, may help ED physicians to identify patients without acute VTE who do not require further examinations. With the purpose of investigating the clinical utility of this approach in the ED, we prospectively evaluated 358 out-patients with clinically suspected DVT and 89 with clinically suspected PE. The standardized clinical probability was calculated and the D-dimer test (semi-quantitative latex assay, Dimertest®, Dade Behring) was performed immediately. Validated objective tests (compression ultrasonography for DVT patients and ventilation/perfusion lung scanning and/or spiral computed tomography and/or pulmonary angiography for PE patients) were applied within 48 hours in all patients. According to the test results, patients were managed as previously described;[9] acute VTE was confirmed in 114 (84 DVT, 30 PE) patients (25.5%, 95% CI 14.9-36.1). The prevalence of VTE was 8.4% (95% CI, 4.3-12.5) in patients with a low SCP, 26% (19.3-32.7) in those with a moderate SCP and 48.3% (43.4-53.2) in those with a high SCP. Table 1 reports the diagnostic accuracy of SCP (alone or in combination with D-dimer). The standardized clinical model was considered *negative* in the case of low probability, positive in all other cases (moderate and high probability). Patients with a negative SCP and negative D-dimer were considered as not having VTE.

We further investigated the accuracy of other combinations and, particularly, that of considering as *negative* patients with low and moderate probability; the sensitivity, specificity, positive and negative predictive values were 50.8%, 87.9%, 68.8% and 78.5%, respectively. After a follow-up of 3 months, none of patients with low SCP and negative D-dimer developed symptomatic recurrent events. The combination of low SCP and negative D-dimer can be safely used as a *triage test* for excluding acute VTE in the emergency department. All other combinations require mandatory objective tests.

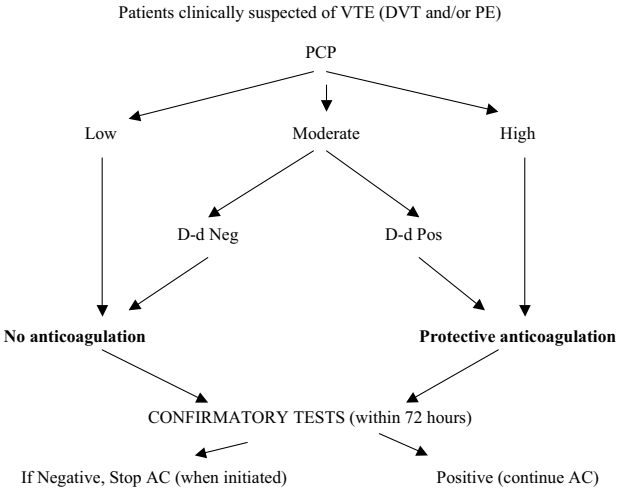
Diagnostic accuracy of D-dimer in relation to clinical variables (age of symptoms, location of dvt and concomitant heparin administration)

The measurement of D-dimer is claimed to have potential value in excluding DVT. New rapid methods have been proposed, but few clinical trials have assessed their performance on an emergency basis. The different accuracy found between the D-dimer assays has been related to the test used (latex or ELISA), but other variables (such as population investigated, thrombus extension, duration of symptoms or concomitant heparin treatment) may be important, even if not sufficiently investigated.

We evaluated the accuracy of a rapid semi-quantitative D-dimer tests (Dimertest®, Dade Behring), with reference to: a) its use in an emergency unit; b) concomitant heparin administration; c) location of venous thrombosis (VT) [in the deep or superficial venous system limited to the greater saphenous vein (GSV)] and d) symptoms older than 14 days.[10] Two hundred and ninety-eight patients suspected of having DVT and 116 with suspected thrombosis of the GSV were investigated. In the DVT patients, the sensitivity, specificity, positive and negative predictive values were 77.4% (95% CI 68.9-85.9), 81.4% (95% CI 76.1-86.7), 65.4% (95% CI 56.5-74.3) and 88.8% (95% CI 84.2-93.4), respectively. Excluding patients on concomitant heparin administration and those with symptoms older than 15 days, the sensitivity and negative predictive value increased to 86.3% (95% CI 78.4-94.2) and 92.8% (95% CI 88.4-97.2), respectively. In patients with GSV thrombosis, the sensitivity, specificity, positive and negative predictive values were 48% (95% CI 34.5-61.5), 90.6% (95% CI 83.2-97.9), 80.6% (95% CI 66.6-94.6) and 68.2% (95% CI 57.8-78.6), respectively. Excluding patients on concomitant heparin administration and those with symptoms older than 15 days, the sensitivity and negative predictive value did not change significantly. Our results show that previous or concomitant heparin administration, non-acute symptoms and thrombosis localized to superficial veins reduced the clinical usefulness of the test because the rate of false negative results was increased.

The use of LMWH as "protective anticoagulation" for up to 72 hours when immediate testing for VTE is not available

Management of patients with suspected DVT or PE can be problematic when diagnostic-imaging is not available, for example after working hours or in poorly equipped institutions. The pre-test clinical probability (PCP) and D-dimer can be used to identify patients for whom empirical, protective



VTE: Venous ThromboEmbolism; DVT: Deep Vein Thrombosis; PE: Pulmonary Embolism; PCP: Pre-test Clinical Probability; D-d: D-dimer, AC: AntiCoagulation

Figure 1. Algorithm of intervention when diagnostic imaging for vte is not immediately available.

Table 1. diagnostic accuracy of standardized clinical probability.

	SCP alone	*D-dimer alone*	*SCP + D-dimer*
Sensitivity	87.7% (81-93.8)	84.2% (61.4-107)	99.1% (98.2-100.9)
Specificity	45.6% (40.3-50.9)	71.7% (67.2-76.2)	37.5% (32.4-42.6)
Positive PV*	64.4% (58.8-70)	49.4% (37.1-61.7)	64.8% (58.6-71)
Negative PV	89.9% (85.2-94.6)	92.9% (89.6-96.2)	99.2% (97.5-100.9)

*Predictive value.

Table 2. Clinical characteristics and events occurring in patients with acute dvt or pe treated in hospital or at home.

	Standard in-hospital		*Home Treatment*	
Number of patients	48 DVT	32 PE	91 DVT	36 PE
Median age (range) in years	68 (37-92)		61 (22-90)	
Proximal DVT	43 (89.5%)	6 (18.7%)	82 (90.1%)	14 (38.8%)
Distal isolated DVT	5 (10.4%)	2 (6.2%)	9 (9.8%)	2 (5.5%)
SVT	0 (0%)	2 (6.2%)	6 (6.5%)	1 (2.7%)
Symptoms of PE	7 (12.5%)	32 (100%)	8 (8.7%)	36 (100%)
Active cancer	10 (20.8%)	7 (21.8%)	8 (8.7%)	6 (16.6%)
Other comorbidity	21 (43.7%)	21 (65.6%)	55 (60.4%)	20 (55.5%)
In-hospital stay	7+2 days	3.1 hours		
Events during the concomitant heparin and warfarin therapy				
Duration of therapy (mean)	8.2 days		6.7 days	
Recurrent DVT	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Recurrent PE	0 (0%)	0 (0%)	1 (1.1%)	0 (0%)
Major bleeding	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Minor bleeding	2 (4.1%)	0 (0%)	1 (1.1%)	0 (0%)
Heparin-induced thrombocytopenia	0 (0%)	0 (0%)	1 (1.1%)	0 (0%)

DVT: deep vein thrombosis; PE: pulmonary embolism: SVT: superficial vein thrombosis (at the saphenous-femoral junction).

anticoagulation is indicated.

In order to evaluate whether the PCP evaluation and the D-d, test together with the use of LMWHs allow the deferral of objective assessment of DVT and PE, 409 consecutive patients with suspected DVT and 124 with suspected PE were investigated.[11] Following the study protocol (Figure 1), patients received a full, protective dose of LMWH only when a high PCP or a moderate PCP with positive D-d were found. In all other cases, patients were discharged without anticoagulation. All patients were scheduled to undergo objective tests for DVT or PE within 72 hours. If VTE was confirmed, standard antithrombotic therapy was administered. Overall, 23.8% (95% CI 20.3-27.3) of patients had confirmed VTE. After the short follow-up (72 hours), only one event had occurred (0.53%, 95% CI 1.17-1.23); at 3 months follow-up, 4 complications (0.7%, 95% CI 0.2-1.4) had occurred in patients in whom the diagnosis was previously ruled out. Ninety per cent of subjects were managed as outpatients. Our study demonstrates that this integrated approach makes the deferral of diagnostic procedures for DVT and PE safe and reduces hospitalization, thus simplifying management.

Home treatment program for DVT and hemodynamically stable PE

Home treatment (HT) for DVT is becoming a widely accepted procedure in low-risk patients. We further tested the feasibility and safety of the HT program for acute VTE in our ED, where 121 consecutive patients (84 with DVT and 37 with PE) were hospitalized for a few hours. According to the previously described algorithm,[7] patients were screened as potentially eligible for HT or for standard in-hospital care. Low-risk patients (n=38) and those high-risk patients who refused hospitalization (n=15) were treated at home (LMWH at therapeutic doses plus warfarin); the remaining high-risk patients (n=68) received the standard hospital care. Patients treated at home were followed at the ED during the period of concomitant heparin and warfarin therapy. The results (Table 2) indicate that there was no difference between hospitalized and HT patients in terms of major outcomes. This

is particularly relevant considering that a subgroup of high-risk patients was treated at home.[12,13]

By 3 months, 2 patients (standard in-hospital care) had died of causes other than VTE. One patient (HT) developed a non-fatal intra-cranial hemorrhage. These preliminary results suggest that our *HT program*, based on a very short hospitalization, is as feasible and safe as standard in-hospital management.

Conclusions

Although diagnosis and therapy of DVT are now well standardized, the management of acute VTE is still sub-optimal when patients are referred during the week-end or the night or to poorly equipped institutions (where VTE consultants may not be immediately available). On the other hand, the extremely high prevalence of VTE and its potential fatality (when the diagnosis is missed) makes it mandatory to provide ED physicians with practical guidelines. However, few data are available about the management of VTE in the emergency setting, and data generated in the setting of clinics may not easily be transferred to the ED, since acute patients represent a different population.

The algorithms and guidelines implemented during a 4-year period at the ED of IRCCS Policlinico S. Matteo show that standardized approaches are safe and well accepted by physicians; our approaches comprised all topics related to the management of the acute phase of VTE (need to reduce invasive tests, non-immediate availability of diagnostic tests, home therapy). Most of our patients were treated at home after a very short hospitalization (4 hours). Although an economic analysis was nor performed, this approach is thought to be cost-effective since the need for prolonged hospitalization was reduced by 90% for DVT patients and by 30% for PE patients.[14]

Can this information be transferred to all other Institutions? Our results offer other hospitals (especially those without immediate testing or consultants) a practical tool for the management of VTE; however, local validation is required to evaluate the safety for patients and acceptance by the ED physicians.

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Thrombosis and malignancy: an underestimated problem

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Malignancy is a thrombophilic condition and there is clinical evidence that patients with cancer have a significantly increased risk of thrombosis. The pathogenesis is multifactorial and, in great part, relies on the capacity of tumor cells to interact with the hemostatic system and activate it in several ways. The association between cancer and thrombosis is clinically relevant because, on the one hand, thrombosis can represent the first symptom of an occult cancer and, on the other hand, thrombotic events in patients with a known malignancy can influence the morbidity and mortality of the underlying disease. Furthermore, it is important to be aware that many factors, such as surgery and chemotherapy, may increase the thrombotic risk in cancer patients. Recently, a number of strategies for prevention and management of thrombosis in cancer have been under evaluation.

The association between cancer and venous thromboembolism (VTE) has been known for over a hundred years. Since the beginning, this association appeared to have a dual significance. First, there is the concept that the occurrence of VTE is a common complication of cancer, as underlined by Armand Trousseau in 1865, who observed that «*in cancer there is a special condition of the blood predisposed to spontaneous coagulation even in the absence of inflammatory reactions*».[1] Second, the possibility of a relation between the clotting mechanism and the development of metastases was postulated as early as 1878 by Billroth, who described cancer cells within a thrombus and interpreted his finding as evidence of the spread of tumor cells by thromboemboli.[2] We here focus our attention mainly on the first aspect.

The mechanisms of thrombus promotion in malignancy include some general host responses to the tumor (acute-phase, inflammation, angiogenesis, etc.) and specific interactions of tumor cells with the clotting/fibrinolysis systems and with blood (leukocytes, platelets) or vascular cells.[3] It is at present difficult to rank the relative weight of these multiple interactions on the risk of clinically overt thrombosis in cancer patients. Moreover, the mechanisms explored so far offer a sound experimental basis to support and explain the hypercoagulable state associated with malignancy.

The wide spectrum of manifestations of the prothrombotic state in cancer ranges from an asymptomatic condition, characterized by abnormal plasma coagulation tests, to massive thromboembolism, when the patient may be seriously ill. Although, deep vein thrombosis (DVT) of the lower limbs is the commonest clinical manifestation in cancer patients, DVT of upper limbs, pulmonary embolism, central sinus thrombosis, migratory superficial thrombophlebitis, as well as syndromes with more systemic involvement of the clotting system, such as disseminated intravascular coagulation or thrombotic microangiopathy, have all been described.

VTE is an important cause of morbidity in patients with malignant disease, but an exact appreciation of the magnitude of the problem of VTE in cancer is not easy. Much of the early information comes from small series, or retrospective analyses, or *post-mortem* studies. Our understanding of the epidemiology of VTE in cancer has only recently become clearer with the advent of large population-based studies, and the data from prospective series describing outcome with regard to VTE. Weighing the magnitude of the problem of VTE in cancer, its relationship to various therapeutic interventions, stage of disease and site of origin of the primary tumor is essential in order to develop strategies to prevent these complications.

Current epidemiological data can help us to address the following questions in patients with cancer and VTE: i) what is the probability of occult cancer in patients with idiopathic or secondary VTE; ii) what is the risk of thrombosis in patients with known cancer and selected conditions; iii) what is the risk of recurrent VTE in cancer patients and in non-cancer patients.

Occult cancer in patients with VTE

The probability of a new diagnosis of cancer within 6-12 months of the diagnosis of idiopathic VTE (including pulmonary embolism) than in the absence of VTE, as well supported by retrospective analyses of large numbers of unselected patients, population-based retrospective cohort analyses from large registries and prospective studies. The odds ratios for a new diagnosis of cancer in these studies are in the range of a 4-7 fold increased risk.

Retrospective studies have shown a rather consistent pattern of a significant difference in the incidence of cancer between patients with secondary VTE (1.8-7.1%) and those with idiopathic VTE (6.5-16.6%).[4] Two very large, retrospective, population-based studies published in 1998 demonstrated that the incidence of cancer was increased during the first year following the diagnosis of VTE, and that this effect persisted for up to 10 years.[4]

Retrospective studies, however, pose several problems. In particular, it is difficult to determine from registry data whether objective criteria were utilized for the diagnosis of VTE and to find the data supporting the

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distinction between primary (or idiopathic) VTE and secondary VTE. Furthermore, documentation of other risk factors (such as congenital thrombophilia, pregnancy, use of oral contraceptives, obesity) is frequently missing, as is the information that the presence of a concurrent cancer had been carefully excluded by comparable criteria. A selection bias may be present unless consecutive patients were admitted to the study.

Data from well-designed, prospective trials are essential to answer the question of whether the risk for occult cancer is significantly increased in patients with idiopathic VTE. In 1992 Prandoni *et al.*[5] published the results of a study of 145 patients with well-documented idiopathic VTE and 105 patients with equally well-documented secondary VTE, all of whom were followed closely for at least 1 year after the diagnosis of VTE. Eleven of the 145 patients with idiopathic VTE (7.6%) developed cancer within 12 months, whereas 2 of the 105 (1.9%) with secondary VTE did so (p=0.043). Patients with recurrent, idiopathic VTE had an even higher risk of developing cancer.[5] Similar results have been reported in other prospective studies. Schulman and Lindmarker have recently provided important corroboration of these findings in another prospective study, albeit with a very different study design.[6]

Thus the question of whether there is an increased risk of occult cancer in patients with well-defined idiopathic VTE clearly has an affirmative answer. A subset question on the likelihood of discovering a tumor in patients with idiopathic VTE has not yet been answered. A prospective, randomized, controlled trial entitled *Screening for Occult Malignancy in Patients with Symptomatic Idiopathic Venous Thromboembolism* (SOMIT), designed to answer this question, has been conducted in Italy and the results are under evaluation.

VTE as a complication of cancer

As already mentioned, the incidence of VTE in cancer patients at *post-mortem* may be as high as 50%. Nevertheless, the optimal study design for determining the true incidence of clinical VTE in cancer patients is a prospective cohort study. In this sense, valuable data are available for selected conditions, i.e. patients exposed to either medical or surgical treatments for cancer.

A retrospective analysis[7] of data derived from randomized clinical trials of therapy in patients with breast cancer, in which data were collected prospectively, was the first attempt to evaluate this risk prospectively. In this setting of breast cancer, the studies demonstrated that therapy with an estrogen receptor agonist (i.e. tamoxifen), chemotherapy, combination therapies (tamoxifen + chemotherapy), the stage of the disease and the menopausal status significantly (though differently) affect the rates of VTE. The rates escalate rapidly with advancing stage of disease and the use of chemotherapy, both of which probably contribute to the hypercoagulability characteristic of patients with more extensive disease.[7] The reported rate of thrombosis in women with stage II breast cancer on chemotherapy varies between 5 and 13%,[8] with the highest rates of thrombosis observed in postmenopausal women. Chemotherapy plus tamoxifen increases the risk of VTE over that of chemotherapy alone and in one study the rate of thrombosis in patients with metastatic breast cancer receiving chemotherapy was 17.5%.

Other patients with advanced cancers who are likely to be at higher risk of thromboembolism include patients with brain tumors receiving chemotherapy, those with locally recurrent rectal cancer receiving radiation, and those with pancreatic cancer or advanced gastrointestinal cancers (particularly adenocarcinomas).[7] However, precise estimates of thrombotic rates in these groups of patients are not available. Von Templehoff *et al.* reported a 10.6% rate of VTE in women with advanced ovarian cancer receiving chemotherapy. Rates of 24% to 60% have been reported in high grade gliomas, and 5-10% in patients with Hodgkin's or non-Hodgkin's lymphoma.[9] In addition, cancer patients with indwelling central venous catheters are at increased risk of thrombosis of the axil-lary/subclavian vein,[9] with the catheters themselves being susceptible to thrombotic occlusion despite the use of routine heparin flushes.

Surgical intervention in patients with cancer increases the risk of postoperative VTE (approximately two fold) in comparison to the risk in non-cancer patients undergoing the same procedures.[10] The risk of VTE in cancer patients undergoing specific types of surgery can be derived from the *no treatment* control arms of trials evaluating prophylaxis of VTE in surgery. Subset analysis has been used, since cancer patients usually constitute approximately 20% of the patients in these studies. The approximate rates for VTE were: general surgery - 29%; gynecological surgery - 20%; urological surgery - 41%; orthopedic surgery - 50-60%; and, neurosurgery - 28%. However, it must be emphasized that many of the thrombi detected were asymptomatic and some of the studies included non-cancer patients, so these rates may not be accurate. Nevertheless, the American College of Chest Physicians has stratified patients with malignancy in the highest risk category of surgical patients and urged routine thromboprophylaxis for these patients.

Turning now to the issue of the distribution of specific cancers associated with thrombotic complications, it appears that the historical association made by Trousseau and others of thrombosis

with gastrointestinal tumors, and with carcinoma of the pancreas in particular,[1] has heavily influenced our views of which types of cancers are linked to thrombophilia. A series of case reports from the literature reported that the most common cancers associated with thrombosis were pancreatic, lung, and stomach cancers. Lieberman, in a retrospective series, reported that the most common cancers associated with thrombosis in males were cancers of the lung and pancreas, while the most common neoplasias associated with thrombosis in females were gynecologic, colorectal and pancreatic cancers. It is likely that the distribu-tion of specific cancers associated with thrombosis follows the frequency of the cancer in the general population, which is once again best determined in patients entered into prospective clinical trials of antithrombotic agents, as illustrated by observation in a study by Levine *et al.*[11] The authors evaluated outpatient therapy with low molecular weight heparin for proximal DVT and found that 103 of the 500 patients entered into the study had cancer. The most common anatomic sites for cancer in men were prostate, colorectal, brain and lung and the most common sites in women were breast, ovary and lung. Again we must consider that this type of retrospective analysis of data from studies not designed to assess prospectively the incidence of thrombosis in cancer prospectively is not ideal. Nevertheless, the data were collected prospectively and get close to an appropriate answer regarding associations of thrombosis with specific types of cancers.

Recurrent VTE

As for post-operative DVT in cancer surgery, the relative risk for recurrence of VTE in the first 3 months after an initial episode in cancer patients treated with heparin and coumadin is about double that in non-cancer patients.

In a prospective cohort study in 355 consecutive patients with DVT treated with heparin followed by warfarin, the risk of recurrent VTE in the 3-month follow up period was higher in cancer patients (10.3%) than in non-cancer patients (4.7%).[12] Hutten *et al.* recently compared the rates of recurrent VTE and bleeding in cancer and non-cancer patients in two randomized trials which compared low molecular weight heparin with standard unfractionated heparin for initial treatment of acute venous thromboembolism.[13] The study included 261 patients with malignancy and 1,038 without cancer. The rates of recurrent VTE were 27% per year versus 9% per year, respectively, $p = 0.003$. These data are supported by the results of a recent population-based cohort study, which compared the outcome of anticoagulation courses in 95 patients with malignancy with the outcome of 733 patients without malignancy.[14] The rate of recurrent thrombosis in cancer patients was 6.8% compared to 2.5% in non-cancer patients, $p = 0.06$. Clinical trials have been initiated to test alternative anticoagulation strategies for the prevention of recurrent VTE in patients with cancer.

Conclusions

In conclusion, analysis of the literature shows that the risk of occult cancer in patients with idiopathic VTE is approximately 4-7 fold, as determined by prospective trials designed to compare the cancer risk in patients with well-defined idiopathic VTE with that in patients with secondary VTE (i.e. due to known causes). This odds ratio rises to perhaps 9 fold when data are examined from patients with *recurrent*, idiopathic VTE. Thus, patients with idiopathic VTE in whom all other causes have been carefully excluded should be followed closely for the development of cancer, particularly during the 6-12 months immediately following the episode of VTE.

It is equally well established that the odds ratio is approximately 2, when comparing the risk for postoperative VTE in cancer patients with that in non-cancer patients undergoing the same surgical procedures, and comparing recurrence of VTE in cancer patients and non-cancer patients.

At present, further studies are needed to collect data prospectively to address the incidence of thrombosis in different types of cancers.

Quantification of the magnitude of the thrombotic risk associated with malignancy and with anti-cancer interventions is indispensable in order develop the optimum anticoagulant strategies to protect cancer patients from thromboembolism.

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Prophylaxis of venous thromboembolism: when to start and how long to treat

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When to start prophylaxis

Thromboprophylaxis significantly decreases morbidity and mortality from venous thromboembolism (VTE). Two key issues remain controversial regarding VTE prophylaxis: the relative efficacy and safety of prophylaxis initiated pre-operatively or post-operatively and the optimum duration of prophylaxis. As regards the initiation of thromboprophylaxis, clinical practice has diverged in North America and Europe. This treatment is given pre-operatively in Europe and post-operatively in North America.

In North America the first dose of low molecular weight heparin (LMWH) is usually administered 12-24 hours after surgery whereas in Europe it is given the evening before the operation (10-12 hours before). Two critical reviews[1,2] and a series of clinical studies in orthopedic surgery have tried to solve the problem of the best time to begin prophylaxis with conflicting results.[3,6] In the first review[1] no difference was found between pre- and post-operative commencement, while the second[2] indicated that pre-operative prophylaxis was more efficient.

In a recent paper on timing based on four clinical studies (all different from each other and, what is more, some using LMWH others oral anticoagulants) Hull *et al.*[7] concluded that if LMWH is begun in close proximity to surgery the rate of deep vein thrombosis (DVT) is 40-50% lower than that when oral anticoagulants are used and the absolute reduction is 11-13%. In contrast, commencement according to classical protocols was not more efficient than oral anticoagulants. After analyzing the data, the authors concluded that the best time to start prophylactic treatment is between 2 hours before surgery and 6-8 hours after. If this approach is adopted the first dose of LMWH should be reduced by half, thus avoiding an increase in the incidence of major hemorrhages.

It is already a widely held opinion that the timing of commencing prophylaxis in surgery is a very important factor influencing the incidence of post-operative VTE. There is good reason to suppose that prophylaxis may be more efficient if administered peri-operatively, with a view to neutralizing, as early as possible, the thrombin generated from the very start of surgery. At the present moment, however, the conclusion of the *Sixth Consensus Conference on Antithrombotic Therapy*[8] is that prophylaxis can be started in the pre- or post-operative phase. For patients at high risk of hemorrhage, prophylaxis should begin 12-24 hours after surgery. The first post-operative dose should, however, be delayed until signs of local hemostasis are detected (via examination of limb and hematic drainage volumes). The *Società Italiana per lo Studio dell'Emostasi e della Trombosi* (SISET) also[9] does not feel it necessary to change its present recommendations regarding the commencement of thromboprophylaxis in orthopedic surgery.

Optimal duration of prophylaxis in orthopedic surgery

In a study in patients undergoing elective hip arthroplasty surgery in California in the period 1993-1996, White *et al.*[10] showed that the number of cases requiring further hospitalization, following post-operative discharge, because of the onset of a new symptomatic episode of VTE gradually rose in the 2-month period after surgery when it then peaked. This shows that the post-operative thromboembolic risk in this type of surgery lasts well beyond the peri-operative period of hospitalization.

Many studies have examined the efficacy of prolonging prophylaxis after hip surgery.[11-18] Some meta-analyses have examined the available studies on prolonged prophylaxis. Eikelboom *et al.*[19] examined studies on both the hip and the knee. They concluded that prolongation of prophylaxis significantly reduced symptomatic venous thromboemboli, with 20 less episodes for every 1000 patients treated. In their meta-analysis of studies on hip surgery,[20] Hull *et al.* confirmed the advantages of prolonged prophylaxis in reducing both symptomatic and asymptomatic episodes.

Very recently the Penthifra-Plus study,[21] which used Fondaparinux as part of the thromboembolic prophylaxis for fractured femur, showed that prolonged administration for up to 28 days (instead of 7 days) produced a 96% reduction in the relative risk of all VTE and an 89% reduction of symptomatic VTE, without any significant difference in the incidence of major bleeding and death. Prandoni *et al.*[22] have recently addressed the issue of the duration of thromboprophylaxis when oral anticoagulants are used after total hip arthroplasty. Patients were randomly assigned to stop taking oral anticoagulants at the time of hospital discharge or to continue for 4 more weeks. The study was premature-

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ly terminated after the inclusion of the first 360 patients because a statistically significant superiority of extended over short-term thromboprophylaxis was observed. Objectively confirmed VTE complications were in fact recorded in 9 (5.1%) out of the 176 control patients, and in only 1 (0.5%) in the group of 184 patients who continued the warfarin treatment.

While prolonged prophylaxis for up to 4-6 weeks after hip surgery is suggested by numerous controlled clinical studies and is accepted by virtually the whole of the scientific community, there is much less evidence as regards knee surgery. Very few studies have addressed the problem of prophylaxis duration in knee surgery. Heit *et al.*[17] treated 723 patients who had undergone knee surgery, administering ardeparin (100 anti-Xa IU/kg body weight) or placebo from the moment of discharge (after 4-10 days of intra-hospital prophylaxis in all of them) for a further 6 weeks. The end-points were clinically evident episodes: DVT, pulmonary embolism or death. The incidence of thrombotic episodes at the end of extra-hospital prophylaxis was 1.4% in the treated group and 1.7% in the placebo group (non-significant difference). The incidence of major hemorrhagies was 0.6% in both groups. This study also showed no benefit of prolonged treatment in hip surgery. It should be borne in mind that only symptomatic episodes were recorded and that overall the prolongation of prophylaxis reduced these episodes by only 2.2%.

Comp *et al.*[18] treated 438 patients who had undergone knee surgery, administering 30 mg x 2 of enoxaparin during bed-rest (7-10 days) and 40 mg once a day or placebo for the next 3 weeks. The incidence of objectively confirmed VTE was 17.5% and 20.8%, respectively, in the group treated with enoxaparin or placebo (this difference is not statistically significant). This same study did, however, show that prolonged treatment was efficacious in hip surgery patients: 8.0% in the group with prophylaxis and 23.2% in the placebo group ($p < 0.001$).

White *et al.*[23] examined the incidence of clinically apparent VTE episodes occurring after hip and knee surgery in Californian hospitals. The average period between operation and diagnosis of thromboembolism was 7 days after knee surgery and 17 days after hip surgery ($p<0.001$). The incidence of thrombotic episodes peaked after 4 weeks in the case of knee surgery but only after about 10 weeks in the case of hip surgery. Overall, the diagnosis of VTE was made after discharge from hospital in 47% of knee cases and in 76% of hip cases. These data confirm that there is a time difference in the development of VET following elective hip and knee surgery, the latter being associated with an earlier risk of thrombosis.

The need to continue antithrombotic prophylaxis at home after major orthopedic surgery has been contested by some authors on the grounds that the overwhelming majority of these late DVT are cases of distal thrombosis, which are not particularly dangerous, while serious thromboembolic episodes are very rare.[24] In a recent multi-center study on 1,984 patients undergoing hip or knee prosthesis surgery, Leclerc *et al.*[25] showed that there are no benefits in prolonging prophylaxis beyond an average of 9 days and that there is no practical use in carrying out a control ultrasound at the moment of discharge.

Other aspects do, however, need to be taken into consideration. First of all, it is perfectly possible that asymptomatic distal venous thrombosis may also prompt symptoms of post-thrombotic syndrome in many patients even if recent data rule out a major incidence of serious post-thrombotic syndrome (presence of trophic ulcers) in subjects who have undergone knee surgery.[26] Second, even those few cases of fatal pulmonary embolism that may occur at home after major orthopedic surgery should, if possible, be prevented, not just for medical reasons, but also for legal ones.

Finally the risk of heparin-induced thrombocytopenia, possibly caused by prolongation of prophylaxis, should be taken into account. It is well known that LMWHs provoke heparin-associated thrombocytopenia to a much lesser extent than unfractionated heparin.[27] Nevertheless, prolonged use of LMWH does raise the question of prevention and the need to monitor for the possible onset (fortunately rare) of this serious complication.

On the subject of the optimal duration of prophylaxis after elective femoral or knee surgery, the ACCP's *consensus conference*[8] admitted uncertainty on the matter and recommended a period of at least 7-10 days (grade 1A recommendation). Longer treatment, which could lower the incidence of clinically significant thromboembolic episodes, is recommended only for patients at higher risk (grade 2A), to the extent that there remains uncertainty as regards cost-benefits. The SISET too recommends similar guidelines in this regard.[9]

The optimal duration of prophylaxis in clinical situations other than orthopedic surgery was recently addressed by Bergqvist *et al.* who considered cancer surgery.[28] In their double-blind, multicenter trial 332 patients undergoing surgery for abdominal or pelvic cancer received 40 mg of enoxaparin subcutaneously daily for 6-10 days and were then randomly assigned to receive either enoxaparin or placebo for another 21 days. The rates of VTE (assessed by bilateral venography) were 12% in the placebo group and 4.8% in the treatment group (p=0.02) at the end of the double-blind phase. A significant difference persisted even at three months (p=0.01). There were no significant differences in the rates of bleeding or other com-

plications. This study showed that patients who undergo surgery for cancer benefit from prolonged VTE prophylaxis.

Prolonged thromboprophylaxis was recently recommended in cancer patients receiving medical treatment. The prophylaxis should usually last for the entire period of treatment, and even longer (4-12 months) in the case of pelvic or cerebral radiotherapy.[29]

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From pharmacologic studies to the improvement of thromboprophylaxis

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Thromboembolic disease is currently a major contributor to morbidity and mortality. Treatment of deep vein thrombosis (DVT) and/or pulmonary embolism (PE) has progressed significantly with the use of low molecular weight heparins (LMWHs). Moreover, home treatment has become a common practice in north America and, to some extent, in Europe as well.[1] Strict recommendations have been made for an appropriate selection of patients who could benefit from such treatment (Table 1). Drug efficacy and safety and patients' improved quality of life are expected when a LMWH is used in home treatment of DVT. However, a switch to oral anticoagulant is an essential part of the strategy in the treatment of DVT and/or PE.

Oral anticoagulants have their own limitations (such as an early decrease in protein C levels and a transient hypercoagulable state during the initiation of the treatment, delayed onset of adequate anticoagulation, requirement of laboratory monitoring and frequent dose adjustment, and interactions with food and drugs).[2] A narrow therapeutic window and an important disturbance of blood coagulation are associated with 1.4% patient/year bleeding episodes and a 0.25% patient/year rate of fatal bleeding.[3]

New antithrombotic drugs have been developed and can be classified according to their mechanism of action into 2 different groups:

- the indirect inhibitors, which act by enhancing the inhibitory activity of antithrombin. Such inhibitors are heparins (unfractionated heparin and LMWH) and the synthetic pentasaccharide (fondaparinux) and the metapentasaccharides (i.e idraparinux);
- the direct inhibitors, which act directly on the target serine protease. Such inhibitors are hirudin, hirulog, agatroban, ximelagatran/melagatran and the DX9065a.

The new antithrombotic drugs are also classified according to their target into:

- *Inhibitors of factor Xa.* Such as the indirect factor Xa inhibitors, fondaparinux and idraparinux. Several other chemical compounds are under development. They belong to a class of direct factor Xa inhibitors. An example is DX9065a which is essentially active by the parenteral route. Moreover, orally active agents are being developed.
- *Inhibitors of thrombin e.g. ximelagatran (Exanta®), which is an orally active direct inhibitor.* This very promising agent has very important advantages such as the absence of drug interference and the fact that laboratory monitoring is not necessary.

The efficacy and safety of fondaparinux, idraparinux and ximelagatran have been studied in controlled trials in patients with DVT or PE. The published phase II and III clinical trials will be briefly presented.

Treatment of venous thromboembolism with fondaparinux (Arixtra®)

Rembrandt, a phase II dose finding study.[4] The relative efficacy and safety of three doses of fondaparinux (5, 7.5 and 10 mg once daily) compared with the LMWH dalteparin (100 IU/kg, twice daily) were examined in patients with venous thromboembolism in a multicenter, randomized trial. The primary outcome measure was the change in thrombus mass determined at baseline and on day 7±1 by ultrasonography of the leg veins (the Marder score) and perfusion lung scintigraphy. An improvement of this outcome was observed in 46/100 (46%), 52/108 (48%), 48/115 (42%) and 56/115 (49%) of the subjects given 5, 7.5 or 10 mg of fondaparinux or dalteparin treatment, respectively. There were eight (2.4%) and six (5.0%) recurrent thromboembolic complications in the fondaparinux and dalteparin groups, respectively. The incidence of bleeding was low and similar among the groups. The anti-Xa plasma activity was well correlated with the administered dose of fondaparinux, but no clear statistically significant evidence of a dose-response relationship for efficacy was found. After examination of all the data the dose of 7.5 mg/day of fondaparinux, a 3-fold higher dose than that used in the prophylaxis, was considered to be appropriate for further evaluation in this setting. It is generally accepted that the ratio between the dose used in the treatment of VTE and that used in prophylaxis of VTE is around 3.

Matisse DVT/PE, a phase III study.[5] Two phase III multicenter randomized trials, were conducted to document that a fixed subcutaneously dose of once-a-day fondaparinux is as effective and equally safe as current initial therapy, administered twice daily and with weight-adjusted dosage in DVT (Matisse-DVT) and in pulmonary embolism (Matisse-PE).[14] Fondaparinux was

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Table 1. Recommendations for early discharge of outpatient therapy in venous thromboembolic disease.[1]

The physician must ensure the following:
• Patient is in a stable condition with normal vital signs
• Low bleeding risk
• Absence of severe renal insufficiency
• Practical system for administration of LMWH and warfarin with appropriate monitoring.
• Practical system for surveillance and treatment of recurrent thrombosis and bleeding complications.

administered for 5 days as a single subcutaneous daily injection of 7.5 mg (or 5 mg if the body weight was < 50 kg and 10 mg if it was > 100 kg). In the DVT study the comparator was enoxaparin (1 mg/kg s.c. twice daily) and in the PE study continuous UFH i.v. The Matisse-DVT had a double blind design whereas the Matisse-PE was open label. All patients received vitamin K antagonists for 3 months. The primary efficacy and safety outcomes in both studies were recurrent symptomatic and objectively confirmed DVT or PE and major bleeding respectively, as assessed by a blinded independent adjudication committee. The duration of the follow-up was 3 months. A total of 2,212 patients were recruited in the Matisse-DVT study and 2,214 were recruited in the Matisse-PE. In the Matisse-DVT study the efficacy of fondaparinux was similar to that of enoxaparin (4.1% versus 3.9%, respectively; $p>0.05$). No significant difference in the rate of PE was found between the two groups (4.1% in the fondaparinux group and 4% in the enoxaparin group; $p>0.05$). Fondaparinux was as safe as enoxaparin, and the incidence of major bleedings was the same in both groups. In the Matisse-PE study the treatment with fondaparinux was as effective and as safe as the treatment with UFH. The incidence of recurrent PE was 3.9% in the fondaparinux group and 5% in the UFH group ($p>0.05$). No significant difference in the frequency of clinically relevant major bleedings was observed between the two groups.

Treatment of venous thromboembolism with idraparinux

PERSIST, a phase II dose-finding study.[6] In this study (PERSIST), after 5 to 7 days of enoxaparin treatment, patients with proximal DVT were randomized to receive 2.5, 5.0, 7.5 or 10 mg of idraparinux subcutaneously once weekly or warfarin (INR 2 to 3) for 3 months. The primary efficacy outcome was the composite of change in thrombotic burden, as assessed by ultrasonography and perfusion lung scanning at baseline and at 12 weeks, and clinical thromboembolic events. This outcome was classified as normalization, no relevant change or deterioration. The safety outcomes were major or clinically relevant bleeding. All outcomes were assessed by a blinded independent adjudication committee. A total of 659 patients were randomized and treated and in 614 patients the primary efficacy outcome was evaluable. The rates of normalization and deterioration were similar in all idraparinux groups and did not differ from those in the warfarin group. There was a clear dose response for major bleeding among patients treated with idraparinux. Patients receiving idraparinux 2.5 mg had significantly less bleeding than warfarin-treated patients(p=0.029). The dose of 2.5 mg of idraparinux administered as a single weekly subcutaneous injection, was chosen to be evaluated in phase III trials for secondary long-term prevention of VTE.

Treatment of venous thromboembolism with ximelagatran (Exanta®)

THRIVE I a phase II dose-finding study.[7] In this randomized, multicenter dose-finding study, the efficacy and tolerability of ximelagatran were compared with those of a LMWH (dalteparin) followed by warfarin in the treatment of lower limb DVT. Patients with acute DVT received oral ximelagatran (24, 36, 48 or 60 mg twice daily) or dalteparin (200 anti-Xa IU/kg subcutaneously, once daily) and warfarin (dose adjusted to achieve INR between 2 and 3). The study drug was administered for 14 days and patients were followed up for a further 2 weeks during which time they were treated according to the local clinical practice. Venograms were obtained prior to the administration of the study drug and at the end of the 2-week study treatment period. Evaluation of paired venograms from 295 patients showed regression of the thrombus in 69% of patients treated with ximelagatran and 69% of patients treated with dalteparin and warfarin. Progression was observed in 8% and 3% of patients, respectively. Changes in thrombus size

according to the Marder score were similar in all groups. There was no statistically significant difference between any of the treatment groups and no significant dose response relationship was observed for the ximelagatran treatment groups. Treatment discontinuation due to bleeding occurred in two patients receiving ximelagatran (24 mg and 36 mg groups) and in two patients receiving dalteparin and warfarin.

THRIVE-II a phase III trial. A double-blind, multicenter, randomized, double placebo-controlled study is being performed to evaluate the efficacy and safety of ximelagatran in the treatment of venous thromboembolism. A total number of 1800 patients with DVT and/or PE are being allocated to receive either ximelagatran or enoxaparin and warfarin for 6 months. In both groups treatment starts at the diagnosis of the thromboembolic episode. The results of this study have not been published yet.

THRIVE-III a phase III trial. This is a multicenter, randomized, placebo-controlled study of the efficacy and safety of prolonged treatment with ximelagatran in patients already treated for 6 months with coumarins for a thromboembolic event. In 612 patients the treatment with ximelagatran (24 mg orally twice daily) started after the discontinuation of coumarins whereas 611 patients received placebo. The duration of the treatment was 18 months. The primary efficacy end-point was the frequency of recurrent venous thromboembolism during the 18 months of observation. Mortality, the frequency of bleeding events and pharmacokinetics parameters were the secondary end-points evaluated in this study. Mature results of this study have not been published yet; however, preliminary results show that the frequency of thromboembolic events (symptomatic and objectively documented) was lower in the ximelagatran group than in the placebo group (1.96% versus 11%, respectively). The mortality rate was similar in both groups (about 1%) but 3 deaths due to pulmonary embolism occurred in the placebo group. The frequency of major hemorrhage was not significantly different between the two groups (0.9% in the ximelagatran group and 0.8% in the placebo group).

Discussion and Conclusion

The search for safer and more effective anticoagulants, by targeting specific steps in the clotting mechanism, has been very successful. These new agents are challenging over multi-target drugs such as heparin and vitamin K antagonists. Fondaparinux is a new synthetic antithrombotic agent developed using a process of rational drug design. It has been classified by the World Health Organization in the class of *Other antithrombotics* (ATC code: B01AX05), differing notably from UFH and LMWH. Indeed, this chemically synthesized molecule is original with regard to its selective target (factor Xa) and its mode of action (via antithrombin). It has a favorable pharmacokinetic profile allowing a convenient once-daily administration without requiring routine monitoring of anticoagulant activity or dose adjustments.[8] The Matisse DVT/PE trials showed that fondaparinux, at the dose of 7.5 mg administered subcutaneously once daily, is as effective and as safe as enoxaparin and UFH for the initiation of the treatment of DVT and PE, respectively.

A new generation of meta-pentasaccharides with improved pharmacodynamics and pharmacokinetics is under development. Encouraging results from the dose finding PERSIST trial raise hope that in the in the near future a single weekly injection of idraparinux might also be an effective and safe option for the treatment of venous thromboembolism. Moreover the results from clinical trials with the synthetic pentasaccharides, demonstrate that a pure factor Xa inhibitor devoid of antithrombin activity can exhibit a powerful antithrombotic activity in man.

Ximelagatran is the first orally active direct synthetic thrombin inhibitor with favorable pharmacokinetics and pharmacodynamics. The pharmacokinetics of ximelagatran is not influenced by other commonly used drugs or by food intake.[8] The trials published so far show that oral administration of ximelagatran is at least as effective and as safe as LMWH treatment for the initiation of anticoagulation after the diagnosis of a thromboembolic event, without any apparent requirement for laboratory monitoring or dose adjustment. Ximelagatran could also be an alternative to coumarins for the secondary prevention of VTE and moreover, is a promising treatment for a long-term secondary prevention of VTE in high risk patients: dose adjustments are apparently not needed.

The forthcoming specific inhibitors are going to enrich the armamentarium against thromboembolic diseases, and raise new questions about the socio-economic impact of the new antithrombotic strategies.

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Clinical evidencies of prophylaxis in major orthopedic surgery: towards optimal results

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Major orthopedic procedures are associated with very high rates of deep venous thrombosis (DVT) and pulmonary embolism (PE). Major limb surgery is so inherently thrombogenic that patients are at a very high risk of VTE, regardless of their accompanying risk factors.[1,2] Without prophylaxis, up to 13% of patients with fracture of the hip die as a result of PE. In the absence of prophylaxis, elective hip replacement and hip fracture are associated with DVT rates of approximately 50%, and rates may be even higher in those undergoing total knee replacement.

Currently available anticoagulants target multiple steps in the coagulation cascade. Classical anticoagulants include the heparins, which act through antithrombin (ATIII) to inhibit thrombin and factors Xa, IXa, XIa and XIIa. Vitamin K antagonists suppress factors II, VII, IX and X and also affect the anticoagulant protein C. Both of these classes of drugs affect a number of different coagulation factors. As a result of this untargeted approach, they have activity that is difficult to predict or control, and their efficacy/safety ratios are less than satisfactory. The focus of recent research has been to develop agents that can modulate the coagulation cascade by acting specifically on a single coagulation factor, with the aim of inhibiting thrombus formation and growth.

New antithrombotics and those currently in development include agents acting through a variety of mechanisms, but most aim at inhibiting only one specific step of coagulation. It is expected that higher selectivity will allow better control of anticoagulation therapy.[3-6]

Tissue factor pathway inhibitors act at an early point in the coagulation cascade to inhibit the tissue factor/factor VIIa complex ($rNAPC_2$). This counters the initiation of the coagulation cascade but may have less impact on its amplification.

Other agents, including the IXa inhibitors, protein C activators and selective factor Xa inhibitors, such as fondaparinux, inhibit the generation of thrombin. As long as they allow some thrombin activity to continue, these agents – though potent – would also be expected to preserve the physiologic feedback mechanisms that operate through thrombin.

Direct thrombin inhibitors, such as hirudin, melagatran and ximelagatran, act directly on thrombin blocking its activity but also that of the feedback mechanisms linked to thrombin.

Fondaparinux

Positioned at the crossroad of the intrinsic and extrinsic coagulation pathways, factor Xa is a central player in the coagulation cascade and ultimately, in the generation of thrombin and the clotting of fibrinogen to fibrin. Indeed, inhibition of one factor Xa molecule leads to inhibition of the generation of many thrombin molecules, leading to effective inhibition of thrombus formation and growth.

The synthetic pentasaccharide fondaparinux is the first in a new class of agents to inhibit factor Xa selectively. Fondaparinux has highly predictable anticoagulant activity, with linear, dose-dependent inhibition of thrombin generation in human platelet-depleted plasma.[3] Within the range of therapeutic plasma concentrations in man (0–2 μg/mL), there is a perfectly linear correlation between fondaparinux concentration and inhibition of thrombin generation. This linearity is the basis for the highly predictable pharmacokinetic and pharmacodynamic profile for fondaparinux.[7]

Fondaparinux, having demonstrated a very favorable efficacy/safety ratio in preclinical studies, has been investigated in major orthopedic surgery. A world-wide clinical development program, the largest ever conducted for thromboprophylaxis in orthopedic surgery, led to four phase III studies: these studies covered all major orthopedic procedures (hip replacement, knee replacement and hip fracture surgery). All used a single regimen of fondaparinux, whatever the type of patient or procedure. These studies were powered to demonstrate superiority over a low molecular weight heparin, currently the most commonly-used treatment.[8-11]

The low inter- and intravariability of fondaparinux's pharmacokinetics allowed a single regimen to be used for all patients included in the phase III trials for VTE prophylaxis. These trials, designed to assess the efficacy and safety of fondaparinux in the prophylaxis of VTE following major orthopedic surgery, are:

- Pentathlon 2000: 2,275 patients undergoing total hip replacement;
- Pentamaks: 1,049 patients undergoing major knee surgery;
- Ephesus: 2,309 patients undergoing total hip

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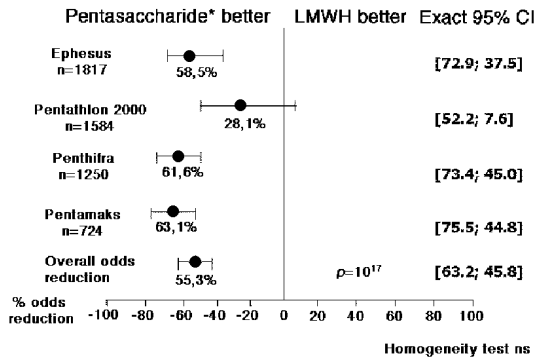

Figure 1. Overall efficacy: fondaparinux vs. LMWH.

replacement;

• Penthifra: 1,711 patients with hip fracture.

All studies were prospective, randomized, and double-blind on two parallel groups (fondaparinux - or enoxaparin-treated), and were designed to assess whether a fixed dose of fondaparinux 2.5 mg daily started post-operatively provides better superior prophylaxis than enoxaparin.

The Pentathlon and Pentamaks studies were performed according to North American preferences, using enoxaparin 30 mg *b.i.d.*, starting post-operatively, as the comparator drug. The Ephesus and Penthifra studies were performed using the European approach, enoxaparin 40 mg once daily, starting pre-operatively, as the comparator drug. The primary efficacy endpoint was VTE up to day 11 after surgery, defined as DVT detected by mandatory bilateral venography and/or documented symptomatic DVT or PE.

The results demonstrated the greater efficacy of fondaparinux vs enoxaparin in the prevention of thromboembolic disease in patients submitted to surgery for hip fracture and in patients submitted to elective hip or knee surgery, with a global relative risk reduction (RRR) in favor of fondaparinux of 55.3%, which was highly significant (p=10-17), as shown in Figure 1.

The greater efficacy of fondaparinux compared to enoxaparin was achieved without an increase in the risk of clinically relevant bleeding. In all four phase III studies of fondaparinux in major orthopedic surgery, major bleeding was the main safety outcome. Major bleeding included four categories: bleeding leading to death, bleeding leading to re-operation, bleeding occurring in a critical organ and bleeding with a bleeding index of 2.0 or more. The bleeding index was calculated as follows: [number of units of packed red blood cells or whole blood transfused] + [(pre-bleeding) – (post-bleeding) hemoglobin (g/dL) values]. The two treatments did not differ in the first three categories of major bleeding, but there was a difference in the bleeding index. Globally there were more patients with a bleeding index ≥2.0 in the fondaparinux group. There were no differences in terms of minor bleedings.

Recently, the Penthifra Plus study assessed the value of prolonged prevention with fondaparinux in hip fracture surgery. After an initial treatment period of 7±1 days patients were randomized to stop prophylaxis or to continue for a total of 21±2 days. Bilateral venography performed at the end of the treatment period showed a significant reduction of all VTE from 35% to 1.4% (RRR 96%). Symptomatic VTE decreased from 2.7% to 0.3% (RRR 89%). These results were achieved without a significant increase in clinically relevant bleeding.[12]

Melagatran and Ximelagatran

Ximelagatran is a novel, oral direct thrombin inhibitor under development for the prophylaxis and treatment of thromboembolic disease. Pre- and post-operative regimens of ximelagatran, and its subcutaneous (sc) form, melagatran, have been evaluated in total hip replacement (THR) and total knee replacement (TKR). The Express study aimed to investigate the efficacy and safety of this thrombin inhibitor started in close proximity to surgery (knife-to-skin). In this randomized, double-blind, parallel-group study, one group received sc melagatran 2.0 mg immediately before surgery followed by sc 3.0 mg in the evening after surgery, and then oral ximelagatran 24 mg *b.i.d.* as a fixed dose (the ximelagatran group). The other group received sc enoxaparin 40 mg once daily, started the evening before surgery (the enoxaparin group). The total duration of active treatments was 8 to 11 days. Bilateral venography was performed on the final day of treatment. Of the 2,764 patients in the ITT population (n=1856 [THR] and n=908 [TKR]), 82% had an evaluable venogram. The majority of patients (92%) started oral therapy the morning after surgery. The rate of proximal vein thrombosis plus pulmonary embolism (the study's primary endpoint) was 2.3% in the ximelagatran group and 6.3% in the enoxaparin group (p<0.0003; RRR 23.6%). Bleeding events were more common in the ximelagatran group than in the enoxaparin group (3.3% vs 1.2%), as were transfusion rates (66.8% vs 61.7%). There were no differences in clinically important bleeding events (fatal bleeding, critical organ bleeding, or bleeding requiring re-operation). The Express study demonstrated that preoperatively initiated sc melagatran followed by oral ximelagatran was more effective than enoxaparin in preventing VTE in patients undergoing THR or TKR.[13]

In a different study, ximelagatran was compared to warfarin to prevent VTE after total knee replacement surgery. Fixed dose (no coagulation monitoring, or dose adjustments) of ximelagatran, 24 or 36 mg b.i.d., or warfarin (target INR 2.5; range 1.8-3.0) and matched placebo were continued for 7-12 days. Warfarin was initiated the evening of surgery, whereas the first dose of ximelagatran was given the morning after surgery. The efficacy of ximelagatran 36 mg *per os b.i.d.* was superior to that of warfarin for the composite endpoint of distal and/or proximal DVT, and/or symptomatic DVT/PE (objectively confirmed), and/or all-cause mortality. Ximelagatran 36 mg per os b.i.d. started the day after TKR for prophylaxis had a greater efficacy than warfarin, did not increase bleeding compared with warfarin, and did not require routine coagulation monitoring or dose adjustments.[14]

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Prevention of venous thromboembolism in high risk abdominal surgery

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Patients who are undergoing major abdominal surgery, essentially for cancer, are at high risk of postoperative thrombosis.[1] The explanation for the high incidence of venous thromboembolism is multifactorial. In addition to their malignancy, when this is the reason for surgery, these patients often have concurrent risk factors that increase their risk of thrombosis, including advanced age, debility, long and complicated surgery, and often a prolonged hospitalization before surgery and a prolonged postoperative hospital stay.

Venous thrombosis after abdominal surgery usually starts in the calf and extends to the proximal veins before it can cause pulmonary embolism. However, screening patients for leg symptoms and signs is an unreliable method for detecting asymptomatic deep vein thrombosis and treating those who actually develop the disease. Indeed, most patients with pulmonary embolism have no symptoms or signs of deep vein thrombosis (DVT) of the lower limbs although venography reveals this disease in 70% of patients with pulmonary embolism. Furthermore, ultrasonography has a low sensitivity for screening asymptomatic deep vein thrombosis. Consequently, primary prophylaxis is the optimal way of preventing postoperative pulmonary embolism, which is not uncommonly fatal.

Kakkar and co-workers were among the first investigators to evaluate the risk of thrombosis following major abdominal surgery using ^{125}I-fibrinogen leg scanning[2] Introduced in the 1970s, this technique was used for outcome assessment in clinical trials evaluating antithrombotic prophylaxis of venous thromboembolism after surgery. This technique is no longer in use because of concern over viral transmission and diagnostic inaccuracy. Kakkar showed that the postoperative rate of DVT was higher in patients with malignancy (41%) than in patients undergoing abdominal surgery for a benign disease (26%). A significantly higher rate of fatal pulmonary embolism following surgery was observed in cancer patients compared to in non-cancer patients. A subgroup analysis of a large, multicenter study with more than 4,000 patients showed that the rate of fatal pulmonary embolism was 1.6% in patients with cancer and 0.5% in those without cancer (p=0.05).[3]

The first step in coming to a decision on whether and what method of prophylaxis should be used after abdominal surgery is risk stratification for postoperative venous thromboembolism.[1] Clinical risk factors include advanced age, prolonged immobility, previous venous thromboembolism, cancer, extensive surgery and heart failure. Pharmacologic prophylaxis has not been proven to be required for patients with a low risk of venous thromboembolism (VTE). Low risk patients are generally less than 40 years of age, have had minor surgery performed, with general anesthesia lasting less than 30 minutes, and do not have clinical risk factors for VTE. They should be mobilized as quickly as possible. Patients with a moderate or high risk of VTE should receive prophylaxis. Unless contraindicated, this will involve the use of an antithrombotic agent alone or in combination with a mechanical method of prophylaxis.

Low-dose unfractionated heparin, at the dose of 5,000 units twice or three times a day, and a low-molecular-weight heparin validated for this indication are adequate regimens when given subcutaneously for prophylaxis of venous thromboembolism after abdominal surgery.[1] There are no randomized trials comparing twice daily dosing with three daily doses of unfractionated heparin, although one meta-analysis showed that unfractionated heparin given every eight hours was more efficacious.[4] A number of clinical trials have compared the efficacy and safety of low-molecular-weight heparin with unfractionated heparin in reducing postoperative venous thromboembolism following major abdominal surgery.[1] Essentially, no significant differences were found in efficacy and safety between these agents. Whether this holds true in both non-cancer and cancer patients is unclear because the results for the two patient groups were not analyzed or reported separately in many of the studies. In the trials of low-dose unfractionated heparin versus low-molecular-weight heparin that did comment on the differences between cancer and non-cancer patients, a higher incidence of thrombotic complications in cancer patients was consistently reported, independently of the prophylaxis agent used. Most of the studies that compared low-molecular-weight heparin with unfractionated heparin had insufficient statistical power to demonstrate a difference in efficacy or bleeding between low-molecular-weight heparin and unfractionated heparin

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Table 1. Randomized trials comparing low molecular weight heparin (LMWH) with unfractionated heparin (UFH) or placebo for the prevention of deep venous thrombosis (DVT) following general surgery.

Study	*LMWH group*	*Control group*	*Patient group*	*N*	*DVT rate in LMWH group (%)*	*DVT rate in UFH group (%)*	*p value*
European Fraxiparin Study group 1988	Nadroparin	UFH	Cancer	704	4.2	5.4	–
			Benign	1,192	2.0	3.9	
Ockelford 1989	Dalteparin	Placebo	Cancer	79	7.1	27.0	<0.001
			Benign	104	1.8	7.8	NS
Nurmohamed 1995	Enoxaparin	UFH	Cancer	516	13.6	8.7	0.09
			Benign	910	4.8	5.0	NS

^{125}I-fibrinogen or impedance plethysmography was used to detect DVT.

in cancer patients. Table 1 summarizes the results from those studies that provided separate rates of deep vein thrombosis for patients undergoing surgery for malignant versus benign disease (Table 1). A distinct advantage of using low-molecular-weight heparins is that they can be administered once a day and are less likely to produce heparin-induced thrombocytopenia.[5]

Only a few studies have specifically focused on prophylaxis in patients undergoing general surgery for malignancy. In one of these studies, Gallus and co-workers randomized 513 patients undergoing elective surgery for intra-abdominal or intrathoracic malignancy to receive perioperative danaparoid, 750 U twice daily, or unfractionated heparin, 5,000 U twice daily.[6] The rates of thrombosis detected by ^{125}I fibrinogen leg scanning, 10.4% and 14.9%, respectively, were not statistically different as were not the rates of bleeding complications. The investigators of the Enoxacan study group compared the low-molecular-weight heparin enoxaparin, 40 mg once daily, with unfractionated heparin, 5,000 U three times a day, in patients having elective curative surgery for cancer of the abdomen or pelvis.[7] Unlike all the previous studies, in which fibrinogen leg scanning or impedance plethysmography was used for outcome assessment, this study used mandatory bilateral venography. The rates of thrombosis were 14.7% in the enoxaparin group and 18.2% in the unfractionated heparin group. This difference was not statistically different. There were also no differences in the rates of major bleeding and the 30-day and 3-month mortality.

Di Carlo and co-workers compared the efficacy and safety of the selective thrombin inhibitor, dermatan sulphate, and unfractionated heparin in the prevention of venous thromboembolism in patients undergoing cancer surgery.[8] Dermatan sulphate was given at the dose of 600 mg intramuscularly on the second day before surgery followed by 300 mg once daily while unfractionated heparin was given at the dose of 5000 U subcutaneously three times a day, starting two hours before surgery. Both treatments were continued until the 7th postoperative day. Bilateral venography was scheduled at the end of treatment. Efficacy was assessed in 521 patients with adequate venography and/or confirmed pulmonary embolism. Postoperative VTE occurred in the 15% of the dermatan sulphate patients and in 22% of those receiving unfractionated heparin.

The issue of extending prophylaxis beyond the hospital stay was addressed by the recently published Enoxacan 2 study.[9] In this study patients undergoing surgery for abdominal malignancy received 1 week of enoxaparin and were then randomized to enoxaparin or placebo for another 21 days. Bilateral venography was performed at the end of treatment. There was a statistically significant reduction in DVT, 12% versus 4.8%.

Physical methods, such as external pneumatic compression and graduated compression stockings, are effective in reducing thrombosis after general surgery. External pneumatic compression, although it is effective, is inconvenient and interferes with early mobilization. It is unclear whether the use of graduated compression stockings in combination with anticoagulant prophylaxis results in further risk reduction. Therefore, clinicians usually reserve these for patients with active hemorrhage or a high risk of bleeding following surgery or for use in combination with unfractionated heparin or low-molecular-weight heparin in patients with a previous venous thrombosis.

The clinical value of the selective factor-Xa inhibitor, fondaparinux, in high-risk abdominal surgery is currently under evaluation in the Pegasus study. Fondaparinux has been shown to be more effective than enoxaparin in four studies on

the prevention of venous thromboembolism after major orthopedic surgery. Pegasus is a randomized double-blind study comparing fondaparinux, given at the dose of 2.5 mg once a day starting 6 hours after surgery, with the low-molecular-weight heparin dalteparin, given at the dose of 5,000 U once a day starting 12 hours before surgery. To assess the primary outcome of the study, venography is being done at the end of the prophylactic regimen. The results of the Pegasus study, the largest venography study ever done in the prevention of venous thromboembolism in high-risk abdominal surgery, are anxiously awaited.

Conclusions

Pharmacologic prophylaxis is effective and recommended for the prevention of venous thromboembolism in abdominal surgery. Although the optimal prophylactic regimen remains undefined in the high-risk group, some general recommendations can be made based on the available evidence. Thromboprophylaxis should be initiated before surgery and continued for at least four weeks (at least in cancer patients). Unfractionated heparin and low-molecular-weight heparin appear to be equally safe and effective. The major advantage of using a low-molecular-weight heparin is the once-daily administration. Graduated compression stockings are effective and may improve the efficacy of pharmacologic prophylaxis. External pneumatic compression is an acceptable alternative in patients with active bleeding or those who are at high risk of bleeding, in which case anticoagulant use is contraindicated. The results of the Pegasus study with fondaparinux are anxiously awaited.

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The treatment of venous thromboembolic disorders: new challenges

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The aim of treating patients with venous thromboembolism (VTE) is to improve outcomes by preventing extension of the thrombosis, embolization to the lungs, and the development of late complications, such as recurrences, post-thrombotic syndrome, and chronic pulmonary hypertension.

The large majority of patients with VTE are currently treated with full doses of unfractionated (UFH) or low-molecular-weight heparin (LMWH) followed by at least three months of oral anticoagulant therapy.[1] Selected patients with critical manifestations of pulmonary embolism (PE) are administered thrombolytic drugs, while intravenal cava filters are confined to patients with either deep vein thrombosis (DVT) or PE who present with serious contraindications to conventional anticoagulation.[1]

Although considerable progress has been made in the treatment of venous thromboembolic disorders, many unanswered questions remain and await proper solution. Furthermore, new opportunities are emerging, which have to potential to modify the therapeutic scenario substantially in the near future. The topics that are worth exploring include home treatment of selected patients with DVT, the treatment of cancer patients with venous thrombosis, renewed interest in thrombolytic drugs in patients with PE, the optimal duration of oral anticoagulant therapy, and the potential of new drugs categories in the initial treatment and secondary prevention of VTE.

Home treatment of DVT

The observation that LMWHs are at least as effective and safe as UFH when administered by fixed-dose subcutaneous injections stimulated the hypothesis that it might be possible to use LMWH preparations to treat selected patients with DVT in an out-of-hospital setting. To test this fascinating hypothesis, two multicenter clinical trials were performed in the second half of the 1990s, one used nadroparin, the other enoxaparin.[2,3] Their conclusions consistently supported the feasibility, efficacy and safety of home treatment of patients with uncomplicated DVT with subcutaneous fixed doses of LMWHs. Furthermore, this strategy was associated with an improvement of quality of life, and a relevant reduction of health care costs. A number of prospective cohort studies have been subsequently performed, supporting the feasibility and safety of home treatment of DVT.[4]

Home treatment of DVT has become daily clinical practice in many countries. There are, however, essential requirements for the success of a home treatment program. Patients need to be educated about what venous thrombosis is, its possible complications and side effects, and need to be instructed on self-injecting the drug or nursing support. Initiation and monitoring of oral anticoagulant therapy are performed entirely on an outpatient basis; thus community facilities should be prepared for this task. A few aspects of home treatment still await appropriate clarification: when and how intensively can patients ambulate? Does the platelet count need to be determined? Might selected patients benefit from drug monitoring ?

The treatment of cancer patients with venous thrombosis

Patients with DVT who also have cancer have a higher risk of recurrent thromboembolism and major bleeding during anticoagulation.[5,6] In a recent prospective cohort study in a wide series of patients with venous thrombosis with or without cancer, the 12-month cumulative incidence of both recurrent thromboembolism and major bleeding during anticoagulation was significantly higher in patients with cancer than in those without cancer.[7] Recurrence and bleeding were both related to cancer severity, occurred predominantly during the first month of anticoagulant therapy but could not be explained by sub- or overanticoagulation.[7] Possibilities for improvement using the current paradigms of anticoagulation seem, therefore, limited and new treatment strategies should be developed. The long-term use of LMWH has recently been shown to be significantly more effective than and as safe as warfarin for the initial treatment and secondary prevention of VTE in cancer patients with venous thrombosis (ASH 2002).

The treatment of pulmonary embolism

Recent studies have put into question the systematic use of anticoagulants alone in the initial treatment of patients with submassive PE. The risk of an unfavourable outcome seems definitely higher in patients with right ventricular dysfunction, as shown by echocardiography.[8,9] The use of thrombolytic drugs,

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which promptly restore the patency of the pulmonary arterial vessels, has the potential to improve the outcome of patients with PE. Recently, two meta-analyses of comparative studies between thrombolysis and heparin in the treatment of acute PE have been published.[10,11] The results of these meta-analyses consistently showed that patients treated with thrombolytic drugs had a more favorable outcome, in terms of prevention of short-term recurrent episodes of PE, than those treated with heparin alone. The difference became statistically significant when a composite endpoint consisting of death/recurrence was calculated.[10] However, patients treated with thrombolytic drugs had a definitely higher risk of hemorrhage.[10,11] In a recent prospective controlled study, a wide series of patients with submassive PE and contemporary right ventricular dysfunction were randomized to receive heparin alone or in combination with alteplase.[12] Patients treated with the combination of heparin with alteplase had a significantly lower rate of in-hospital death and clinical deterioration, while the hemorrhagic risk was similarly low in the two treatment groups. The results of this study have the potential to expand the use of thrombolysis in patients with acute PE, at least in those with right ventricular dysfunction.

The optimal duration of anticoagulant treatment

After the publication of an impressive series of prospective cohort studies,[13,14] population-based studies,[15] and randomized clinical trials,[16-19] we know that:

- 5-10% of patients with secondary DVT from transient risk factors have a recurrent VTE after three months of oral anticoagulant therapy;
- 15-30% of patients with idiopathic DVT have a recurrent VTE after three months. This rate will not change by prolonging OAT up to 6-12-24 months;
- The role of thrombophilia is controversial.
- The annual incidence of major bleeding from oral anticoagulant therapy is 1.5-2.0%. The *case-fatality rate* of an episode of major bleeding is four times as high as that observed in patients with recurrent VTE.

To optimize the long-term treatment of VTE, new strategies and new drugs are currently under investigation. The former include the evaluation of the benefit-to risk of reducing the intensity of oral anticoagulants, and that of tailoring the duration of anticoagulants according to residual vein thrombosis, as shown by repeat leg vein ultrasonography, and/or the behavior of the D-dimer test. The latter include the evaluation of new categories of drugs, i.e., pentasaccharides and thrombin inhibitors such as melagatran and derivatives (see below).

Beyond heparins

Selective factor Xa inhibitors. Fondaparinux, a pentasaccharide, is the first of a new class of synthetic antithrombotic agents designed specifically for a single physiologic target in the coagulation cascade. This compound is identical to the pentasaccharide sequence in heparin with high affinity for antithrombin. It selectively binds to antithrombin and induces a conformational change of its molecule that increases the anti-Xa activity of antithrombin by almost 300 times. This compound has recently been approved for prophylaxis of VTE in patients undergoing major orthopedic surgery.

In a phase II study published in 2000, this compound appeared to be as effective and safe as dalteparin across a wide range of doses also for the treatment of established DVT.[20] According to the results of two large phase III multicenter clinical trials, the once daily subcutaneous administration of 7.5 mg of fondaparinux is as effective and safe as enoxaparin for the treatment of DVT, and as least as effective and safe as UFH for the treatment of PE (ASH 2002). Furthermore, the once weekly administration of 2.5 mg of a long-active formulation of pentasaccharide (idraparinux) has recently been shown in a phase II study to be at least as effective and safe as warfarin for the secondary prevention of DVT (ASH 2002).

Direct thrombin inhibitors. The direct thrombin inhibitors include hirudin, bivalirudin, and active-site inhibitors (such as argatroban and melagatran). Agents that directly inhibit thrombin have several advantages over (LMW)heparins, including the inhibition of fibrin-bound thrombin, a dose response that is more predictable because there is no binding to plasma proteins, and a lack of potential to produce immune thrombocytopenia. Among these preparations, ximelagatran (an oral prodrug that is converted to melagatran and does not require laboratory monitoring) show promise for the prophylaxis and treatment of VTE. According to the results of a recent, randomized clinical trial, the oral administration of fixed doses of ximelagatran is more effective than and as safe as placebo for the prevention of recurrent VTE following the administration of six months of warfarin in patients with DVT (ASH 2002). A phase III clinical trial of ximelagatran for the initial treatment of DVT has recently completed recruitment.

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The contribution of Italian cardiology to the knowledge of acute coronary syndromes

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Great progresses in the understanding of the pathophysiology and therapy of acute coronary syndromes (ACS) have been made in the last quarter of the 20th century. In this field the contribution of Italian Cardiology has been particularly remarkable, with original research activities in the understanding of mechanisms as well as in the assessment of therapies in ACS.

The GISSI story

In the mid 1980s the GISSI 1 study[1] introduced a true revolution in the treatment of patients with acute myocardial infarction (AMI) by demonstrating the efficacy of intravenous thrombolysis. The demonstration of the benefit of streptokinase compared with placebo was clearcut. The GISSI 1 trial also assessed the effectiveness of thrombolytic treatment, because this therapeutic intervention was tested in a large number of patients enrolled in a large number of unselected Coronary Care Units (CCUs) from throughot Italy.

The GISSI 1 national megatrial was organized in 1984 by the Italian Association of Hospital Cardiologists (ANMCO) and the Mario Negri Institute with a modest financial investment. This was the first of a series of GISSI studies which became an international model for collaborative clinical research. According to Robert Califf,[2] "*the broad scale clinical research collaboration initiated by the ISIS and GISSI groups has changed the fate of cardiovascular medicine*".

As finely stated by Luigi Tavazzi[3] in the honorary lecture on Population Sciences at the 2002 ESC Congress, "*the GISSI 1 trial was born and perceived as a collective identity card of a whole professional society, which agreed to transform the routine clinical activity into an experimental exercise, and to become a cooperative, public-health oriented network.*"

Following the success of the GISSI 1 trial, which led to the regulatory approval of streptokinase in AMI by the Food and Drug Administration, a number of GISSI studies were run within the ANMCO network involving more than 200 CCUs in Italy.

In summary, the main results of the GISSI trials, which represent a substantial contribution to the treatment of AMI, are the following:

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GISSI 1 (1984-1985).[1] Thrombolysis is effective and safe in AMI.

GISSI 2 (1988-1989).[4] No major differences in efficacy between thrombolytics of 1st and 2nd generation in AMI patients.

GISSI 3 (1991-1993).[5] ACE-inhibitors are effective and safe in AMI; systematic nitroglycerin infusion in absence of clinical indications is neutral.

GISSI Prevention (1993-1996).[6] N-3 PUFA are safe and effective in preventing sudden death in postinfarct patients; vitamin E is not effective.

In the 20 years since their beginning, the GISSI studies have obtained a wide recognition in the international world of Cardiology and are considered a methodological landmark. Most importantly, thanks to the active participation of the national cardiology community as *investigators*, the results of the GISSI studies were rapidly incorporated into the clinical practice. In general, transfer of the results of the scientific literature to clinical practice takes place very slowly. GISSI has accelerated this process: the use of thrombolytics and ACE inhibitors in patients with AMI is widespread and surely more common than that observed in CCUs in the USA and in Canada.

The GISSI studies not only led to a change in the global care of patients with AMI, but through the huge database constituted a golden source of information of clinical epidemiology, pharmaco-epidemiology and were generators of new algorithms of prognostic stratification and decision-making processes.

Relevant byproducts derived from the GISSI database include:

- systematic incorporation of post-AMI prognostic stratification (echocardiography, exercise test, Holter monitoring): from GISSI 2;
- focus on left ventricular dysfunction as major prognostic marker: from GISSI 3;
- the GISSI chart of risk for secondary prevention: from GISSI-Prevention.

Beside the GISSI studies the contribution of the ANMCO to research in ischemic heart disease includes surveys, outcome studies and registries. The surveys performed in the setting of ACS include:

- the avoidable delay in early care of AMI patients (1990) focusing on time and pathways from onset of symptoms to treatment;
- GISSI-Prognosis (1996) focusing on decision-mak-

ing processes leading to prognostic stratification and therapy after AMI;
- EARISA survey (1996) focusing on the in-hospital processes and outcome of patients with AMI, stable or unstable angina, heart failure, and supraventricular arrhythmias;
- BLITZ (2001) focusing on the pre-hospital or in-hospital management of ST-elevation and non-ST elevation ACS.

The outcome studies and registries performed in the setting of ACS include:
- two *appropriateness* studies on coronary revascularization procedures in Lombardy (1995, 1997), focusing on appropriateness of indications for revascularization procedures, rates and times of performance;
- MISTRAL (1998) focusing on the therapeutic approach to high-risk AMI patients (ST elevation) at admission to selected centers with and without primary PTCA facilities;
- SPS Registry (2000 - ongoing) focusing on the implementation of preventive measures in patients with ischemic heart disease.

Coronary vasospasm

Many individuals have contributed to the vast amount of accumulated literature on coronary artery spasm, but Attilio Maseri and his group have provided the most elegant pathophysiologic investigations in patients presenting with angina at rest. In the first study performed in Pisa, which subsequently became a historical article and classical citation, Maseri *et al.*[7] described characteristic features of 138 patients with *variant angina*. They provided evidence that coronary vasospasm can result in myocardial ischemia, which can occur in the presence or absence of coronary atherosclerosis of varying degrees with or without a previous myocardial infarction and with or without exertional angina. They also indicated that vasospastic origins of myocardial ischemia can be associated with ST-segment depression rather than ST-segment elevation, and finally, they indicated that myocardial ischemia secondary to vasospasm can be asymptomatic and in a few instances evolve into AMI and sudden cardiac death.

Another important observation made by Maseri *et al.*[8] was that vasospastic angina can occur in the presence of extremely variable degrees of coronary atherosclerosis and in any phase of ischemic heart disease, possibly evolving into AMI and sudden death.

Through these original investigations Maseri put emphasis on primary angina rather than on vasospastic angina, writing a new chapter in the pathophysiology of myocardial ischemia. Maseri and his group proved conclusively that not all episodes of acute myocardial ischemia are due to fixed critical coronary artery stenosis that limits coronary bood flow under conditions of increased myocardial oxygen demand. The new concept that also non-critical coronary stenoses can lead to acute myocardial ischemia represents a pioneering contribution to the discovery of mechanisms of ACS.

Inflammation

Among the different factors involved in the development of ACS, a growing role is being attributed to inflammation. This is now recognized as a well-established evidence. In this moving field much original research has been contributed by Italian investigators, mainly from Florence and Rome.

Neri Serneri *et al.*[9] proposed that unstable angina is associated with an acute transient burst of inflammation, with lymphocyte activation triggered by unknown factors. The results of this original investigation demonstrated that blood clotting activation with increased thrombin formation in unstable angina patients is due to the expression of tissue factor-like activity by activated monocytes. The same authors reported that enhanced lymphocyte activation is associated with the worst prognosis.

Following these original investigations, a rich wealth of information relating to the role of inflammation in ACSs was provided in the last decade by the brilliant group of Maseri at the Catholic University of the Sacred Heart in Rome.

Histological evidence of inflammation in atherosclerosis clearly differentiates stable from unstable forms of IHD. Not only have activated inflammatory cells been found in the plaques, but more interestingly also circulating activated inflammatory cells as well as elevated levels of systemic markers of inflammation have been described. Among these, C-reactive protein (CRP) is of clinical value, as its levels are associated with outcome.

In 1994 Liuzzo *et al.*[10] reported on the prognostic value of CRP, a prototypic acute phase reactant, in severe unstable angina patients with normal troponin T levels. More recently the same group has shown that elevated CRP levels at discharge are associated with a recurrence of ischemic events, including death and myocardial infarction, at 1 year[11] and that inflammation is important also in triggering the mechanisms of restenosis after percutaneous transcoronary angioplasty (PTCA).[12]

Platelets and aspirin

In the early 1980s the discovery that aspirin can simultaneously inhibit thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) synthesis, a potent antiaggregating and vasodilating agent, raised the so-called *aspirin dilemma*. The assumption was made, and popularized, that to achieve antithrombotic efficacy, the inhibitory effect of aspirin on platelet

cyclo-oxygenase should be retained, while that on the vascular enzyme should be minimized.

Many clinicians were fascinated by this *aspirin dilemma* and urged pharmacologists to solve it rapidly. In this field the Italian contribution was relevant, mainly due to a number of pharmacological research studies by Neri Serneri, De Gaetano, Patrono and Mannucci.[13-15] Biochemical selectivity of aspirin in relation to platelet and vascular cyclo-oxygenase was the target of *in vitro* and *in vivo* experimental studies. A better knowledge of the pharmacokinetics of aspirin and salicylate was achieved thanks to the productive research of Giovanni De Gaetano and his group at the Mario Negri Institute.[16] The low-dose aspirin concept was subsequently supported by the results of large clinical trials showing a dose-unrelated beneficial effect of aspirin in secondary prevention after AMI.

Left ventricular thrombosis

Many researchers in the mid 1980s started to study intracardiac thrombosis. In the pre-thrombolytic era the phenomenon of left ventricular thrombosis after AMI was particularly important for the catastrophic effects of embolism on the clinical course of the patients. In this field the contribution of Carlo Vecchio and his fine group in Genova was outstanding. In a series of studies they assessed the prognostic significance and natural history of left ventricular thrombi in patients with AMI, along with the morphologic features of embolic potential as a useful tool for identifying candidates for more aggressive antithrombotic treatment.[17]

Conclusions

The above mentioned Italian contributions to the knowledge of ACS represent only a part of the studies carried out on this field in our country in the last 25 years. What are the perspectives for the future ? A solid cardiological framework has been definitely established in Italy, including 882 Cardiology centers and 380 CCUs. This allows an efficient network for randomized controlled trials testing new therapeutic strategies in ACS, clinical-epidemiological surveys, observational studies on diagnostic-therapeutic pathways or resource utilization and , very importantly, for outcome studies to translate scientific results into clinical practice.

This is the bright face of the moon. The dark face is that a further advance in the knowledge of ACS requires pathophysiological studies to be performed in selected research centers. At present this is an unmeet need, as few such programs are in progress. A major obstacle is the paucity of funding from public sources, as private sources, mainly pharmaceutical companies, are now supplying the vast majority of the budget for cardiovascular research. It is unlikely that companies will be interested in supporting orphan studies, physiopathological investigations or studies finalized at circumscribing a certain therapeutic strategy to specific subgroups of responders.

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Acute coronary syndromes: new trends in blood anticoagulation

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The role of coronary thrombosis in the development of acute coronary syndromes with either persistent ST-segment elevation (STEMI) or non-persistent ST-segment elevation (NSTEMI) is so important that targeting therapy to platelets, blood coagulation and the fibrinolytic system has become the rule in the management of most clinical situations.

The majority of patients receive multiple antithrombotic drugs, with different combinations for different indications. This entails the need for careful evaluation of the trade-off between efficacy and safety in a growing population of patients at high risk of both cardiovascular events and bleeding outcomes, due to advanced age and concomitant illness. The whole subject is further complicated by the very frequent use of invasive procedures, requiring specific adaptations of antithrombotic regimens.

Targeting blood coagulation is central to the treatment of virtually all patients with acute coronary syndrome, because optimal anticoagulation will reduce both ischemic and hemorrhagic complications. Therefore, cardiologists must become familiar with the coagulation system, with drugs that affect its components and with new achievements in the field.

Patients with persistent ST-segment elevation (STEMI)

All patients with chest pain of less than 12 hours' duration are candidates for reperfusion therapy with either primary angioplasty or thrombolytic therapy. Primary angioplasty is the *preferred* treatment according to the European guidelines.[1] In this context it is recommended that a lower heparin dosage is used in the catheterisation laboratory when adjunctive GP IIB-IIIA inhibition is contemplated, in order to avoid bleeding complications.

When thrombolytic therapy is undertaken (i.e. when primary angioplasty cannot be performed within 90 minutes or when thrombolysis is administered in the pre-hospital setting), the use of a lower-intensity heparin regimen, such as that combined with tenecteplase in the ASSENT-2 study, results in significantly fewer major bleeding complications.[2]

In the context of thrombolysis, the trade-off between efficacy and safety may be further improved by switching from unfractionated to low molecular weight heparin (LMWH) (Table 1). LMWHs do not increase early coronary patency (i.e. patency observed 60-90 minutes after treatment), but they decrease coronary reocclusion and in-hospital reinfarction, achieving higher late patency rates. One thing that is still not clear is whether an initial intravenous bolus of LMWH is needed during thrombolysis. This initial bolus did not improve early patency in the ENTIRE-TIMI 23 study,[3] but may be responsible for some excess in intracranial hemorrhage that was observed in the ASSENT-3-PLUS study. For these reasons the exact role and the modality of LMWH anticoagulation after thrombolysis remains to be fully elucidated by new large scale trials currently ongoing, such as the EXTRACT-TIMI 25 study.

It seems clear, however, that in conjunction with the results observed in patients with acute coronary syndromes without ST-elevation (*see below*), early blockade of the coagulation cascade is more efficient than downstream control of a much larger number of activated molecules. In this connection, although preliminary, the results of the PENTALYSE trial are extremely promising.[4] In this study 333 patients with acute myocardial infarction treated with alteplase received concomitant heparin or different amounts of fondaparinux, a synthetic sulphated pentasaccharide derived from the factor-Xa-binding moiety of unfractionated heparin. Fondaparinux selectively binds to antithrombin III (AT III), inducing a conformational change that increases the antifactor-Xa of AT-III by more than 300 times, resulting in dose-dependent inhibition of factor-Xa. Fondaparinux inhibits thrombin generation without any direct effect on the thrombin molecule itself. The prolonged and dose-independent fondaparinux half-life (15-18 hours) allows single-daily subcutaneous injections. A recent meta-analysis showed that fondaparinux treatment (2.5 mg) results in greater than 50 % reduction (compared with twice-daily enoxaparin), in the odds of developing venous thromboembolism following major orthopedic surgery without increasing the risk of *clinically relevant* bleeding.[5]

In the PENTALYSE trial 4-12 mg fondaparinux doses in conjunction with thrombolysis (first dose intravenously) achieved better TIMI-3 flow rates at day 5 to 7 (86% vs 75%, $p = 0.10$), less reocclusion from TIMI-3 to TIMI-0,1 (0.9% vs 7.0%, $p = 0.065$) and less revascularization rates (39% vs 51%, $p = 0.054$). Again, there was no difference in early patency rates between fondaparinux and unfractionated heparin.

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Table 1. Clinical studies on the use of low molecular weight heparin in conjunction with thrombolytic therapy in acute myocardial infarction.

Study (patients)	*Drug*	*1° end-point*	*LMWH*	*UFH*	*p*
HART-II (400)	Enoxaparin + rt-PA	90 ' patency*	53%	48%	NS
BIOMACS-II (101)	Dalteparin + SK	20-28 hrs patency*	68%	51%	N
BAIRD (300)	Enoxaparin + SK	90-day D/MI/R	25%	36%	0.04
TIMI-23 (483)	Enoxaparin + TNK	60 ' patency *	51%	50%	NS
AMI-SK (496)	Enoxaparin + SK	8-day patency*	70%	58%	0.01
ASSENT-PLUS (434)	Dalteparin + rt-PA	4-7-day patency *	69%	62%	NS
ASSENT-3 (4078)	Enoxaparin + TNK	D°/ MI#/RI#	11.4%	15.4%	0.0002
ASSENT-3-plus(1639)	Enoxaparin + TNK‡	D°/ MI#/RI#	14.2%	17.4%	0.08

*TIMI-3 flow; °at 30 days; #in-hospital; ‡pre-hospital administration of thrombolysis; D: death; MI: non-fatal myocardial infarction; R: re-hospitalization; RI: refractory ischemia; SK: streptokinase; TNK: tenecteplase.

Perhaps clinical trials may help in elucidating the mechanisms of coronary thrombosis. Thus, the fact that neither unfractionated heparin, nor LMWH nor pure anti-Xa agents such as fondaparinux actually affect the immediate efficacy of thrombolysis negates an important role for thrombin activity inhibition. Thrombin generation appears as the true target for achieving the important role of re-infarction prevention.

With the above considerations in mind it is not difficult to understand the narrow therapeutic index of direct thrombin inhibitors, such as desulfatohirudin or bivalirudin. Both agents form a 1:1 stoichiometric complex with thrombin. However, binding is irreversible for hirudin and recombinant hirudins only. The fact that bivalirudin has a shorter half-life than hirudin (25 vs 60 minutes), coupled with its clearance being mainly through extrarenal mechanisms, makes bivalirudin more attractive from the safety profile. In the GUSTO-2B study des-hirudin reduced death/myocardial infarction at 24 hours following thrombolysis (OR 0.61, 95% CI 0.46-0.81), but the result was no longer significant at 30 days (OR 0.89, CI 0.79-1.00).[6] In TIMI-9B there were no outcome differences between patients treated with hirudin or heparin following thrombolysis at 30 days.[7] More recently bivalirudin adjunctive treatment in patients treated with streptokinase resulted in a small reduction of 96-hour reinfarction rate (0.7% absolute reduction), but no decrease in death or death/myocardial infarction; bivalirudin tended to increase major hemorrhages (0.2% absolute excess) but not intracranial hemorrhages.[8] Thus, trials of direct antithrombin treatment confirm that it is extremely difficult to prevent re-thrombosis after thrombolysis by direct anti-thrombin agents.

Patients without persistent ST-segment elevation (NSTEMI)

Mural thrombosis is the key pathogenetic finding in patients with acute coronary syndromes without persistent ST-elevation.[9] Due to the high-shear forces involved in the coronary circulation, platelets play a major role in the process of thrombus growth. Potent antiplatelet therapy with aspirin, clopidogrel and GP-IIB-IIIA inhibitors is recommended by the European guidelines.[10] However anticoagulation is still neeeded, even in the presence of the potent intravenous infusion of tirofiban.[11] This is usually achieved by unfractionated heparin but the use of low molecular weight heparin is more evidence-based (Table 2),[12] with

Table 2. Short-term rate of death/non-fatal myocardial infarction in trials of low molecular weight heparins in acute coronary syndromes without persistent ST-segment elevation.

Study (drug)	*Timing of the end-point*	*LMWH*	*UFH*	*OR (95 % CI)*
FRIC (dalteparin)	0-6 days	3.9%	3.6%	1.07 (0.63-1.80)
ESSENCE (enoxaparin)	14 days	4.6%	6.1%	0.75 (0.55-1.02)
TIMI-11B (enoxaparin)	14 days	5.7%	6.9%	0.81 (0.63-1.05)
ESSENCE + TIMI-11B	14 days	5.2%	6.5%	0.79 (0.65-0.96)
FRAXIS (nadroparin)	14 days	4.9%	4.5%	1.08 (0.72-1.62)
Overall				0.86 (0.72-1.02)

LMWH: low molecular weight heparin; UFH: unfractionated heparin.

preliminary findings indicating their expanding role also in combination with GP IIB-IIIA inhibitors.[13,14] Direct thrombin inhibition by hirudin, bivalirudin, argatroban efegatran, or inogatran results in a small 9 % reduction in death/myocardial infarction at 30 days, but at the expense of an increase in major hemorrhages (OR 1.28, 95 % CI 1.06-1.55).[15] Again, the very minor yield of directly targeting thrombin is confirmed.

On the other hand, hinging the treatment upon exclusive factor-Xa inhibition by fondaparinux seems to add value to that achieved by a mixture of anti-Xa and anti-IIA activity; in a phase-II study (PENTUA) investigating different fondaparinux doses against the usual enoxaparin regimen, the 30-day cumulative end-point (death/myocardial infarction/recurrent ischemia) was reduced by fondaparinux (once-daily injection of 2.5 mg) from 43.6 % to 33.8 % ($p < 0.05$).[16] Surprisingly, there was no relation between fondaparinux dose and clinical outcome; based on these findings the drug is presently being investigated in a large-scale phase-III trial (OASIS-5).

Not only are subcutaneous LMWHs/fondaparinux at least as effective as intravenous unfractionated heparin (and probably better), but their use may be prolonged beyond the usual 48-hour window usually recommended for intravenous heparin. So far, there is no compelling evidence that treatment with LMWH should be maintained long-term.[12] However, in the FRISC-II study long-term dalteparin was associated with a reduction in the 3-month composite endpoint of death/myocardial infarction/revascularization (29 % vs 33 %, $p = 0.031$) and with a reduction in the 30-day primary end-point of death/myocardial infarction ($p = 0.002$). In retrospect, the 3-month primary endpoint was reduced by dalteparin in troponin-positive patients.[17]

These data, along with biochemical evidence of persistent, ongoing thrombosis as a risk factor for further cardiovascular events[18,19] support the concept of long-term anticoagulation in NSTEMI. This concept is substantiated by the clinical efficacy of adding oral anticoagulants (with intended INR levels > 2.0) to aspirin[20] or using oral anticoagulants alone (with intended INR levels > 2.5).[21] One interesting finding of the WARIS-2 trial is that event curves continue to diverge literally for years after the index admission, demonstrating the life-long propensity for coronary thrombosis in these patients and the potential role of continuing blockade of the coagulation system.

On the other hand, oral anticoagulants are difficult to manage, with a large fraction of patients resulting *non-compliers*[22] and with some inherent risk of major bleeding. The fact that oral anticoagulants represent a real option for only a minority of patients does not mean that new long-term potentiated antithrombotic regimens should not be investigated and, actually they are. Although ineffective on thrombosis markers,[23] combined aspirin plus clopidogrel treatment is effective following the first month of treatment[24] and should be considered in spite of the small, yet significant increase in bleeding rates. At present it is not clear what to do once the 1-year treatment period with the aspirin-clopidogrel combination intended in the CURE trial has elapsed, because no data are available in the very long run, as they are for the combination of aspirin plus oral anticoagulants.

One further interesting strategy is the use of oral antithrombin drugs. So far, ximelagatran compared favorably with warfarin (EXULT-A) and enoxaparin (EXPRESS) in the short-term after orthopedic surgery and has been shown to be useful compared to placebo, in the out-of-hospital long-term treatment of patients with previous venous thromboembolism (THRIVE-3). Trials in cardiology are currently ongoing.

Conclusions

Optimal anticoagulation is a key treatment of acute coronary syndromes. All the available evidence indicates that thrombin generation is the true target. Factor-Xa inactivation is presently the best strategy according to published trials. Thrombin inactivation appears less crucial in the acute phase, although room exists for this approach in the long-term setting. Long-term anticoagulation is conceptually worthwhile but needs confirmation in the framework of all available options.

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Atherothrombosis: different localizations, a unique disease. Is this still valid from an ethiopathologic point of view?

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Atherosclerotic disease affects mainly the medium and large-sized arteries, and is a focal pathologic phenomenon characterized by thickening and obstruction of the arterial lumen. Although atherothrombosis is a generalized disease throughout the entire arterial tree, lesions involving the coro-nary, extracranial cerebral, and lower extremity circulation have the most clinical relevance. Epidemiological and experimental studies have identified several risk factors that are relevant in patients with atherosclerotic disease.[1] Some of these causative risk factors demonstrate affinities to particular arterial domains. Thus, cigarette smoking is particularly associated with atherothrombotic involvement of the pelvic and lower limb arteries, whereas arterial hypertension is associated with intracranial cerebral artery disease. Plasma hyperlipidemia appears to play a major role in atherogenesis while thrombotic complications are thought to be the major trigger of acute events in the coronary, cerebral, and peripheral arterial circulation. The proportion of ischemic arterial events that is due to atherothrombosis varies ac-cording to the vascular bed in which the event occurs, from near-total dependency for the lower limbs (intermittent claudication) to less than 50% for cerebrovascular events (ischemic stroke).[2]

Arterial thrombosis is triggered by disruption of an atherosclerotic plaque. In addition, there is clear evidence indicating that mural thrombosis, also at the site of plaque rupture, is an important mechanism in the progression of atherosclerosis even when symptoms are absent. Thrombin formation through both the intrinsic (surface-activated) and extrinsic (tissue factor-dependent) coagulation pathways clearly contributes to disease manifestations and progression. This concept of vascular injury as a trigger of the platelet-coagulation response is important to the understanding of the pathogenesis of thrombosis and its inhibition.[3]

Most platelet aggregation agonists seem to act through the hydrolysis of platelet membrane phosphatidylinositol. The exposed matrix of the vessel wall and thrombin generated by the activation of the coagulation cascade are powerful platelet agonists. Adenosine diphosphate (ADP) is a platelet agonist that may be released from hemolyzed red cells in the area of vessel injury. Each agonist stimulates the discharge of calcium from the platelet-dense tubular system and promotes contraction of the platelet, with subsequent release of its granule contents. Arachidonate, which is released from the platelet membrane in response to the stimulatory effect of collagen, thrombin, ADP, and serotonin, is another platelet ago-nist. Arachidonate is converted to thromboxane A2 by the sequential effects of cyclooxygenase and thromboxane synthase. Thromboxane A2 not only promotes further platelet aggregation, but is also a potent vasoconstrictor.[3]

The initial recognition of damaged vessel wall by platelets involve adhesion, activation, adherence to recognition sites on the thromboactive substrate, spreading of the platelet on the surface, and aggregation with other platelets to form a platelet plug or white thrombus. The efficiency of the platelet recruitment depends on the underlying substrate and local geometry.

Atherosclerotic lesions tend to develop in lesion-prone areas, such as in arterial bifurcations, which are subject to repeated mechanical stresses, such as oscillating shear forces.[4,5] The endothelium at these sites is dysfunctional and characterized by increased permeability leading to an influx of low-density lipopro-teins (LDL) and other plasma proteins into the intima.[6] Depending on the size of the branch sites, the endothelial microfilaments are organized differently, which may reflect differences in endothelial function which are essential in maintaining endothelial integrity at these sites.[7] Recent pathologic evidence suggests that the lipid rich core originates primarily from lipoprotein trapping and binding to matrix proteins such as glycosaminoglycans, collagen and fibrinogen,[8,9] a process that results in focal collections of lipid-laden foam cells. The unequal distribution of glycosaminglycans, such as chondroitin 4/6 sulphate (enriched in 6-sulphated disaccharide units) through the arterial tree might imply that glycosaminglycans has a role in the multifactorial mechanisms that modulate the differential localizations of atherosclerotic lesions.[10] The initial lipid and macrophage driven process is subsequently accompanied by smooth muscle cell activation, migration, and proliferation, followed by extracellular matrix deposition and further lipid accumulation. This gives rise to more mature and clinically significant atherosclerotic plaques.[11]

Arteries are generally diffusely involved by confluent plaques carpeting the vessel wall.[12] However, individual plaques vary greatly in composition. A significant atheromatous core is present in the majority of unstable plaques, whereas fibrous plaques are stable and

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often resistant to disruption. A vulnerable plaque consists of a lipid-rich core separated from the arterial lumen by a fibromuscular cap. This atheromatous core is mostly avascular, hypocellular (except for macrophage foam cells), devoid of supporting collagen, rich in free cholesterol esters and very soft.[13] Plaque rupture frequently occurs when the fibrous cap is thin, and most heavily infiltrated with foam cells and macrophages.[13] This region of the plaque is also subjected to peak stress loading as all physical forces acting here are greatest. When the plaque is disrupted, exposure of the contents of the necrotic core to the blood may result in thrombus formation and subsequent lumen narrowing or occlusion. High degrees of stenosis and roughness of the substrate are associated with larger platelet-thrombus formation[14] as a consequence of increased local shear rate conditions.

There is a marked heterogeneity in the composition of human atherosclerotic plaques that can be found in the same individual. Therefore, disruption of different plaques exposes different vessel wall components to blood. Data on the thrombogenicity of disrupted atherosclerotic lesions are limited.

In a comparative study on the thrombogenicity of different human atherosclerotic plaques (normal intima, fatty streaks, sclerotic plaques, fibrolipid plaques, and atheromatous lipid rich core) exposed to flowing blood, we demonstrated that the atheromatous plaque, characterized by the presence of a lipid core abundant in cholesterol crystals, had the highest thrombogenicity.[15]

Tissue factor antigen and activity have been reported in human atherosclerotic plaques,[16] and we have recently reported a positive correlation between human plaque thrombogenicity and the tissue factor content of the plaques.[17] This observation suggests that tissue factor is an important determinant of thrombogenicity af-ter spontaneous or mechanical disruption of human atherosclerotic plaques. Therefore, new therapeutic approaches specifically directed towards the tissue factor pathway of coagulation may offer promising new tools for preventing thrombotic occlusion in patients with unstable angina, or for preventing re-occlusion after successful thrombolysis or percutaneous coronary angioplasty.

Platelet deposition increases significantly with the degree of stenosis, indicating shear-induced cell activation. In addition, analysis of the axial distribution of platelets suggests that the severity of the acute platelet response to plaque disruption depends in part on the sudden changes in the degree of stenosis following the rupture.[14,18] Furthermore, fibrin(ogen) and platelet deposition were maximal at the apex of the stenosis where shear rate is extremely high, and parallel streamlines deformed. However, fibrin(ogen) deposition seems to be significantly less dependent on high shear rates than is platelet deposition, and the pattern is not influenced by time.[19] In this respect, fibrin(ogen) deposition was predominant in the thrombus layers adjacent to a severely damaged vessel wall regardless of the local shear stress levels and flow conditions.[20]

In summary, blood hemodynamic characteristics and vessel wall composition vary according to their local distribution through the arterial tree. Systematic studies should reveal whether these intra-individual variations reflect different etiopathologic processes in atherothrombotic disease. A better understanding of the pathogenesis of atherothrombosis is essential in order to develop new strategies for prevention and effective treatments based on tissue-location and site-specific characteristics.

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Aspirin resistance

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Antiplatelet therapy, devised to prevent acute thrombotic occlusion, is a mainstay of cardiovascular therapy in various cardiovascular diseases. Aspirin is by far the most widely used antiplatelet drug, and has been tested in patients across the entire spectrum of severity of atherosclerotic vascular disease, from healthy low-risk individuals to patients presenting with acute myocardial infarction or acute ischemic stroke. The recently completed Antithrombotic Trialists' Collaboration has compiled a meta-analysis of 65 trials using aspirin in high-risk patients, and found a 23% odds reduction in vascular events in the aspirin treated groups.[1] However, the effectiveness of aspirin is undoubtedly limited, since 10-20% of patients with arterial thrombosis who are treated with aspirin still experience a recurrent vascular event during a long-term follow-up. Moreover, both the proportional size of aspirin's effects and its absolute benefits are somewhat heterogeneous in different clinical settings. There is some evidence, for instance, that the relative efficacy of aspirin (i.e. not only its absolute efficacy, obviously depending on the baseline risk of the population) is greater in patients with unstable angina than in those with post-myocardial infarction and in other types of patients, possibly reflecting a greater contribution of aspirin-sensitive mechanisms to the pathogenesis of vascular events in these subgroups.[1] Consistent with observations of some clinical variability in responses to aspirin, studies examining platelet function after aspirin treatment have demonstrated a wide variability in its antiplatelet effects.[2] On the basis of clinical and laboratory evidence of a reduced or absent response to aspirin in some individuals, the concept of *aspirin resistance* has been originated. Discovery of aspirin resistance in individual patients might be important in order to devise better antiplatelet strategies and improve our ability to prevent acute thrombotic occlusion.

Mechanisms of the action of aspirin: how aspirin's biochemical interaction with platelet cyclo-oxigenase translates into a clinical benefit

The best characterized mechanism of action of aspirin is mainly related to its capacity to permanently inactivate the cyclo-oxygenase (COX) activity of prostaglandin (PG)H synthase-1 and PGH synthase-2 (also referred to as COX-1 and COX-2) in various tissues.[3] These isozymes catalyze the first step in prostanoid biosynthesis. COX-1 is a predominantly constitutive enzyme, whereas COX-2 is predominantly induced by inflammatory and mitogenic stimuli. The antiplatelet effect of aspirin, specifically, is due to the inhibition of platelet COX-1-dependent synthesis of thromboxane (TX) A2, a powerful inducer of vasoconstriction and of platelet aggregation, from arachidonic acid.[4]

The molecular mechanism for the permanent inactivation of COX activity by aspirin is related to the acetylation of a strategically located serine residue (*i.e.*, Ser529 in the human COX-1 and Ser516 in the human COX-2) that prevents substrate access to the catalytic site of the enzyme.[5] Aspirin has a short half-life in the human circulation and is much more potent at inhibiting platelet COX-1 than monocyte COX-2,[6] so that it appears to be ideally suited to act on anucleate platelets, inducing a permanent defect in TXA_2-dependent platelet function. Moreover, since aspirin probably also inactivates COX-1 in relatively mature megakaryocytes, and since only 10% of the platelet pool is replenished each day, once-a-day dosing of aspirin should be able to maintain virtually complete inhibition of platelet TXA2 production. In contrast, inhibition of COX-2 needs higher doses of aspirin, because COX-2 is less sensitive than COX-1 to aspirin, (about 170 times less so),[7] and a shorter between-dose interval, because nucleated cells rapidly resynthesize the enzyme.

Minimum effective dose of aspirin

Randomized trials have shown that aspirin in an effective antithrombotic agent when used at doses ranging between 50 and 100 mg/day. Aspirin at a dose of 75 mg/day was shown to be effective in reducing the risk of acute myocardial infarction (MI) or death in patients with unstable angina and chronic stable angina, reducing stroke or death in patients with transient cerebral ischemia and reducing the number of post-operative strokes after carotid endarterectomy.[8] The lowest effective dose of aspirin for these various indications is shown in Table 1.

Platelet function tests

The determination of platelet aggregation according to Born 9 has been by far the most widely used of *ex vivo* test of platelet function. This test monitors the for-

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Table 1. Vascular disorders for which aspirin has been shown to be effective and the minimum effective dose.

Disorder	*Minimum effective daily dose (mg)*
Men at high cardiovascular risk	75
Hypertension	75
Stable angina	75
Unstable angina*	75
Acute MI	160
TIA and ischemic stroke*	50
Severe carotid artery stenosis*	75
Acute ischemic stroke*	160

*Higher doses have been tested in other trials and not found to confer any greater risk reduction.

mation of platelet aggregates in calcium-deprived platelet-rich plasma in response to a variety of agonists. However, Born's aggregometry system has limited sensitivity to the effect of aspirin, which is often, on this basis, considered a *weak* antiplatelet agent.

In vivo tests include the widely used bleeding time, with its recent *in vitro* variation, the so-called PFA-100 test. Bleeding time, which is significantly prolonged by aspirin, has been successfully used as a measure of platelet response to aspirin;[10] however its clinical use has serious problems of methodologic standardization and the test is believed to be of limited value in predicting hemostatic competence.

The platelet function analyzer (PFA)-100™ test is a system developed as a means of rapidly assessing platelet function using whole blood. This assay uses a cartridge containing a small aperture coated with collagen and epinephrine or ADP. Aspirin prolongs the collagen/epinephrine closure time, but does not prolong the collagen/ADP closure time.[11]

On the basis of conventional tests with platelet aggregation, some authors have suggested that a difference between aspirin *responders* and *non-responders* is attributable to variations in the platelet reactivity to collagen evaluated by platelet aggregation tests[12] or to the type and strength of aggregating triggers.[13] It is well recognized that platelets can be activated by TXA_2-independent pathways that are not blocked by aspirin.[14,15] Moreover, it has been described that *in vitro* cell-cell interactions may modify the response of aspirin-treated platelets to various agonists such as ADP.[16] A recent study suggests that, among patients with coronary artery disease, a subset of aspirin-resistant patients have platelets that are more sensitive to ADP than those of a control group.[17] Finally, in a recent trial, aspirin resistance was defined on the basis of optical aggregation or the PFA-100.[11]

Non-compliance, aspirin failure and aspirin resistance

One obvious reason for which some patients apparently on aspirin might have thrombotic events is inadequate compliance to aspirin. No matter how this issue might be underestimated, this cannot explain most cases of inadequate suppression of platelet function in patients putatively treated with aspirin, on the basis of salicylate measurements.[18]

In patients definitely being treated correctly with aspirin, the occurrence of a vascular event can be broadly defined as aspirin failure. Some such cases are certainly due to intrinsic limitations of aspirin as an antiplatelet drug. Overcoming aspirin's effects by a high dose of platelet agonists and the simultaneous stimulation of platelets with more than one natural agonist,[19] together with the only partial dependence of vascular events on thrombosis[20] are reasonable explanations for some vascular events may not be prevented despite the full aspirin antiplatelet effect. Direct confirmation that aspirin's effects on thrombotic endpoints are certainly limited and not maximal has come from intervention studies showing the benefit of adding another drug (e.g. a second antiplatelet agent like clopidogrel[21] or an oral anticoagulant[22]) on top of aspirin in some categories of patients at risk of vascular event. Therefore, however broad it may be, the definition of *aspirin failure* will certainly encompass true intrinsic limitations of aspirin as an antiplatelet drug. The term *aspirin resistance* should more properly be applied to conditions in which – despite adequate administration – aspirin fails to achieve the biochemical and functional antiplatelet effects which it is documented to produce in the majority of cases and for which it is given to patients at risk of thrombotic occlusion.

Aspirin resistance: prevalence, findings and classification

Initial evidence that some patients may be resistant to aspirin came from a study by Metha *et al.*[23] who showed that 30% of patients with coronary artery disease had minimal inhibition of platelet aggregation after a single 650 mg dose of aspirin. Subsequent studies attempted to estimate the prevalence of aspirin resistance in patients with cerebrovascular disease,[24] peripheral arterial disease[25] and ischemic heart disease.[10] Aspirin-resistant patients were found in the Warfarin-Aspirin Reinfarction Study (WARIS)-II[22] and among healthy volunteers.[26] The overall range of estimated prevalences of aspirin resistance in these studies varied from 8% to 45% and this wide range clearly depends – at least to some extent – on the variable definition of the entity. There will probably never be universal agreement on what can be termed aspirin resistance, because of the multiple parameters

Table 2. Evidence for aspirin resistance.

Population studied	*ASA dose (mg/day)*	*Method*	*Criteria for ASA resistance*	*% ASA resistance*	*Ref.*
CABG patients (n=40)	325	Bleeding time	No prolongation of bleeding time above baseline	43%	10
AMI patients (n=143)	75-160	Platelet aggregation ratio (PAR)	PAR≤0.82 after ASA PAR≤0.82 after additional ASA	9.8% 1.4%	47
Healthy young adults (n=31)	325	Whole blood assay: samples incubated with arachidonic acid until aggregation occurred	Aggregation time before and after ASA. Mean response after ASA was doubling of aggregation time, but a highly variable response was seen	Not determined	26
Stroke patients (n=180)	500	Platelet reactivity (PR): aggregation induced by blood collection	Normal PR index (<1.25) at 2 or 12 hours = resistance PR index >1.25 at 2 and 12 hours = expected response	36%	24
PVD patients (n=100)	100	Corrected whole blood aggregation using ADP and collagen agonists	Platelet aggregation after agonist compared to baseline values (>40% of baseline after ASA dose was considered resistance)	60%	25
Patients with stable CAD (n=325)	325	Optical platelet aggregation by ADP and arachidonic acid	Normal ADP induced aggregation and arachidonic acid induced >20% after ASA = resistance	5.5%	11
		PFA-100® using collagen/ADP and collagen/EPI	PFA-100®. Normal (<193s) collagen/EPI closure time after ASA = resistance	9.5%	

ADP: adenosine diphosphate; AMI: acute myocardial infarction; ASA: aspirin; CABG: coronary artery bypass grafting; CAD: coronary artery disease; EPI: epinephrine; PFA-100®: platelet function analyzer-100®; PVD: peripheral vascular disease.

used to assess platelet function. The complexity of this definition is shown in Table 2.

Since the best measurable biochemical reflection of the direct effect of aspirin on platelets is platelet TX production, and since this would translate into a functional effect on platelets, which may be traditionally measured by Born's aggregometry, on the basis of variable combinations of aspirin lacking effect on either or both such parameters, Weber *et al.*[27] proposed three categories of aspirin resistance, as explained in Table 3.

A complementary classification of aspirin resistance was proposed by McKee *et al.*,[2] who divided mechanisms of aspirin resistance in extrinsic (to platelets) and intrinsic. A modification of such a classification is reported in Table 4, and will now be discussed in greater detail.

Extrinsic mechanisms

Insufficient aspirin dosing?

Whether aspirin doses used in various trials are adequate in all patients has been highly debated over the past several years: if the effect of aspirin were dose-dependent, this could explain, at least in part, aspirin resistance, which could be overcome by increasing the daily dose. However, the outcome of clinical trials conducted with various doses of aspirin seems unaffected by the use of either low or high doses of aspirin in a range between 50 mg and over 1000 mg per day.[1]

Increased platelet turnover

An increased platelet turnover may explain a defective platelet inhibition by aspirin. In patients undergoing coronary bypass surgery increased platelet turnover may overcome most of the effects of daily aspirin dosing, leading to only 30-50% inhibition of TX production compared to the 94% inhibition occurring in the healthy volunteers.[28] Moreover, both COX isoforms likely contribute to prostanoid formation during human megakaryocytopoiesis, and COX-2-derived PGE_2 and TXA_2 may play some role in inflammatory and hemostatic responses in clinical syndromes associated with high platelet turnover.[29]

Cigarette smoking

Recent data have shown that cigarette smoking accentuates the formation of a platelet thrombus in a way that is not inhibited by aspirin.[30,31] Therefore, although more clinical trials are needed to

Table 3. Aspirin resistance classified on the basis of variable combinations of aspirin's lack of effect on either or both Born's aggregometry and platelet TX production.

Type I
Pharmacokinetic type
Normal responses occurring in ex vivo aggregation (by low concentrations of collagen in their study) despite in vivo aspirin use. Inhibition when further aspirin is added *ex vivo*.

Causes
Such conditions can be defined as due to variations in aspirin pharmacokinetics. It would be mimicked by non-compliance.

Type II
Pharmacodynamic type
Aggregation despite aspirin, even after further *in vitro* addition of aspirin, in the presence of impaired inhibition of platelet TX formation.

Causes
Possible causes would include production of TX by other probably extra-platelet sources (such as COX-2 in monocytes or other nucleated cells), or the feeding of prostaglandin endoperoxides PGG2/PGH2 from non-aspirin inhibited extraplatelet sources to platelets, bypassing aspirin inhibition of platelet cyclo-oxygenase.

Type III
Pseudo-resistance
Permanence of aggregation despite the *in vitro* addition of aspirin, but in the presence of inhibited TX production.

Causes
This would indicate the presence of other platelet agonists coming into play, possibly including isoprostanes, and would indicate a variable (in this case diminished) TX dependence of collagen-induced aggregation.

Table 4. Proposed mechanisms of aspirin resistance.

Extrinsic Mechanisms
i. Accentuation of platelet thrombosis by exogenous substances (i.e. cigarette smoke).
ii. Drugs, such as non-steroidal anti-inflammatory drugs, that may interact with aspirin's acetylation of cyclo-oxygenase-1.
iii. Increased platelet turnover overcoming once-daily aspirin dosing.
iv. Inadequate aspirin dosing.

Intrinsic Mechanisms
i. Inducible cyclo-oxygenase-2 that is not adequately inhibited by low-dose aspirin, thereby allowing for platelet thromboxane A_2 production despite inhibition of cyclo-oxygenase-1.
ii. Polymorphisms in the cyclo-oxygenase-1 gene that alter the structure of the native site and prevent acetylation by aspirin.
iii. Regenerated, uninhibited cyclo-oxygenase-1 in nucleated cells such as macrophages and vascular endothelial cells producing prostaglandin H_2 that is shunted into platelets, thereby bypassing platelet cyclo-oxygenase-1.
iv. Polymorphisms in the glycoprotein IIb/IIa receptor complex that confer varying degrees of platelet responsiveness to aspirin.

elucidate the relationship between aspirin and the effects of smoking on platelet aggregation, smoking-induced, aspirin-insensitive platelet aggregation may be considered one of the mechanisms of aspirin resistance. However, in the first trial designed to determine the prevalence of aspirin resistance,[11] there were significantly more current smokers in the aspirin-sensitive group than in aspirin-resistant patients, as measured by Born's aggregometry, and there were no significant differences between aspirin-resistant and aspirin-sensitive patients, as measured by the PFA-100 test.

Co-administration of aspirin and other non-steroidal anti-inflammatory drugs

The co-administration of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) can lead to pharmacodynamic interactions between the two, leading to an attenuation of the antiplatelet effect of aspirin.[32,33] Because the ability of aspirin to acetylate a critical serine residue at the bottom of the COX channel is dependent on its initial binding to arginine-120,[5] a common docking site for all NSAIDs, the presence of non-aspirin NSAIDs may preclude aspirin from permanently modifying platelet COX-1.[8]

Intrinsic mechanisms

Platelet production of thromboxane despite aspirin inhibition of COX-1

Platelets may produce TXA2 despite aspirin's effect on COX-1. Polymorphisms and/or mutations in the COX-1 gene affecting Ser529 may be the structural basis for aspirin resistance in some patients,[34] although this hypothesis remains to be tested. Circulating platelets from healthy subjects may also express COX-2 protein and messenger RNA.[35] Since low-dose aspirin is an ineffective inhibitor of COX-2, this mechanism, although disputed,[36] may represent a factor in aspirin resistance. Alternatively, platelets can produce TXA2 through a pathway bypassing platelet COX,

through PGH2 provided by nucleated cells where *de novo* synthesis of COX-1 may occur. Nucleated cells may themselves synthesize their own TXA_2,[14] and can also produce PGH2 through COX-2,[37] which is not inhibited by low-dose aspirin. Therefore, increased COX-2 expression may contribute to aspirin resistance, especially in patients with ischemic heart disease, in whom inflammatory phenomena have been repeatedly and consistently demonstrated.[38] COX-2 induction in plaque monocytes/macrophages or activated endothelial cells may contribute to aspirin-insensitive TXA_2 biosynthesis, occurring in some patients with unstable angina despite >95% suppression of platelet COX-1 activity,[39] by generating PGH2 as a substrate for the TX-synthase of the same cell (*constitutive* biosynthesis) or by providing PGH2 to the TX-synthase of aspirinated platelets (*transcellular* metabolism).

Platelet-erythrocyte interactions

The presence of erythrocytes induces an increase in platelet TXA2 synthesis, and release of serotonin, b-thromboglobulin and additional ADP, indicating that erythrocytes modulate platelet eicosanoid formation.[40] This may affect platelet reactivity and possibly contribute to aspirin resistance by promoting platelet release reactions, eicosanoid synthesis, and platelet recruitment.[41] The clinical importance of these phenomena remains to be established.

Non-enzymatic production of isoprostanes

Aspirin resistance may also be linked to the production of isoprostanes from arachidonic acid through a non-enzymatic process of lipid peroxidation catalyzed by oxygen free radicals.[42] Recent data suggest that urinary excretion of 8-iso-$PGF_{2\alpha}$, a marker of *in vivo* lipid peroxidation, is abnormally elevated in the vast majority of patients with severe unstable angina compared to in patients with stable angina, despite aspirin therapy.[43] In these patients, elevated urinary excretion of 8-iso-PGF2α correlates with 11-dehydro-TXB_2 excretion, an index of *in vivo* TXA_2 biosynthesis. Thus, both COX-2–derived TXA_2 and non-enzymatic F2-isoprostane formation might represent two eicosanoid mechanisms that contribute to aspirin-insensitive platelet activation in unstable angina.

Platelet glycoprotein IIIa polymorphisms

Genetic variation in the IIIa subunits of the glycoprotein IIb/IIIa receptor has been identified, with patients being $PI^{A1,A1}$ or $PI^{A2,A2}$ homozygous or $PI^{A1,A2}$ heterozygous. It has been shown that carriers of the PI^{A2} allele have more reactive platelets than carriers of the PI^{A1} allele, and show enhanced thrombin formation and a lower threshold for activation, α-granule release, and fibrinogen binding.[44] However, studies implicating the PI^{A2} allele as a risk factor for coronary artery disease have been inconclusive.[45] Moreover, most studies indicate that PIA2 carriers are less responsive to the antithrombotic effects of aspirin,[44] but there are no studies correlating the presence of PI^{A2} and aspirin resistance in the general population. It is likely that there are additional, so far unidentified genetic factors contributing to aspirin resistance.

Clinical significance

Recent studies suggest that aspirin resistance may be clinically important. In a study of post-stroke patients, aspirin resistance, defined as normal platelet function after aspirin administration, was present in 30% of patients,[46] and in these patients an 89% increased risk for a subsequent vascular event after a 2-year follow-up was observed.[24] The results of this and other smaller studies in stroke patients have suggested that aspirin resistance may contribute to a lack of response to treatment (i.e., recurrent ischemic events while receiving antiplatelet therapy). However, the uncontrolled nature and small sample size of these studies make results difficult to interprete. Similarly, among patients undergoing peripheral arterial angioplasty only 40% demonstrate appropriate inhibition of platelet function after 100 mg aspirin, and aspirin *non-responders* (assessed by corrected whole blood aggregometry upon stimulation by arachidonic acid, ADP and collagen) had an 87% increased risk of arterial re-occlusion during follow-up.[25] A recent case-control sub-study from the population of the *Heart Outcomes Prevention Evaluation* (HOPE) trial found that, among aspirin treated patients, those with higher urinary excretion of 11-dehydro-TXB_2, the presence of which suggests an aspirin-insensitive biosynthesis of TXA_2, had a two-fold higher risk of myocardial infarction and a 3.5-fold higher risk of cardiovascular death.[34] Although this was a non-randomized, open, *post-hoc* evaluation, these findings suggest the possibility that elevated urinary 11-dehydro-TXB_2 levels identify patients relatively resistant to aspirin who may benefit from antiplatelet therapies that block *in vivo* TX production or activity more effectively.

Conclusions

Although resistance to the antithrombotic effects of aspirin in vascular patients has been reported several times, many questions remain unanswered. While a definition of aspirin resistance should probably not be solely based on clinical outcomes, which laboratory evidence has to be chosen is – and likely will remain - a matter of dispute, and no universal criteria for distinguishing true resistance from therapeutic failure will probably ever be available in the individual patient.

Numerous tests have been used to measure platelet aggregation with varying methodologies, sensitivities, and specificities. Criteria for normal or abnormal responses have not been clearly defined or correlated with clinical outcomes. Patient-specific factors that may increase the risk of resistance have not been identified. The underlying mechanisms are not completely known and are likely multifactorial. Guidelines for *Antithrombotic Therapy from the American College of Chest Physicians* acknowledge the possibility of aspirin resistance;[8] however, because the prevalence and clinical relevance remain unknown, the *Guidelines* recommend a daily aspirin dose of 50–325 mg, without the need to monitor platelet function. In high-risk patients or those with recurrent thromboembolic events despite aspirin therapy, consideration should be given to alternative antiplatelet drugs or combination therapy with another antiplatelet agent, such as clopidogrel, or with oral anticoagulants, on the basis of evidence from the CURE[21] and the WARIS-II study[47] in non-ST elevation acute coronary syndromes and post-myocardial infarction, respectively.

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Primary and secondary prevention of atherothrombosis: is there a limit?

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Definition of atherothrombosis

Atherothrombosis is a diffuse disease. From a pathophysiological standpoint, the available literature suggests that a similar process occurs at the level of the plaque, irrespective of the territory involved (slow growth of the plaque, inflammation, rupture and/or erosion, thrombosis, embolization). The consequences of atherothrombosis may, however, vary from one territory to another; this may be related to differences in arterial vasculature or in cell tolerance to ischemia or in adaptative mechanisms such as collateral circulation.

The initially silent progression of the plaque, prompted by classical atherosclerotic risk factors, such as cigarette smoking, hypertension, diabetes mellitus, and dyslipidemia, is followed by a phase of acute or chronic progression towards an increasing degree of stenosis caused by thrombosis. In this phase, hemostasis-related risk factors, i.e. factor VII, fibrinogen, PAI-1 and platelets play a crucial role. The *morphologic pathway* of the lesion begins with an early lipid streak, followed by a fibrous plaque, by a plaque with various degrees of (in)stability, and finally by the acute complications of the plaque itself, leading to different clinical events in the coronary, cerebral and peripheral vasculature. The unifying pathophysiologic concept of atherothrombosis identifies the coexistence of atherosclerosis in several districts and the formation of a superimposed thrombus determining relevant clinical pictures (ischemic heart disease, cerebrovascular disease, peripheral obstructive arterial disease).[1]

Demographic and pathologic features of the largest study in the field of atherothrombosis, the CAPRIE study (19,185 patients, mean age 62 years) shows that the above mentioned clinical pictures (coronary, cerebral and peripheral) tend to overlap, and that about 25% of the patients enrolled have clinical evidence of multidistrict atherosclerotic disease.[2]

Another important trial about atherothrombosis is the HPS recent study (*MRC/BHF Heart Protection Study*).[3,4] This *large simple trial*, designed according to the model proposed some years ago by the USA Food and Drugs Administration, enrolled patients in both primary and in secondary prevention with increased risk of CHD death due to prior disease (Figure 1):

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– Myocardial infarction or other coronary heart disease;
– Occlusive disease of non-coronary arteries; or diabetes mellitus or hypertension.

Prevention of atherothrombosis

This definition of atherothrombosis explains why a patient with atherothrombosis may be symptomatic for one vascular bed, and asymptomatic for another one, in which this disease may be diagnosed only by means of instrumental evaluation. As a matter of fact when considering a patient with acute myocardial infarction, the possible involvement of other vascular beds should be looked for, and the same should be done when treating a patient with stroke or transient ischemic attack (TIA), or with peripheral arterial disease. Therefore, primary prevention of atherothrombosis in one vascular bed may represent secondary prevention for another vascular site.

Non-pharmacologic strategies for cardiovascular prevention

The reduction of risk factors (Table 1)[5] is a priority for prophylaxis in a patient with atherothrombosis, both in primary and in secondary prevention, and also in the acute phase, although the benefits of their correction have different weights depending on the most symptomatic vascular bed of the individual patient. The treatment of hypertension can reduce the risk of stroke by 42% and the risk of acute myocardial infarction by 30%, while it does not appear to be of major importance in patients with peripheral arterial disease. The reduction of hypercholesterolemia through diet or physical activity and the reduction of body weight are very important preventive measures.

Homocysteine

Homocysteine (Hcy) is a sulfhydryl amino acid derived from the metabolic conversion of methionine dependent on vitamins (folic acid, B12 and B6) as co-factors or co-substrates. In 1969 McCully[6] reported for the first time the presence of severe atherosclerotic lesions in patients with severe hyperhomocysteinemia and hypothesized the existence of a pathogenic link between hyperhomocysteinemia and atherogenesis. Some case-control and cross-sectional studies confirmed McCully's initial hypothesis, showing that even moderate hyperhomocysteinemia is associated with an increased risk of occlusive arterial disease. Less consistent results have been reported in prospective cohort studies of subjects who

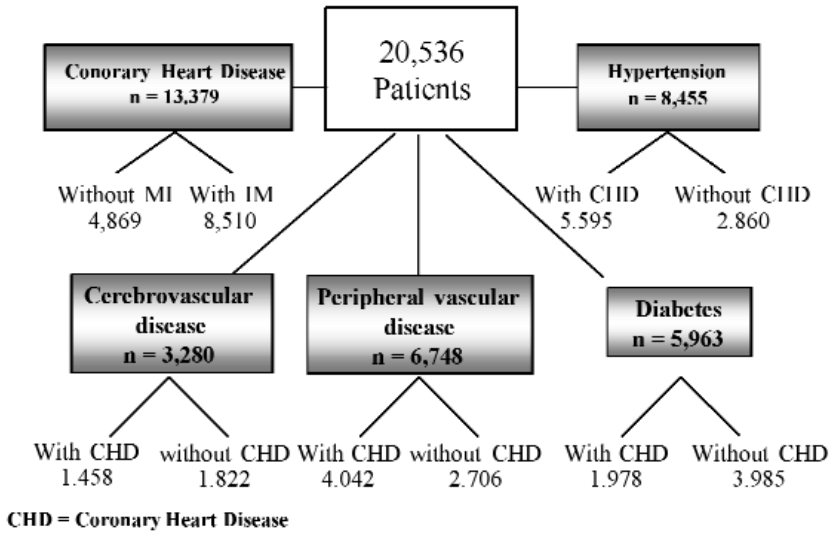

Figure 1. Patients in the HPS study.

Table 1. Cardiovascular risk factors. Evidence supporting their association with disease.

Risk factors	*Epidemiology*	*Clinical trials*
CATEGORY I (risk factors for which interventions have been proved to lower cardiovascular risk)		
Cigarette smoking	+ + +	+ +
LDL cholesterol	+ + +	+ + +
High fat cholesterol diet	+ + +	+ +
Hypertension	+ + +	+ + + (stroke)
Left ventricular hypertrophy	+ + +	+
Thrombogenic factors	+ + + (Fibrinogen)	+++ (Aspirin, warfarin)
CATEGORY II (risk factors for which interventions are likely to lower cardiovascular risk)		
Diabetes mellitus	+ + +	+
Physical inactivity	+ + +	+ +
HDL cholesterol	+ + +	+
Triglycerides; small density LDL	+ +	+ +
Obesity	+ + +	–
CATEGORY III (risk factors associated with increased cardiovascular risk that if modified might lower risk)		
Psychological factors	+ + +	–
Lipoprotein (a)	+	–
Homocysteine	+ +	–
Oxidative stress	+	–
No alcohol consumption	+ + +	–
CATEGORY IV (risk factors associated with increased cardiovascular risk, but which cannot be modified)		
Age	+ + +	–
Male gender	+ + +	–
Low socioeconomic status	+ + +	–
Family history of early-onset cardiovascular disease	+ + +	–

+ = weak evidence, somewhat consistent evidence; ++ = moderately strong, rather consistent evidence; +++ = very strong, consistent evidence; – = evidence poor or non-existent (5).

Table 2. Summary results from the MTHFR studies and the prospective studies on the risk of ischaemic heart disease, deep vein thrombosis with or without pulmonary embolism, and stroke associated with serum homocysteine concentration.

Study type	*No. of studies*	*No. of cases*	*5 µmol/L increase in homocysteine*		*3 µmol/L decrease in homocysteine*	
			Summary odds ratio (95% CI)	*Combined odds ratio (95% CI)*	*Odds ratio (95% CI)*	*Risk reduction (95% CI)*
Ischemic heart disease						
MTHFR	46	12 193	1.43 (1.11 to 1.84)	1.33 (1.22 to 1.46)	0.84 (0.80 to 0.89)	16% (11% to 20%)
Prospective*	16	3 144	1.32 (1.19 to 1.45)			
Deep vein thrombosis						
MTHFR	26	3 439	1.60 (1.15 to 2.22)	0.75 (0.62 to 0.92)	25% (8% to 38%)	
Stroke						
MTHFR	7	1 217	1.65 (0.66 to 4.13)	1.59 (1.30 to 1.95)	0.76 (0.67 to 0.85)	24% (15% to 33%)
Prospective*	8	676	1.59 (1.29 to 1.96)			

*Prospective studies adjusted for regression dilution bias and for age, sex, blood pressure, and serum cholesterol concentration in all studies except one[w106] (adjusted for only age and sex).

were healthy at the time of their enrollment, whereas prospective cohort studies of patients with overt coronary artery disease or other risk conditions consistently confirmed the association between moderate hyperhomocysteinemia and the risk of cardiovascular morbidity and mortality.[7] A meta-analysis of methylenetetrahydrofolate reductase (MTHFR) studies has recently been published in the British Medical Journal.[8] This meta-analysis showed a significantly higher risk of both ischemic heart disease and deep vein thrombosis (with or without pulmonary embolism) in people with the MTHFR mutation. A meta-analysis of prospective studies documented a significant association between homocysteine concentration and ischemic heart disease, comparable in size to that expected from the results of the MTHFR studies and a significant association with stroke. A decrease in serum homocysteine of 3 µmol/L (achievable through a daily intake of about 0.8 mg of folic acid) should reduce the risk of ischemic heart disease by 16%, deep vein thrombosis by 25%, and stroke by 24% (Table 2).[9] The mainstay of treatment of hyperhomocysteinemia is folic acid, alone or in combination with vitamins B12 and B6. Although it is clear that vitamins effectively reduce total Hcy plasma levels, whether they decrease the risk of vascular disease is not known.[7]

Table 2 shows the odds ratios for a 5 µmol/L increase in homocysteine concentration and the odds ratios for a 3 µmol/L decrease in homocysteine (the maximal effect of folic acid, achieved through a daily dose of about 0.8 mg).[9,10] Folic acid could be taken in tablets by high risk patients, and possibly supplied to the general public through food fortification or a combination of both.

Pharmacologic prevention

Antiplatelet therapy

Aspirin was the first antiplatelet drug used in different dosages in these clinical contexts. A number of studies established that aspirin is a clinically effective, safe and relatively well tolerated agent in primary and secondary prevention of cardiac and cerebral ischemic event.

The *Primary Prevention Project* showed that low-dose aspirin has no significant influence on blood pressure in treated hypertensive subjects.[11] This appears important in the light of the results of the HOT study,[12] which provided the first documentation of the efficacy of low dose aspirin in preventing major cardiovascular events in hypertensive patients.

Patients at moderate to high cardiovascular risk (those with chronic stable angina, prior myocardial infarction or stroke/TIA) should be prescribed low-dose aspirin (75-100 mg daily) because its potential benefit clearly outweighs the risk of serious bleeding complications (Figure 2).[13,14] Patients at low cardiovascular risk (those without a prior vascular event) are not likely to be prescribed low-dose aspirin because of the uncertain benefit/risk profile of such a strategy in this setting (Table 3). The role of aspirin and other platelet-active drugs in the treatment and prevention of atherothrombosis was reviewed at the *Sixth ACCP Consensus Conference on Antithrombotic Therapy* (Table 3).[13,15]

Many trials have been conducted in the last few years on the primary prevention of atherothrombosis; the results of the studies reviewed above do not justify the use of a daily dose of aspirin of >75 mg when primary prevention with aspirin is considered in the setting of individual clinical judgement by

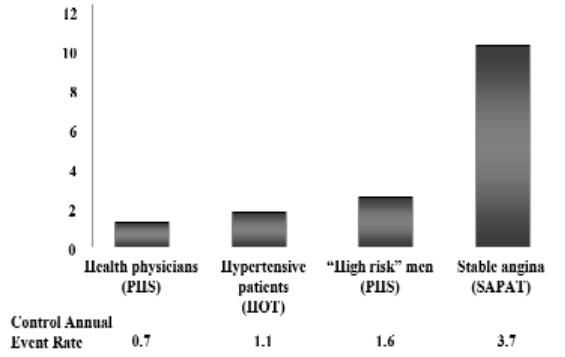

Figure 2. Absolute benefit of aspirin in primary prevention.[13]

health care providers.[13]

In the *Primary Prevention* trials reported by Patrono[13] the absolute excess of major bleeding complications ranged between 0.3 and 1.7 per 1,000 patient-years.

Recent evidence on the efficacy and safety of antiplatelet treatment has been provided by the last collaborative meta-analysis of 266 secondary prevention trials, prepared by the *Antithrombotic Trialists' (ATT) Collaboration*.[14] This analysis extends the direct evidence of benefit from antiplatelet therapy to a much wider range of patients at high risk of occlusive vascular disease. Antiplatelet therapy reduced the risk of serious vascular events (non-fatal myocardial infarction, non-fatal stroke or vascular death) by about 25%, not only among the patients with unstable angina, acute myocardial infarction, stroke, or TIA, but also among other patients with coronary or peripheral arterial disease and among those at high risk of embolism. Overall mortality was also significantly reduced in these high risk patients, and, compared to these benefits, the absolute risk of fatal and major non-fatal bleedings was limited.

Recent trials have shown that the use of antiplatelet therapy has been increasing during the last few years but that a substantial proportion of high risk patients still do not receive it. For example, only about 50% (or fewer) of all patients with a history of myocardial infarction, angina, or peripheral arterial disease are currently receiving antiplatelet therapy, and rates are lower in older people despite their higher absolute risk.[3,16,17] The use of aspirin among patients with diabetes is even more limited; a recent survey suggests that fewer than 25% of those with a definite history of coronary artery disease were taking aspirin regularly[16] and another study has demonstrated that only about 7% of the subjects without a history of coronary artery disease were taking aspirin.[3] Similarly, only about 33% of patients with atrial fibrillation receive oral anticoagulant therapy, which is the most effective treatment for the prevention of strokes in this condition.[18,19] This may due to the associated risks of bleeding and the need for anticoagulation monitoring; however, fewer than 50% of such patients who were not taking oral anticoagulant therapy received antiplatelet therapy despite the high risk of stroke (especially in elderly people).[20,21] These results reinforce the value of routinely considering antiplatelet therapy with 75-150 mg aspirin daily (or some other effective antiplatelet regimen) in all patients at high or intermediate risk of occlusive vascular events (>2% a year), irrespective of history of a major vascular event. Whether it is possible to identify particular

Table 3. Vascular disorders for which aspirin has been shown to be effective, minimum effective daily dose[13] and benefit/risk ratio of antiplatelet prophylaxis with aspirin in different settings[15] (modified from Patrono).

Clinical setting	*Minimum Effective Daily Dose, mg*	*Benefit** Number of pts in whom a major vascular event is avoided per 1,000/year*	*Benefit°° Number of pts in whom a major vascular event is avoided per 1,000/year*
Men at high cardiovascular risk	75	1-2	1-2
Essential Hypertension	75	1-2	1-2
Chronic stable angina	75	10	1-2
Unstable angina*	75	50	1-2
Prior MI	100	20	1-2

*Higher doses have been tested in other trials and not found to confer any greater risk reduction. **Benefits are calculated from randomised trial data reviewed by Patrono;[13] °°risks of upper gastrointestinal bleeding are estimated from a background rate of 1 event per 1000 per year in the general population of non-users and a relative risk of 2.0 to 3.0 associated with aspirin prophylaxis.

groups of apparently healthy people who may be at increased risk of myocardial infarction or stroke and for whom the benefits of daily aspirin outweigh the hazards is still an unanswered question, and is currently being investigated in an analysis of primary prevention trials. For the majority of healthy individuals (for whom the risk of a vascular event is likely to be substantially less than 1% per year) daily aspirin may be inappropriate.

With regard to the secondary prevention of atherothrombosis, clopidogrel, a newly licensed ADP receptor antagonist, proved to be the only antiplatelet agent superior to aspirin in the reduction of major ischemic events (myocardial infarction, ischemic stroke, vascular death) in patients whose initial manifestation of atherothrombosis was one of the three main clinical pictures of the disease (recent ischemic stroke, myocardial infarction, established peripheral arterial disease). In the CAPRIE study, clopidogrel (75 mg) provided increased benefit over aspirin (Relative Risk Reduction 8.7%, p=0.043) in secondary prevention in atherothrombotic patients, including those with diagnosed peripheral arterial disease, it reduced the risk of all major events (among which myocardial infarction) (Figure 3) offering better gastrointestinal safety and tolerability if compared with aspirin (325 mg). A growing body of evidence is now available on the favorable effect of clopidogrel in association with aspirin on the outcome of patients undergoing coronary stenting for acute coronary syndromes.[2]

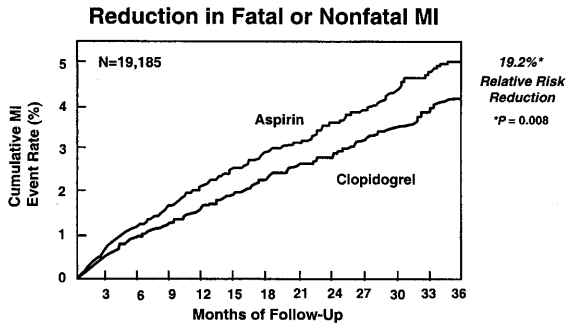

Figure 3. Relative-risk reduction and 95% CI by disease subgroup. ***MI=myocardial infarction (Reprinted from Cannon CP, Am J Cardiol 2002; 90:760-2).***

Non-antiplatelet strategies

Antihypertensive treatment and lipid-lowering drugs represent two major non-antiplatelet strategies. Several systematic reviews of randomized controlled trials (RCTs) found that pharmacological treatment reduced the risk of fatal and non-fatal stroke, coronary events and death in primary prevention; the most remarkable benefit was reported in patients with the highest baseline risk. In fact one systematic review (8 RCTs, 15,693 people) found that, in people aged over 60 with systolic hypertension, the treatment of systolic pressures higher than 160 mmHg decreased total mortality and the incidence of fatal and non-fatal cardiovascular events.[22] Absolute benefits were greater in men than in women, in people aged over 70 years, and in those with prior cardiovascular events or wider pulse pressure. The relative hazard rates associated with a 10 mmHg higher initial systolic blood pressure were 1.26 (p=0.0001) for total mortality, 1.22 (p=0.02) for stroke, but only 1.07 (p=0.37) for coronary events (active treatment reduced total mortality).[22] A RCT (HOT study) (18,790 people, mean age 62 years, diastolic blood pressures between 100–115 mmHg) was aimed at evaluating the effects on cardiovascular risk of target diastolic blood pressures of 90, 85, and 80 mmHg.[23] However, the achieved mean diastolic blood pressures were 85, 83, and 81 mmHg, which limited the power to detect differences between groups. No significant differences in major cardiovascular events were found between the three groups. Two systematic reviews found that initial treatment with diuretics, angiotensin-converting-enzyme inhibitors, or β-blockers reduced mortality and morbidity, with minimal adverse effects. RCTs failed to find significant differences in morbidity or mortality attributable to these different agents.

Blood pressure reduction achieved with β-blockers and diuretics (conventional treatment) is the best intervention to date for prevention of cardiovascular morbidity and death in patients with hypertension. One single blind RCT (10,985 people, aged 25–66 years) found that an angiotensin-converting-enzyme (ACE) inhibitor (captopril) was no more effective than conventional treatment in reducing cardiovascular morbidity or mortality.[24] One systematic review compared different antihypertensive regimens, and failed to find significant differences in outcome among people initially treated with β–blockers, diuretics, or ACE inhibitors.[25] However β blockers or diuretics were more effective than calcium-channel-blockers in reducing coronary events although there was no significant difference for all cause mortality. ACE inhibitors did not significantly affect all cause mortality or stroke rate compared with calcium-channel-blockers, but decreased coronary events (OR for ACE inhibitor versus calcium-channel-blockers 1.03, 95% CI 0.91 to 1.18 for all-cause mortality; 1.02, 95% CI 0.85 to 1.21 for stroke; 0.81, 95% CI 0.68 to 0.97 for coronary events).[25] A double blind RCT (335 high risk subjects with hypertension) failed to find differences in coronary heart disease outcomes among patients treated with dox-

azosin, β-blockers, or chlorthalidone. However, doxazosin was less effective than chlorthalidone in reducing the total number of cardiovascular events and, in particular, increased congestive heart failure.[26]

In the HOPE (*Heart Outcomes Prevention Evaluation*) study, ramipril significantly reduced the rates of death, myocardial infarction, and stroke in a broad range of high-risk patients with low ejection fraction or heart failure.[27]

In the LIFE study, losartan prevented cardiovascular morbidity and mortality more effectively than atenolol in spite of similar reductions in blood pressure, and was better tolerated. Losartan seems to confer benefits beyond reduction in blood pressure.[28] In secondary prevention the results of the PROGRESS study showed that ACE-inhibitors (such as perindopril) are effective in reducing stroke or TIA in patients who have had a prior stroke.[29]

Strong evidence is available about the efficacy of the lipid-lowering drugs, the statins, in reducting cardio-cerebrovascular events both in primary prevention and in secondary prevention. As demonstrated in the *MRC/BHF Heart Protection Study (HPS)*, 40 mg simvastatin treatment (5 years) produced benefits across all groups of patients regardless of age, gender or baseline cholesterol value and proved to be safe and well tolerated, both in primary prevention and in secondary prevention.[4]

The *HPS,*[4,30,31] including more than 20,500 subjects, is the largest trial of statin therapy ever conducted. It was a prospective, double-blind randomized, controlled trial with a 2×2 factorial design investigating prolonged use (>5 years) of simvastatin and antioxidant vitamins. The aim of the HPS trial was to assess the impact of simvastatin 40 mg daily on the prognosis of patients considered to be at high global risk of mortality because of cardiovascular disease, and provided definitive evidence about women, elderly people, diabetics, people with low baseline cholesterol and those with prior occlusive non-coronary vascular disease. The results showed a 12% reduction in total mortality, a 17% reduction in vascular mortality, a 24% reduction in CHD events, a 27% reduction in all strokes and a 16% reduction in non-coronary revascularizations. Overall, simvastatin produced an approximately 25% reduction in the rate of major vascular events irrespective of previous or co-existing disease (CHD, peripheral vascular disease and diabetes), irrespective of age (<65 to > 75 years) and irrespective of gender.

Pravastatin has proven to be effective in primary prevention. It reduces the risk of stroke in patients with a wide range of lipid values and documented coronary disease. This effect is due to a reduction in non-fatal non-hemorrhagic strokes.[32]

Conclusions

The boundary between primary and secondary prevention is subtle, and is related to the potential of clinical ischemia. The available armamentarium for cardiovascular prevention is complex, including cardiovascular risk factor reduction, lifestyle changes, antiplatelet drugs, and cholesterol lowering drugs (statins). When evaluating a patient with atherothrombosis, asymptomatic or symptomatic, the novel cardiovascular risk factors, such as homocysteine, for which strong evidence of effective treatments is available, should also be remembered.

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Antiplatelet agents in perspective

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In discussing antiplatelet agents, it is important to recognize that approximately 1011 platelets are produced each day under physiological circumstances, a level of production that can increase up to tenfold at times of increased need.[1] Platelets form by fragmentation of megakaryocyte cytoplasm and have a maximum circulating life span of about 10 days in man.[1] Thus, platelets are anucleate blood cells that provide a circulating source of chemokines, cytokines and growth factors that are preformed and packaged in storage granules. Moreover, activated platelets can synthesize prostanoids [primarily, thromboxane (TX)A_2] from arachidonic acid released from membrane phospholipids, through rapid coordinated activation of phospholipase(s), cyclooxygenase (COX)-1 and TX-synthase.[2] Newly formed platelets also express the inducible isoforms of COX (COX-2) and PGE-synthase, and this phenomenon is markedly amplified in association with accelerated platelet regeneration.[3] Although activated platelets are not thought to synthesize proteins *de novo*, they can translate constitutive mRNAs into proteins, including interleukin-1β, over several hours.[4] Thus, platelets may have previously unrecognized roles in inflammation and vascular injury, and antiplatelet strategies may be expected to impact on platelet-derived protein signals for inflammatory and/or proliferative responses.[4,5]

An ideal antiplatelet agent is one that would exploit the unique metabolic features of platelets noted above through a *hit-and-run* mechanism of action, ie by permanently inactivating a platelet protein (an enzyme or receptor) that cannot be resynthesized during a 24-hour dosing interval, through a short-lived active moiety, thus limiting the extent and duration of any potential extra-platelet effect(s). Two currently available antiplatelet drugs, ie acetylsalicylic acid (aspirin) and clopidogrel, meet these requirements (Table 1).[6]

At least four distinct platelet proteins represent the target of reversible inhibitors with variable antiplatelet effects, ie COX-1, glycoprotein (GP)IIb/IIIa, the PGH_2/TXA_2 (TP) receptor and the ADP receptor P2Y12.[7] Whether incomplete, reversible inhibition of platelet COX-1 by traditional nonsteroidal antiinflammatory drugs (NSAIDs) is associated with clinical benefits has not been tested adequately in randomized trials. Two population-based observational studies failed to demonstrate an association between non-aspirin NSAID prescription and reduced risk of developing cardiovascular events[8,9] despite the well known association with increased risk of upper gastrointestinal (GI) bleeding. The incomplete and reversible inhibition of platelet GPIb/IIIa by oral blockers is also not associated with clinically detectable benefits, despite dose-dependent increase in bleeding complications.[6] This apparent paradox may be reconciled by considering that persistent high-grade blockade of these platelet proteins may be required to prevent thrombosis in response to sudden fissuring of an atherosclerotic plaque as opposed to transient inhibition of the same target potentially causing bleeding from a pre-existing GI lesion.[5] The successful utilization of intravenous, high-grade blockade of GPIIb/IIIa by commercially available antagonists of this receptor (abciximab, tirofiban, eptifibatide)[6] is consistent with these mechanistic considerations.

The role of aspirin and other platelet-active drugs in the treatment and prevention of atherothrombosis has been reviewed by the *Sixth American College of Chest Physicians* (ACCP) Consensus Conference on Antithrombotic Therapy (available on line at *www.chestnet.org*).[6] Moreover, updated information on the efficacy and safety of antiplatelet therapy is provided by the collaborative meta-analysis of 287 secondary prevention trials, prepared by the Antithrombotic Trialists' (ATT) Collaboration (available on line at *www.bmj.com*).[10] A similar analysis of individual patient data is currently being performed on primary prevention aspirin trials.

The *Seventh ACCP Consensus Conference on Antithrombotic Therapy* is scheduled on April 11-12, 2003 and will publish its recommendations in early 2004. The new data concerning antiplatelet agents can be summarized as follows. The role of aspirin in primary prevention has been the subject of recent recommendations based on the assessment of cardiovascular risk;[11-13] however, establishing a *threshold* of cardiovascular risk of 1% to 1.5% per year is quite arbitrary and does not take into proper consideration the *historical* nature of cardiovascular risk assessment and its downward trend in recent years; moreover, average estimates of bleeding risk associated with low-dose aspirin need to be tailored to the individual patient, based on consideration of age, prior history of gastrointestinal complications and use of other gastrotoxic drugs. An increasing number of reports

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Table 1. Main features of aspirin, clopidogrel and oral GPIIb/IIIa antagonists for chronic therapy.

Feature	*Aspirin*	*Clopidogrel*	*GPIIb/IIIa antagonists*
Targeted platelet protein	COX-1	P2Y12	aIIbb3
Reversibility of the effect	no	no	yes
Desirability of saturation of the target	yes	yes	no
Half-life of the drug or active metabolite	min	min	hours
Need for monitoring	no	no	?
Need for dose-titration	no	no	?

Modified from ref. 6.

suggest a substantial interindividual variability in the response to antiplatelet agents and various phenomena of *resistance* to the antiplatelet effects of aspirin and clopidogrel have been described.[14] The benefit/risk profile of currently available GPIIb/IIIa antagonists is substantially uncertain for patients with acute coronary syndromes who are not routinely scheduled for early revascularization.[15-17] There is an expanding role for the combination of aspirin and clopidogrel for the long-term management of high-risk patients,[18,19] and at least 8 randomized clinical trials in approximately 75,000 high-risk patients are currently ongoing to further evaluate the efficacy and safety of this combination; use of the lowest effective dose of aspirin (75-100 mg daily) and avoidance of known drug interactions, e.g. aspirin and ibuprofen;[20] clopidogrel and some statin[21] appear to be critical for improving the benefit/risk profile of this combination.

The *European Society of Cardiology* is currently developing guidelines for the use of antiplatelet agents in patients with atherosclerotic cardiovascular disease. The purpose of these guidelines is to integrate a mechanistic understanding as to why some antiplatelet drugs work and some don't, with an evidence-based definition of categories of patients for whom the benefits of antiplatelet therapy clearly outweigh the risk of bleeding complications.

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How to identify the high-risk patient: an expanding paradigm

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In recent years it has become highly popular to assess vascular risk on the basis of chart-based procedures, so as to outline the risk profile in patients in whom coronary disease is not (yet) manifest. Three main categories are established: i) subjects at low risk, with no risk factors, for whom the frequency of future events is likely to be lower than 0.5%/year over the next ten years; ii) subjects at high risk, likely to have a 2-3% annual risk over the next ten years; and iii) subjects at intermediate risk.

For the first category it is proposed that the risk profile should be reassessed periodically – every five years. For the second category every effort must be made to eliminate or reduce all modifiable factors. For the intermediate category, however, it is not at all clear how to proceed.[1] One solution, suggested by the AHA guidelines,[2] would be to measure the ankle-brachial pressure index (ABI), especially in patients aged over 50. This is a simple, inexpensive and highly sensitive and specific method for detecting and diagnosing peripheral arterial disease (PAD). If the ABI is less than 0.9 the patient has PAD. Most people with PAD have no symptoms, but the cardiovascular prognosis is still not good, regardless of whether they have symptoms. Therefore, a patient classified as at intermediate risk on account of major risk factors but who also has an abnormal ABI should be moved up into the higher-risk class, with the appropriate consequences regarding management of risk factors.

Various non-invasive approaches have been proposed to detect asymptomatic atherosclerosis; these include intima-media thickness, coronary calcium score by electron beam computed tomography and coronary magnetic resonance imaging scans. However, the only one that can be recommended in clinical routine – i.e. outside clinical research projects – is the ABI, although most physicians still have only limited familiarity with it.

The vascular risk is high in PAD patients, even among those with no symptoms;[3] the relative risk is 6.3 for cardiovascular mortality, 4.8 for coronary mortality and 3.1 for overall mortality, in comparison with the risk in people of the same age, without PAD. Therefore abnormal ABI findings in an asymptomatic person should, in any case, suggest the need for secondary prophylaxis.

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Attempts are being made to stratify the risk for subjects who already have signs of coronary disease, but the aims are different from those of the algorithms proposed to identify *primary* risk. The main aim of a *secondary* risk map is to identify patients at very high risk – more than 5% – in the short-term, i.e. within four years. These subjects need vigorous preventive measures. Indicators of risk, incuding secondary risk, in such cases include PAD but the focus tends to be more on symptoms such as claudication. Even so, while it is clear that symptoms indicate the severity and extent of atherosclerotic lesions of the lower limb arteries, they also reflect the patient's attitude to walking, and any non-cardiovascular morbidity. There is, in any case, evidence that in patients with manifest coronary disease, both symptomatic and asymptomatic PAD have the same discouraging prognosis at five years.[4]

Patients with asymptomatic PAD outnumber those with symptoms by at least four to one. It might, therefore, be useful, with an eye to prevention, to expand the risk paradigm to include these asymptomatic subjects, who could be identified through systematic ABI screening. The PATHOS project (*Polyvascular Atherothrombosis Observational Survey*) includes an observational study to assess the prevalence of PAD in subjects admitted to hospital for myocardial infarction/unstable angina or stroke/transient ischemic attack (TIA), and follow their clinical course for one year. Departments of cardiology (48%), neurology (30%) and internal medicine (22%) in 54 centers throughout Italy are participating. Patients in hospital because of acute myocardial infarction, unstable angina, stroke or TIA as index events were investigated before discharge for the coexistence of PAD using the ankle-arm blood pressure Index (ABPI) and San Diego claudication questionnaire. A 12-month follow-up was planned to record any recurrences of vascular events.

So far 1,800 patients have been enrolled and the data reported here relate to the first 1,400. Overall, 69.5% are male and 30.5% female, and their mean ages are respectively 72.1 years (±10.9 SD) and 65.3 years (±11.3 SD). Of these patients, 57.3% cases (519 patients) had suffered myocardial infarction or unstable angina and 42.7% (387 patients) had had a stroke or TIA. PAD, diagnosed by an abnormal ABPI of 0.9 or less, was found in 28.7% of cases, specifically in 28.5% of those with acute coronary syndromes and in 28.9% of those with acute cerebral ischemia.

This is the first assessment of the prevalence of PAD

in a large population of patients with atherothrombotic disease. The proportion of patients with disease in both locations is considerably higher than that reported in previous studies. This combination could involve a group of patients at very high risk for subsequent vascular complications. The follow-up phase of the survey, currently in progress, will help clarify this issue.

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How guidelines have changed since the CURE results

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The treatment of acute coronary syndromes (ACS) without ST elevation has changed completely in the last few years. Until just a few years ago, the medications used in this condition were aspirin and heparin, but now several new antithrombotic drugs have been shown to be effective in this clinical condition, including the antiplatelet GP IIb/IIIa blockers and low molecular weight (LMW) heparin. Both these types of drugs have been demonstrated to be able to reduce clinical events, specifically cardiovascular death and myocardial infarction, in patients with ACS.

However, GP IIb/IIIa blockers are generally given intravenously and can, therefore, only be used as acute in-hospital treatment. Further, *post hoc* analyses of trials testing this therapeutic approach consistently showed that the benefit is limited to high-risk patients, specifically those with positive markers of ischemia or those undergoing a percutaneous coronary intervention.

LMW heparins are administered by subcutaneous injection and could, therefore, be used on a relatively chronic basis, but trial results have favored restricting their use to short-term treatment only. Thus, the only medication used for long-term treatment of unstable angina is aspirin, and even with the use of aspirin, 10-15% of patients still die or have a myocardial infarction (MI) within 1 year, and 20% of patients are readmitted to hospital with unstable angina.

It is, therefore, reasonable to hypothesize that long-term administration of an antiplatelet/antithrombotic regimen more powerful than just aspirin should result in a further reduction in events. Several medications have been investigated for the long-term, chronic treatment of ACS, in particular oral GP IIb/IIIa blockers. However, these new drugs have produced very disappointing results, with a paradoxical increase in event rates, which has led to the belief that they may be acting as partial agonists at the platelet receptor.

Clopidogrel has been shown to be effective in reducing events in patients with vascular disease (in the CAPRIE trial) and in reducing thrombosis when used in combination with aspirin in patients undergoing intracoronary stent implantation.

The combination of aspirin plus clopidogrel has also been tested in the CURE trial. The CURE trial evaluated the efficacy and safety of the antiplatelet agent clopidogrel, given with aspirin in patients with acute coronary syndromes without ST elevation. The 12,562 patients who presented within 24 hours after the onset of symptoms were randomized to receive clopidogrel (300 mg immediately, followed by 75 mg once daily) or placebo in addition to aspirin for 3 to 12 months.

The first primary outcome - a composite of death from cardiovascular causes, non - fatal myocardial infarction, or stroke - occurred in 9.3% of the patients in the clopidogrel group and 11.4% of the patients in the placebo group ($p<0.001$). The second primary outcome - the first primary outcome or refractory ischemia - occurred in 16.5% of the patients in the clopidogrel group and 18.8% of the patients in the placebo group ($p<0.001$). The percentages of patients with in-hospital refractory or severe ischemia, heart failure, and revascularization procedures were also significantly lower with clopidogrel.[1]

In agreement with the pharmacologic data that show the possibility of obtaining a prompt antiplatelet effect with high dosages of clopidogrel, the use of 300 mg of clopidogrel on the first day of treatment was associated with a significant reduction of the co-primary composite end-point in the first 24 hours from the onset of symptoms.

There were significantly more patients with major bleeding in the clopidogrel group than in the placebo group ($p=0.001$), but there were not significantly more patients with episodes of life-threatening bleeding or hemorrhagic strokes. When the bleeds were evaluated according to both the TIMI and GUSTO criteria, no significant differences were observed between the patients allocated to clopidogrel plus aspirin and those allocated to aspirin alone.

One of the most interesting findings of the trial is the fact that the benefit, in terms of reduced clinical events, was consistently seen in subgroups of patients at different risk levels: the direction of the effect favored clopidogrel in diabetics and not diabetics, in patients with non-Q AMI and in those with unstable angina, in patients with and without ECG modifications, and in patients with and without positive markers of ischemia. A recent *post hoc* analysis showed that the benefit from clopidogrel was similar in the different risk groups stratified according to the most widely used method in the field of ACS, the TIMI score.

The significant reduction of clinical events was obtained irrespective of the background therapy

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(heparin, beta-blockers, calcium-channel blockers, GP IIb/IIIa inhibitors, different dosages of aspirin, ACE-inhibitors, statins). A higher rate of bleeding was observed in those patients who received GP IIb/IIIa inhibitors, but no significant difference was observed among those patients allocated to clopidogrel plus aspirin with respect to those allocated to aspirin alone.

With respect to the patients who underwent a revascularization procedure, the CURE study showed that:

- the benefit from clopidogrel was similar in patients treated or not with percutaneous coronary interventions (PCI), performed according to the clinical indications;[2]
- the rate of major bleeds was similar in the patients receiving clopidogrel or not;
- in patients treated with coronary artery bypass grafting (CABG), a non-significant excess of major bleeds was observed in patients treated with clopidogrel.

However, if it was possible to discontinue the clopidogrel treatment for at least 5 days before the intervention, differences in terms of bleeding were no longer observed.

Conclusions

The antiplatelet agent, clopidogrel, has beneficial effects in addition to those of aspirin in patients with ACS without ST-segment elevation. The clinical event reduction was observed starting from the first day of treatment and continued for the whole period of the study (3 to 12 months). The risk of major bleeding was increased among patients treated with clopidogrel, although there was not a significant difference in term of life-threatening events. The benefit was observed in all subgroup of patients with different levels of risk, background therapy and use of percutaneous revascularization procedures.

On the basis of these results, both US and European guidelines suggest the use of clopidogrel, in association with aspirin, in all cases of a definite diagnosis of ACS without ST elevation.[3,4]

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Clopidogrel: from CURE to new studies in cardiology

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Patients with cardiovascular disease present at different points on the continuum of risk from asymptomatic to acute ischemic event. Patients at high risk of ischemic events (cardiovascular death, myocardial infarction or stroke) may benefit from an appropriate therapy that is able to reduce this risk with acceptable safety. Thrombosis, caused by rupture or erosion of an atherosclerotic plaque , represents the common pathogenetic factor in acute coronary syndromes. Antiplatelet therapy, principally with acetyl-salicylic acid (ASA), has been shown unequivocally to reduce ischemic events when used as secondary prevention. In more than 150 trials, as summarized in the 2002 *Antithrombotic Trialists' Collaboration* meta-analysis, antiplatelet therapy, largely ASA, confers an approximately 25% reduction in cardiovascular death, MI, or stroke across a wide variety of subjects.[1] Given these dramatic effects of ASA in reducing both mortality and non-fatal events in subjects with cardiovascular disease, the search has been on for more effective antiplatelet agents. The intravenous glycoprotein IIb/IIIa inhibitors have been a significant advance in percutaneous interventions (PCI) and in unstable angina (UA) non-ST-elevated myocardial infarction (NSTEMI). In contrast the oral GPIIb/IIIa inhibitors have been a distinct failure in these populations. A third class of oral antiplatelet agents, however, has shown great promise: the thienopyridines (clopidogrel and ticlopidine). These agents inhibit the adenosine diphosphate (ADP) pathway by blocking the P2Y12 receptor. There are at least two types of ADP receptors: the first is a low affinity type 2 purinergic receptor that is G-protein coupled and results in mobilization of calcium from internal stores (P2Y12). This is followed by a conformational change in and activation of the GPIIbIIIa receptor complex with subsequent fibrinogen binding and platelet aggregation. The second type of ADP receptor (P2Y1) is a high affinity receptor that is necessary for platelet shape change or calcium influx. Because clopidogrel and ticlopidine do not interfere with platelet shape or calcium influx, it is thought that they interfere with the P2Y12 receptor. This interference with the ADP-dependent step of platelet activation and activation of the glycoprotein GPIIbIIIa complex results in markedly decreased platelet aggregation and thus impairs thrombus formation.

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Prior studies with clopidogrel

Clopidogrel was first tested and approved for long-term secondary prevention in a broad population of subjects with atherosclerosis. Three groups of patients were enrolled in the CAPRIE study;[2] patients with recent ischemic stroke, acute myocardial infarction or symptomatic peripheral artery pathology. A significant 8.7% reduction in re-hospitalization for ischemic stroke, myocardial infarction or vascular death during long term follow-up was found in patients treated with clopidogrel (1,502 patients) compared to in the group treated with ASA (1,673 patients). The CAPRIE study showed the superiority of clopidogrel versus aspirin, and moreover suggested the rationale for long-term associated therapy in cardiovascular disease.

The combination of clopidogrel + ASA was, therefore, studied in the CURE trial[3] (*Clopidogrel in Unstable angina to prevent Recurrent Events*). A total of 12,562 patients with acute coronary syndromes were randomized to receive clopidogrel or placebo on top of ASA). The group of patients treated with clopidogrel has shown a significant risk reduction of 22% within the first 30 days after randomisation and 17% between 30 days and the end of the study (the mean follow-up was 9 month) in the primary endpoints of death, myocardial infarction, and stroke. The reduction was seen in all subgroups, including patients with or without ST-segment depression or patients with or without positive cardiac markers. The divergence of the Kaplan-Meier curves starts in the first hours and remains stable during the course of the therapy.

The combination of clopidogrel plus aspirin was associated to a higher incidence of haemorrhagic complications (3.7% vs 2.7%).[4]

PCI-CURE[5] is a sub-group of 2658 patients, approximately 25% of patients enrolled in the CURE study, undergoing percutaneous revascularization. From the above-mentioned group, one was randomised to clopidogrel (pre-loading dose 300 mg), while the other group to placebo, both in association with aspirin. During the following 28 days of follow-up, both groups were treated with thienopiridinic agents and in the 12 months thereafter, they resumed again therapy with clopidogrel or placebo, as the initial randomization. Even in this substudy the Kaplan-Meier event rates began to show a reduction in events (death, myocardial infarction and urgent revascularization) starting two hours after randomisation; the benefit was then maintained, so that at the 400 days of follow-up. the risk was reduced by 31%

in patients receiving clopidogrel. Based on these results clopidogrel is indicated for secondary prevention in patients having suffered an acute coronary syndrome for at least 12 month. The PCI-CURE study was the first suggestion that pre-treatment with clopidogrel significantly reduces major cardiovascular events (in particular IMA pre-PTCA) in patients with unstable angina and non-Q wave myocardial infarction undergoing PTCA.

The CREDO (*Clopidogrel for Reduction of Events During Observation*) was directly designed to answer many question not answered by the PCI-CURE. This trial was designed to determine whether more complete platelet inhibition with a thienopyridine at the time of a percutaneous revascularization is beneficial, as well as the most effective duration of clopidogrel treatment in patients after PCI. In the CREDO study[7] 2261 patients who had to undergo elective PCI, were randomised to receive 300 mg of clopidogrel (loading-dose), or placebo on top of aspirin, within 3 to 24 hour before the invasive procedures. Fifty-one percent of patients treated with clopidogrel received the loading dose between the 3rd and the 6th hour before PCI, and 49% between the 6th and the 24th hour before PCI. The 230 patients treated between the 6th and the 12th hour and the 621 treated between the 12th and the 24th hour before PCI, had shown a significant relative reduction of combined risk from 35,5% and 40,1%, whereas no significant benefit was observed in patients receiving clopidogrel in the six hours preceding intervention.

Perhaps, the most important question facing the interventional cardiologist regarding clopidogrel pre-treatment , which are unanswered by the PCI –CURE are whether pre-treatment with clopidogrel remains beneficial if a platelet GPIIbIIIa inhibitor is administered or whether the benefit of aspirin plus clopidobrel plus a GPIIb/IIIa antagonisis are additive without an unacceptable bleeding risk. In the *Intracoronary Stenting and Antithrombotic Regimen-Rapid Early Action for Coronary Treatment* (ISAR-REACT) study, patients who had symptoms of coronary artery disease and scheduled to undergo coronary angiography and who are extremely unlikely to require CABG within day of angiography will receive a loading dose of 600 mg clopidogrel for at least two hours before the procedure; those with insulin-dependent diabetes mellitus and those with positive biomarkers are excluded. Patients receiving this large dose of clopidogrel will be randomised to receive either abciximab and reduced dose heparin or standard dose heparin and placebo. The primary end-point of the study is the composite rate of death, MI, and urgent target vessel revascularization.

In conclusion, although there is strong support for the benefit of clopidogrel pre-treatment in patients undergoing PCI, still the results cannot be consideret definitive especially settings in which early angiography and the use of GPIIbIIIa inhibitors is the standard and in which a large number of patients (approximalely 35-40%) of patients undergoing angiography are expected to undergo CABG in a few days.

Future studies

Given the excellent results of studies in acute coronary syndromes and in patients undergoing interventional procedures, there are two major areas in which the efficacy of clopidogrel is going to be assessed: in primary prevention and in acute myocardial infarction.

Following results of the CURE trial, which demonstrated the additional benefit of long-term treatment with clopidogrel in combination with ASA in patients with recent acute coronary syndromes without ST elevation, the purpose of CHRISMA is to extend demonstration of a similar benefit to a broad high-risk population of patients receiving ASA. Since the recognized therapeutic dose of ASA in this population is between 75 and 325 mg, and since recent observations from CURE suggest a dose effect for bleeding, but not for efficacy within this range, the trial has incorporated a lower dose of ASA(75-165 mg). The primary objective of the study is to assess whether clopidogrel 75 mg daily is superior to placebo in preventing the occurrence of major ischemic complications (stroke, MI, cardiovascular death) in high-risk patients aged 45 year or older, who are receiving low-dose ASA or other background therapies, at a 40-month follow-up. The secondary objective is to evaluate the safety of clopidogrel, i.e., the incidence of fatal bleeding. A patient is considered to have a high risk in the presence of atherothrombotic risk factors, documented cerebrovascular disease, coronary artery disease or symptomatic peripheral arterial disease.

The second area of investigation is ST elevation myocardial infarction (STEMI). STEMI is typically due to coronary artery plaque rupture resulting in an occlusive intracoronary thrombus. Platelet activation and aggregation are central to this process and inhibition with ASA has been shown to be highly effective therapy in association with fibrinolytic therapy. Given the early effects of clopidogrel in CURE, it is hypothesized that an early benefit on reperfusion will be observed. Furthermore, given the reduction in MI seen in CAPRIE and CURE, it is expected that the reocclusion will be reduced in the setting of fibrinolytic therapy, and thus infarct-related artery patency will be improved.

The primary objective of another ongoing study is to demonstrate, in subjects with acute STEMI treated with fibrinolytic therapy, that, compared with results achieved with ASA alone, the combination of clopidogrel plus ASA will reduce the pro-

portion of patients who have an occluded infarct-related artery (TIMI flow 0 to 1) on the predischarge angiogram or who die or have a recurrent MI by hospital discharge. For subject who do not undergo angiography the end point will be death or MI by day 8.

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State-of-the-art in the treatment of cerebrovascular patients

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The evaluation of an epidemiologic hypothesis must take in account all the scientific evidence, including basic research, observational epidemiologic studies, and randomized clinical trials. Each of these strategies provides unique and complementary information for the totality of evidence by which rational decisions may be taken both for the care of the single patient and for the general health service. Even though much progress has been made in the past twenty years we still need greater knowledge about the prevention and therapy of stroke. The population epidemiology, the natural history of disease and the stroke registries change points of view, with the force of the new observations. Gorelik says that, at present, about half the cardiovascular disease risk is explained by conventional risk factors and that, studying atherosclerosis, many others will be discovered. Among these, markers of inflammation, coagulation/platelet-related factors, lipoproteins, the renin-angiotensin system and the amino acid homocysteine are first-rate factors.[1] Some clinical trials, such as those that have used statins or ACE-inhibitors, seem to confirm the value of these hypotheses.[2,3]

Unlike other vascular territories, the brain damage may be ischemic or hemorrhagic and consequently the pathophysiologic mechanisms in the brain and coronary/peripheral circulation are not completely superimposable. Chronically raised blood pressure is by far the most powerful risk factor for stroke in general, whether ischemic or hemorrhagic. In many cases, it is chronic hypertension that underlies the degenerative change in small perforating arteries and ultimaly leads to their rupture in the basal ganglia, cerebellum or brainstem, or less often in the subcortical white matter arteries. Few prospective studies have assessed the different risks of increasing blood pressure for hemorrhagic stroke and ischemic stroke. But arterial hyertension, sometimes associated with diabetes or hyperhomocysteinemia, also causes hyaline degeneration of the small perforating arteries, considered end arterioles, which produces lacunar infarction by progressive stenosis or occlusion. About one quarter of all ischemic strokes are lacunar, not easy recognizable in the acute phase and seldom responsive to thrombolytic drugs. In the last few decades another disorder has been recognized as a cause of primary intracerebral hemorrhage, particularly of lobar hemorrhages, in approximately 30% of people aged over 70 years. The abnormality consists of patchy deposits of amyloid in the muscle layer of small and medium-sized cortical arteries, which is not a part of a process of generalized amyloidosis. Typically the hemorrhage is at the border of the grey and white matter of the occipital, parietal or frontal lobes, in very different sites from those of hypertensive hemorrhage.[4]

Even if about 50% of ischemic strokes and transient ischemic attacks (TIA) are emboli left from ruptured or inflamed plaque, the substances that form them are very different. In certain cases they are lipid debris, more frequently aggregated platelets and fibrin, sometimes with the addition of red blood cells. The treatment of a thrombus, which may have been caused by different pathologic activations such as aggregation or coagulation, demands a specific drug or drug association that acts on the basic process. The clinical choice of antiplatelets drugs or anticoagulants, in specific situations, needs diagnostic exactness, and could profit from the results of ongoing clinical trials. Among these I would like remember the study on atrial fibrillation with a direct thrombin inhibitor (melagatran) and the one studying the association of ASA and clopidogrel on non-cardioembolic stroke or TIA. The consequences of atherothrombosis may, however, vary from one territory to another, even if from a pathophysiologic standpoint the process of slow growth of the plaque, inflammation, rupture and erosion, thrombosis and embolization are the same. This may be related to differences in arterial circulation or in tolerance to ischemia or in adaptative mechanisms such as collateral growth. The CAPRIE study, comparing ASA 325 mg to clopidogrel 75 mg, in 19,815 patients divided into three subgroups according to whether they are cerebral, peripheral or coronary recent disease showed that the subset of patients with symptomatic peripheral disease had the most benefit from clopidogrel therapy on the composite end points of stroke, myocardial infarct and vascular death. The reasons for these differences are not fully explained.[5] Finally the prevention of stroke should be considered at the same time as other vascular complications in patients with high vascular risks. This is the case of patients with type 2 diabetes, presenting with hypertension and dyslipidemia associated with hyperglycemia: these patients have a risk of death from cardiovascular disease that is two to six times higher than that among people without diabetes. Several studies showed that intensive therapy of hyperglycemia

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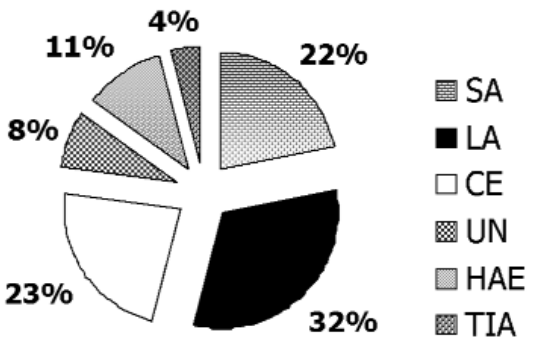

Figure 1. Vicenza SU Stroke subtypes in 1000 patients. ***SA: small arteries 22%; LA: large arteries 32%; CE : cardioembolic 23%; UN: undetermined 8%; HE: hemorrhage 11%; TIA: Trans. isch. attack 4%.***

reduced the macro and microvascular complications less significantly intensive than did therapy of all the risk factors associated. The UKPDS 38 study about hypertension,[6] the HOT study that linked aspirin to good control of hypertension[6] and the 4S study on the control of cholesterol levels with simvastatin[6] demonstrated significant reductions of stroke. A recent study concluded that a target-driven, long-term, intensified intervention aimed at multiple risk factors in patients with type 2 diabetes and microalbuminuria reduces the risk of cardiovascular and microvascular events by about 50 percent.[6] Physicians and other healthcare professionals who treat stroke patients or stroke-prone persons need to be aware of new guidelines for stroke prevention and effective ways to implement the recommended prevention and treatment measures. However for statin agents and ACE-inhibitors the evidence base has been strengthened by recent results. Statin agents are now recommended for prevention of stroke in people with CHD and symptomatic carotid artery disease. Furthermore ACE-inhibitors have been shown to reduce stroke risk in high-risk subjects with vascular disease or diabetes mellitus plus other risk factors and in those with ischemic or hemorrhagic stroke and elevated or normal blood pressure.[2,3]

Acute phase therapy

Therapeutic approaches to stroke have been centered on two distinct approaches, one primarily vascular and one primarily neuronal. Reperfusion is theoretically attractive since it is perfusion failure that underlies all the ischemic strokes, and relief of the initiating event should prevent all the consequences of the neuronal ischemia. Strategies for reperfusion have included thrombolytic drugs to promote dissolution actively, anticoagulants to prevent propagation of thrombus and a variety of therapies designed to increase regional cerebral blood flow or alter the rheological characteristics of the blood. The alternative approach has sought to prolong the viability of neurons subjected to ischemia and is therefore known as neuroprotection. The worsening during the first few hours often has quite different explanations from that occuring in the following hours (12 to 48). There are mainly three different broad categories of worsening: i) medical complications, especially febrile illness, which affect the patient systemically and may also lead to increased ischemia; pneumonia by inhalation in dysphagic patients; deep venous thrombosis and consequent pulmonary embolism. Intensive nursing in the Stroke Unit reduces these complications and improves mortality and morbility; ii) brain edema, a complication of most large strokes, especially hemorrhages, can be counteracted by the use of diuretics or osmotic agents, with some results only on mortality; iii) gradual or stepwise increases in focal deficits while the patient remains alert and free of medical complications. It is this last category, which usually begins during the first day of admission, that makes it worth selecting the patients.[7] Progression occurs in different patterns and with different time courses depending on stroke subtype. Although there may be no problems in distinguishing hemorrhagic from ischemic stroke, it is hard to recognize the different subtypes of ischemia within the time-limit. Similar clinical pictures are given by cardioembolic, atherotrombotic and lacunar infarcts and the help of neuroradiological examinations in distinguishing the different subtypes is scarse. On the other hand recent studies, using newer magnetic resonance technology, show that patients whose perfusion-weighted images (PWI) show a larger area of involvement than diffusion-weighted images (DWI), i.e. who have occlusive lesions on resonance magnetic angiography and do not reperfuse, develop larger infarcts than those with open arteries and no PWI>DWI mismatch.[8] The factors predicting and explaining worsening are the presence of a severe flow-reducing arterial lesion supplying the ischemic zone, chronic hypertension and a diminished frequency of transient ischemic attacks preceding the stroke. The obvious solution is to try to increase bood flow, to use the drugs able to reduce coagulation (either heparins or antiplatelet drugs) and to employ neuroprotective agents. The last two strategies will, of course, be ineffective if the blood flow is still deficient. Improving blood flow can be accomplished in two broad ways: opening arteries or augmenting collateral blood flow by systemic strategies. Opening arteries can be achieved mechanically (by surgery or stenting) or by thrombolysis. Pharmacologically raising blood pressure and expanding the circula-

tion in experimental animals using albumin infusion have been shown to limit the size of brain infarcts. The same effect in humans has been achieved only in lacunar strokes, augmenting blood flow by giving a volume expander (intravenous hetastarch).[9] Systemic thrombolytic therapy, indicated in the first three hours after cardioembolic and atherothrombotic stroke, needs a specialized team and a well-equipped hospital. Locoregional thrombolysis, which requires special clinical and instrumental abilities to select patients and the presence of an interventional neuroradiologist in the hospital, is yet more demanding and suitable for only a few cases.

Studies of the pathophysiology of stroke in humans suggests that the duration of a *therapeutic time window* in some types of patient may be quite short, perhaps an hour or less, but much longer, perhaps even 24 hours or more, in others. Beside the utility to distinguishing brain attacks on the basis of their physiopathology, we should consider that the size of necrotic core depends on a lot of conditions, including the various development of an adequate collateral blood flow to the ischemic zones, which makes the incomplete ischemia responsible for the spatial and temporal dynamics of cerebral infarction. With ischemic progression, the endothelial tissue loses its action on mechanisms of platelet adhesion and on the coagulation cascade and, because of the subsequent inflammation, underlies the irreversibility of the process together with the no-reflow phenomenon produced by deposition of platelets and fibrin in the distal arterioles and veins. There is little doubt that succesful thrombolytic treatment of carefully selected patients with acute ischemic stroke can result in much reduced disability in survivors, in spite of hemorrhagic transformation.[10] Nevertheless, the arterial recanalization produced by the thrombolytic drugs does not enhance collateral blood flow and is ineffective on the occlusion produced by platelets. Better results could be achieved by acting contemporaneously on many mechanisms, such as the manipulation of the systemic circulation, the use of powerful drugs preventing fibrinogen binding to the GP IIb/IIIa receptor antagonist, the discovery of active neuroprotectants, and suppression of the inflammatory response. All these actions are intended to increase the tissue's defence against ischemia.

Finally we must act corageously and forcefully against the nihilism that considers the strokes to be too numerous, that they hit elderly people and that the costs of caring are too expensive.

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High hematocrit values and prognosis of patients with a first-ever ischemic stroke

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Hemorheological changes may play an important role in the complex hemodynamic situation leading to ischemic stroke. Recent studies showed that enhanced erythrocyte aggregability was the strongest indicator of hemorheological impairment in patients with ischemic stroke.[1] Chronic hyperviscosity, which is largely influenced by hematocrit, was also shown to be important in the development of ischemic stroke.[2] Hemorheological factors may also mediate the effects through which conventional risk factors, such as cigarette smoking and dislipidemia, favor ischemic stroke.[2]

According to previous studies, high hematocrit values should be considered as an independent risk factor for ischemic stroke, associated with high morbidity and mortality.[4,5] In the Framingham study the risk of stroke was increased in subjects with high values of hematocrit and fibrinogen.[6] Other studies suggested that in subjects with high hematocrit values the risk of stroke was weak and confounded by cigarette smoking, arterial hypertension, and plasma fibrinogen levels.[7]

The aim of this study was to investigate the prognostic role of high hematocrit values at the onset of a first-ever ischemic stroke.

Methods

From among the residents of the L'Aquila district, all patients with a first-ever ischemic stroke occurring between January 1994 and December 1998 were identified and included. Pearson χ^2 test was used to compare groups and logistic regression analysis was applied to evaluate the association between high hematocrit values (≥45%) and the presence of other vascular risk factors. Survival curves were estimated by the Kaplan-Meier method. Cox regression analysis was used to evaluate independent predictors of 30-day mortality.

Results

A total of 3,481 patients with available hematocrit values at the onset of their first-ever ischemic stroke were included in the study. High hematocrit values were found in 778 subjects (22.3%); 464 were men and 314 were women with a mean age±SD of 74.5±10.7 years. Patients with high hematocrit values were more frequently men (OR 1.90; 95% CI 1.62-2.24), aged under 65 years old (OR 1.32; 95% CI 1.06-1.64), with higher proportions of hypercholesterolemia (OR 1.41 95% CI 1.19-1.68; p=0.0001), cigarette moking (OR 1.37; 95% CI 1.15-1.64; p=0.0005), and coronary heart disease (OR 1.20 95% CI 1.01-1.44; p=0.04). At the multivariate logistic regression analysis chronic atrial fibrillation (p=0.007) emerged as a further risk factor independently associated with high hematocrit values together with hypercholesterolemia (p<0.0001) and coronary heart disease (p=0.04).

Thirty-day mortality, as shown in Figure 1, was higher in subjects with high hematocrit values than in those with lower values (23.1% vs 18.8%; p=0.007) and mostly depended on a higher frequency of cerebral deaths (73.3% vs 69.8% p=0.42). The Cox regression analysis confirmed that high hematocrit values were independent predictors of 30-day mortality, together with age over 65 years, diabetes mellitus, coronary heart disease, and chronic atrial fibrillation, while hypercholesterolemia and cigarette smoking were associated with low mortality.

Discussion

In our study 22.3% of subjects with a fist-ever ischemic stroke had high hematocrit values at stroke onset. According to our data, the association between high hematocrit values and ischemic stroke might have been mediated by conventional risk factors such as male sex, hypercholesterolemia, cigarette smoking, coronary heart disease, and chronic atrial fibrillation. Other large studies, looking at mutual correlations between risk factors, are necessary to clarify whether high hematocrit values may be considered as an independent risk factor for ischemic stroke. As shown in our study, high hematocrit values were associated with younger age possibly because of a higher proportion of cigarette smokers among younger subjects.

Data on the association between high hematocrit values and stroke mortality are lacking. In our study, high hematocrit values were strong predictors of high 30-day mortality, indicating that direct and indirect control of this parameter may play an important role in improving survival after an ischemic stroke. The association between high hematocrit and 30-day mortality was independent of age, gender, and other risk factors. However, despite negative results in trials evaluating hemodilution in the acute phase of stroke, careful con-

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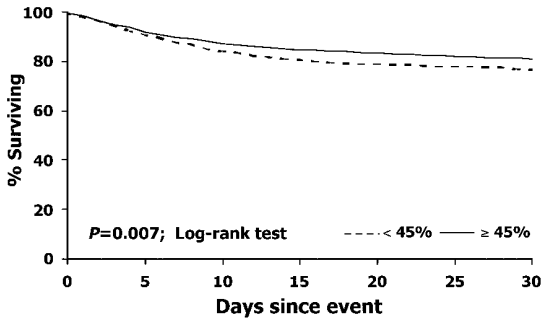

Figure 1. Thirty-day survival after a first-ever ischemic stroke, according to hematocrit values over and under 45%.

trol of high hematocrit values should be considered in treating acute stroke patients.

In our opinion, high hematocrit values might play a relevant role as a risk factor for stroke and have important short-term prognostic implications, thus deserving proper consideration in future studies.

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SELECTED ABSTRACTS

TOTAL PLASMA HOMOCYSTEINE AND METHYLENETETRAHYDROFOLATE REDUCTASE C677T POLYMORPHISM IN PATIENTS WITH COLORECTAL CARCINOMA

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Purpose. To investigate the pattern of total plasma homocysteine levels (tHcy) and its genetic determinants in patients with cancer, tHcy and genotype for the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) were determined in a group of patients with early stage colorectal cancer. *Methods.* Eighty-eight consecutive patients (48 men, mean age 66 years) affected by colorectal adenocarcinoma were enrolled in the study. One-hundred healthy subjects, matched for age and sex, served as a control group. tHcy was quantified by Abbott IMx immunoassay. Screening for MTHFR 677C_T substitution was performed by PCR and restriction analysis. *Results.* The frequency of C/T and T/T genotype of the MTHFRC677T polymorphism was not different between the groups. tHcy was statistically higher in patients than in controls carrying the same C/C or C/T genotype whereas it was not different between the T/T homozygous carriers of the two groups. tHcy was statistical significantly higher in T/T than in C/C genotype in both groups. Conclusions: The statistically significant increase of tHcy observed in C/C and in C/T genotype carriers of our patients could be related to tumor cells proliferation rate and, through homocysteine procoagulant activity, may act as a permissive factor for thrombosis in concert with the thrombophilic cancer state.

DEEP VEIN THROMBOSIS OF THE LOWER LIMBS: AN ALTERNATIVE APPROACH TO HOSPITAL ADMISSION

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In cases of deep vein thrombosis (DVT), it is essential to start anticoagulation immediately with adequate doses of heparin. The level of anticoagulation achieved with low molecular weight heparins (LMWH) does not need to monitored continuously so patients with DVT can also be treated as out-patients. *Aim.* A complementary out-patient service was set up within the Accident and Emergency (A&E) Department with the aim of reducing the number of admissions by using a diagnostic pathway offering alternatives to access to A&E for a series of pathologies including DVT. The specific aim was to provide rapid confirmation of a DVT clinically suspected by the patient's general practitioner (GP). During a series of preliminary meetings between participating GPs and personnel of the Diagnostic Ultrasound Service, an organizational/diagnostic pathway was established and the ways of the patients' access to the hospital were agreed. *Results.* During 2002, the GPs participating in the project sent 110 patients to our out-patients to undergo echocolor Doppler of the veins of the lower limbs. The clinical suspicion of DVT was based on signs and symptoms and the presence of certain risk factors for DVT. A venous thrombosis was found in 56 patients (51%): in 26 patients the DVT was proximal while in the remaining 30 patients the thrombosis was distal. The GP was then contacted in order to decide the subsequent management: immediate initiation of treatment with LMWH and return to home or admission to hospital in the presence of contraindications to home treatment. Four of the patients in the group with a distal DVT were admitted to hospital because of contraindications to home treatment; the other patients in this group were referred back to their GP. Of the 26 patients with proximal DVT, 16 were admitted to hospital (8 with medical contraindications, the others because of social problems). Subsequent follow-up 3-6 months later did not reveal significant events. *Comment.* The medical literature reports that a clinically suspected DVT is confirmed in only 30% of out-patients. Our series, albeit of limited size and collected over a relatively short period, shows that more effective hospital-community integration can contribute to reducing admissions for a pathology known to be associated with inappropriate admissions.

TT HOMOZYGOSITY FOR THE C677->T POLYMORPHISM OF METHYLENE-TETRAHYDROFOLATE REDUCTASE PREDISPOSES TO HYPERHOMOCYSTEINEMIA AND CORONARY ATHEROSCLEROSIS ONLY WHEN THERE ARE LOW PLASMA LEVELS OF FOLIC ACID

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High levels of plasma homocysteine (Hcy) are an independent risk factor for cardiovascular disease. Homozygosity for the C677->T polymorphism of methylenetetrahydrofolate reductase (MHTRF-C677->T) is associated with high levels of Hcy when there is a lack of folates. AIM. The aim of this research was to investigate the relationship between the extent of coronary atherosclerosis (CATS), Hcy, folic acid (FA), and MHTRF-C677->T. *Methods.* Ninety-five patients, mean age 52±6.2 years (median 51); 13 females (F), consecutively admitted for acute ischemic heart disease 13±2 days after the acute event underwent blood tests for Hcy, (nv: <15 µmol/L, <14 in women), FA (nv: 1.6-12.2 ng/mL), evaluation of MHTRF-C677->T, and coronarography to assess the extent of CATS, quantified using Gensini's carscore (GCS). *Results.* Mean Hcy: 15.5±7.9 µmoli/L; mean FA: 5.9±3.7 ng/mL, median: 5 ng/mL (no patient had values of FA below those of the normal range). Hcy levels were normal in 59 (62%) patients and raised in 36 (38%). There were 22 (23.1%) homozygotes for the MHTRF-C677->T (TT) mutation, 47 (49.5%) heterozygotes (TC), and 26 (27.4%) wild type homozygotes (CC). The TT patients had higher values of Hcy than did the TC and CC ones (TT: 22±12 µmoli/L, TC: 13.3±4.1 µmoli/L, CC: 14.1±6.5 µmoli/L, ANOVA-Bonferroni: $p<0.05$). The patients with FA concentrations above the median had higher values of Hcy than those with FA concentrations below the median 17.8±9 µmoli/L: 13.5±6.4 µmoli/L, t-test: $p<0.01$). The patients with hyperhomocysteinemia had a higher GCS than the patients with normal levels of Hcy (37.2±24.8 vs 21.5±19.3: t-test $p<0.001$). The GCS was not statistically different between patients with FA levels above or below the median. The TT homozygotes had a higher GCS than did the TC+CC subjects, but the difference was not statistically significant. Nevertheless, the TT patients with an FA concentration below the median had a worse GCS than that of all the other patients (TT with FA below the median: 45±29.5, TT with FA above the median: 17.1±16.9, TC+CC: 27.3±21.8, ANOVA-Bonferroni: $p<0.05$). The level of Hcy was also higher in TT patients with FA below the median than in all the other patients (TT with FA below the median: 26.6±13.5; TT with FA above the median: 17.4±8.4, TC: 13.3±4.1, CC:

14.1±6.5, ANOVA-Bonferroni: p<0.05). *Conclusions.* High levels of Hcy are associated with more extensive CATS. Among TT homozygotes, those with low FA, even though this was within the normal range, had significantly higher levels of Hcy and more extensive CATS. This suggests that these patients could benefit from dietary folic acid supplementation.

A 62-YEAR OLD PATIENT WITH LOWER LEG DEEP VEIN THROMBOSIS: CONCOMITANT PRESENCE OF FOUR COAGULATION DEFECTS (THREE CONGENITAL AND ONE ACQUIRED)

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Introduction. An idiopathic deep vein thrombosis (DVT) is often indicative of an underlying thrombophilic state which may be congenital, acquired or mixed. At present, thanks to studies started after the discovery of AT III deficits, we know that the most important causes of congenital thrombophilia are: 1) resistance to activated protein C (aPCR), which in 95% of cases presents with a mutation in the gene coding for factor V (RR for DVT 2-8, heterozygotes); 2) the mutation in the G20210A gene which codes for prothrombin (RR for DVT 2-3); 3) the C6771 mutation in the gene coding for methylenetetrahydrofolate reductase (RR not increased). The risk of a first thrombotic event increases in the presence of a combination of more than one of these factors. This has been shown by various family studies in which the carriers of several thrombophilic traits had a higher prevalence of thromboembolic events than that among carriers of only one defect. The concomitant presence of hyperhomocysteinemia and factor V Leiden or prothrombin G20210A has been associated with a 20 to 50-fold increase in the risk, although the data are conflicting. While a substantial number of carriers of factor V Leiden have a first spontaneous event after the age of 45 years, the risk of thromboembolism in patients with the G20210 polymorphism seems to increase significantly with age, reaching a 19-fold increase in patients over 60 years old. Is there an age limit for screening for thrombophilia, and should family studies always be carried out? Can the finding of one or more defects influence the management of the oral anticoagulation? *Methods.* A 62-year old patient presented with a suspected DVT of the lower left leg. The clinical suspicion (Wells' score: 3) was confirmed by both D-dimer and echoDoppler: thrombosis of the superficial femoral vein, and of the popliteal and the subpopliteal branches. Given the negative family history, the negative personal history for recent or past thromboembolic events and the absence of triggering factors, the DVT was classified as *idiopathic*. Investigations for possible underlying neoplastic or autoimmune disease were negative. The patient therefore underwent tests to identify a possible state of congenital or acquired thrombophilia. *Results.* Lupus anticoagulant (Lac): positive; activated protein C resistance (functional test): 0.66 (n.v. >0.80); R506Q mutation of factor V (FV Leiden): positive (heterozygote); G20210A mutation of the gene for FII: positive (heterozygote); A223V mutation of the methylenetetrahydrofolate-reductase (MTHFR) enzyme: positive (heterozygote). The investigations were therefore extended to family members: a sister and a son were positive heterozygotes for FV Leiden and the G20210A mutation of FII, a granddaughter was positive for the A223V mutation of the gene for MTHFR, while another grandson was a positive heterozygote for the G20210 mutation of the FII gene and for the A223V mutation of MTHFR. *Conclusions.* 1) There is no age limit to carrying out screening for thrombophilia in *idiopathic* DVT. 2) In all positive cases the study should be extended to other family members. 3) The finding of one or more defects definitely influences the management of the oral anticoagulant treatment. 4) Individual evaluation of each case is nevertheless crucial.

ACTION AGAINST THROMBIN OF LOW-DOSE ACETYLSALICYLIC ACID IN BLOOD TESTIFIED BY SERUM TAT COMPLEXES CONCENTRATION

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It is known that low dose of acetylsalicylic (ASA) is effective in therapy of coronary heart disease (CHD), although it has not yet been clarified all pathways it exerts. Large randomised trials have showed that patients affected by chronic coronary insufficiency benefit from therapy with low dose of ASA. ASA, in fact, remains the first choice for antithrombotic treatment in CHD, due to its relative safety and well documented efficacy. Moreover, ASA is more effective at daily lower doses than those required for other actions. It is generally assumed that antithrombotic properties as well as antiplatelet action of ASA is mediated by irreversible acetylation of platelet cycloxigenase which leads to impairment of platelet function through inhibition of thromboxane A2 synthesis (*Patrono et al., 1994*). Other studies refer to an antithrombotic effect of ASA by decreased thrombin generation (*Szczeklik et al., 1992*). However, it is generally believed that the mecahanism whereby ASA exerts its antithrombotic action involves decrease of thrombin formation although it was shown that ASA treatment does not have any effect on dilution-induced enhancement of coagulation (*Ruttmann et al., 1999*). Here we report that treatment of patients affected by CHD based on administration of 100 mg daily of ASA does not attenuate thrombin generation (serum F 1+2: 124 nM in untreated patients vs serum F 1+2: 123 nM in ASA 100 mg/daily treated patients; $p > 0.05$, ns), but reduces free thrombin by favouring of thrombin-antithrombin complexes (TAT) formation (serum TAT 25 nM in untreated patients versus serum TAT 53 nM in ASA 100 mg/daily treated patients; p: <0.05, s). Data were expressed as media; statistical analysis was performed with the Bonferroni test; differences were considered to be significant if $p < 0.05$. This results are caused by increased activation of AT III make by inhibition of platelet factor 4 (PF4) release from platelet _-granules, thus leading to higher heparin availability. If the action of PF4 is impaired, hindering platelet _-granules release, it follows that an increased amount of TAT can form, because the increased heparin availability generates hyperactivation of AT III.

PROPHYLAXIS WITH LOW DOSE WARFARIN IN CANCER PATIENTS WITH CENTRAL VENOUS CATHETERS FOR POLYCHEMOTHERAPY

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Aim. To evaluate the safety and efficacy of prophylaxis with warfarin 1.25 mg/die in cancer patients with central venous catheters (CVC) being treated with cycles of polychemotherapy. *Methods.* Fifty-five patients were evaluated. There were 34 women (62%) and 21 men (38%); their mean age was 60 years (min 30, max 75). All had a port-a-cath in situ for infusion of polychemotherapy for treatment of advanced solid tumors (53 patients) or for adju-vant therapy (2 patients). The

types of cancer were: colon 44%, breast 27%, rectal or sigmoid 26%, others 3%. The most frequent sites of metastasis were: liver 40%, lung 22%, peritoneum 16%, bone 15%, lymph nodes 11%, others 16% (more than one site was involved in 40% of the patients). The drugs administered were: fluorouracil, adriamycin, cyclophosphamide, oxaliplatinum, taxol, irinotecan, navelbine and trastuzumab. The total duration of the treatments was 446 months (min 1, max 26; median 6). The total number of cycles was 641 (min 3, max 34; median 9). Twelve patients (22%) died. The total number of INR assays carried out during the treatment was 492. Three patients were already receiving anticoagulation for previous deep vein thrombosis (and had maintained a target INR between 2 and 3 without any problems - 49 determinations). Of the remaining 443 determinations, concerning 52 patients, 350 (79%) showed INR values between 1.01 and 1.50. In 53 determinations (12%), concerning 12 patients, the INR was between 0.91 and 1.00; in 28 (6%) determinations in17 patients, the INR was between 1.51 and 2.00 and in 12 (3%), from 10 patients, the INR was >2.00. *Conclusions.* One patient (1.9%; 95% CI: 0.0005-0.1026) had deep vein thrombosis involving the CVC while the INR =1.09. In 2 patients (3.8%; 95% CI: 0.0047-0.1327) prophylaxis was interrupted because of the development of hematoma (a 74-year old woman with liver metastases from cancer of the cecum - INR 8.31) and persistent, disturbing epistaxes (a 54-year old male with colonic carcinoma and liver metastases - INR 2.48). In one case (1.9 %) the dose of warfarin was reduced (1.25 mg on alternate days) because of an INR of 2.53 without any side effects (a 71-year old woman with lung metastases from rectal cancer); in one case of epistaxis (INR 1.40) changes of drug dose were not considered necessary. Age, sex, type of cancer, sites of metastases, duration of therapy, drugs used and number of cycles did not statistically influence the INR values.

EVIDENCE AND SCREENING FOR THROMBOEMBOLIC DISORDERS

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The Department of Laboratory Medicine and Environmental Hygiene considers a screening program if it satisfies three criteria: 1 - efficacy: that is, there must be a benefit for those undergoing the test; 2 - the benefit must outweigh any possible negative effects; 3 - the benefit must justify the resources used. *Materials and Results.* a) Hyperhomocysteinemia in identifying coronary artery disease. The data concern plasma/serum concentrations of homocysteine in relation to atherosclerotic damage in case-control studies, there being few prospective studies. The cost-benefit analysis shows that, at present, there is insufficient evidence to plan a screening program. The results certainly demonstrate an association, but not a causal relationship, between homocysteine and risk of coronary events. In particular, it should be highlighted that concentrations of homocysteine and associated metabolites (methionine, methylfolate) vary in different districts, so plasma levels may not be good indicators of intracellular processes and thus may not accurately reflect thromboembolic risk. Nevertheless, from the 45,000 patients enrolled in 12 randomized studies, it seems to be confirmed that vitamin supplements (folic acid, vitamin B6 and B12) can, by decreasing homocysteine levels, reduce cardiac damage. *b) Hyperferritinemia and coronary artery disease.* An Iranian study has shown that increased levels of ferritin in adult males, by increasing LDL oxidation, are associated with atherosclerotic cardiovascular disease. The patients in the highest quartile of ferritinemia had an OR of 1.62 (95% CI, 1.12-2.42; p<0.01) compared with those in the lowest quartile. This was particularly true for patients under the age of 50 years and in diabetics. No association between ferritin and coronary artery disease was found in women. At the same time there are reports that when this parameter is correlated with other markers, such as C-reactive protein, malondialdehyde and related to sex and age, it loses its significance; an angiographic evaluation showed poor correlation between increased ferritin and damage to the coronary vessels. Thus, at present, the results appear to be conflicting and not such as to strengthen clinical evidence. c) *Environmental pollution* and cardio-cerebrovascular damage. Korean studies have noted that high atmospheric concentrations of ozone, PM(10), NO_2, CO, and SO_2 are significantly associated with the incidence of ischemic stroke, particularly in women (increase in the RR). The European APHEA project has shown an association between PM(10)/exhaust gas and cardiovascular disease only in patients over 65 years old. At present, there is not evidence to support widespread monitoring of atmospheric pollution. *Conclusions.* The necessary evaluation of evidence must favor systematic reviews, large-sized, randomized, controlled studies, and prospective studies over retrospective studies. Furthermore, it should be remembered that a good quality study is to be preferred over experts' opinions.

DERMATAN SULPHATE IN SIX PATIENTS WITH HEPARIN-INDUCED THROMBOCYTOPENIA AND ACUTE VENOUS THROMBOEMBOLISM

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Type II heparin-induced thrombocytopenia (HIT) is the most common form of immunologic thrombocytopenia caused by drugs and can have severe clinical consequences, in particular venous and arterial thromboembolic events. The treatment of such complications is difficult; heparin administration must be stopped immediately and an alternative antithrombotic therapy initiated. Dermatan sulphate (DS, Mediolanum Farmaceutici) is a selective inhibitor of thrombin characterized by a low probability of cross-reaction with heparin. We describe the cases of six patients with acute venous thromboembolism (VTE) and type II HIT who were successfully treated with DS.

Six patients (1 male, 5 females), mean age 57.5 years (range 6-85) were included in this study. All the patients had had normal platelet counts before the administration of heparin and had developed HIT during treatment with unfractionated heparin (3 cases) or low molecular weight heparin (3 cases). Five patients had been receiving prophylactic doses of heparin and developed acute, symptomatic VTE at the same time as the diagnosis of HIT; in one patient the HIT developed during administration of heparin at therapeutic doses for deep vein thrombosis of an upper limb. At onset the mean platelet count was 52,500/mm3 (± 22,600) and the diagnosis of HIT was confirmed by a platelet aggregation test (4 cases) or positive ELISA test for anti-PF4-heparin antibodies (2 cases). The heparin was immediately withdrawn from all patients and continuous intravenous infusion of DS was initiated. The mean dose of DS was 0.618 (± 0.17) mg/Kg/h and the infusions were continued for a mean of 174 (± 58.7) hours (range 60-216), producing a mean aPTT of 48 seconds (±14.7). The level of anticoagulation achieved by the DS was stable, making only minor dose adjustments necessary during the infusion. The platelet count rose within a few days in all patients, thus allowing treatment with warfarin to be commenced. During the treatment with DS no patient had recurrent thromboembolism, bleeding complications or other side effects. In con-

clusion, in our series DS was a safe and effective antithrombotic agent, allowing a rapid rise in the platelet count. The use of DS should be considered in the treatment of thromboembolic complications during type II HIT.

EVALUATION OF BLEEDING RISK FROM THE ASSOCIATION OF ANTIAGGREGANTS AND ANTICOAGULANTS

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Aim. The association of antiaggregants and anticoagulants has always been a difficult therapeutic choice because of the possibility of increasing the risk of hemorrhage in the patients. This combination of drugs frequently seems to be inevitable in ischemic heart disease associated with disorders managed with oral anticoagulants. Recent studies (Warfarin, aspirin or both after myocardial infarction. Hurlen M et al. NEJM 2002,vol 347,n 13) confirm the increased risk of hemorrhage when these two drugs are associated. The aim of our study was to evaluate the incidence of major and minor bleeding events (defined according to FCSA criteria) in patients treated with both these types of drugs. *Methods.* We selected 46 patients (32 males, 14 females), aged between 47 and 89 years old, who were attending our anticoagulation clinic with various conditions. Of these patients, 38 were being treated with warfarin and 8 with acenocoumarol. All were also contemporaneously receiving antiaggregants (42 ASA and 4 ticlopidine). All the patients regularly attended their appointments which were never more than 3 weeks apart. A computer program (GESTAO2000) was used to calculate the total days of therapy and the number of days that anticoagulation was within the therapeutic range for each patient. *Results.* A total of 15291 days of treatment were considered: of these about 65% were passed with the patient in the correct therapeutic range (the planned target INR was 2.5 for 42 patients and 3 for 4 patients. Two cases of minor bleeding occurred (one epistaxis and one macroscopic hematuria) equivalent to 4.3% of events per patient per year. There were no major bleeding episodes. In both cases with minor bleeding, the INR was within the therapeutic range at the time of the event. *Discussion.* Although this is a limited series, the results suggest that in the context of continuous and punctual monitoring of anticoagulant therapy, the association between antiaggregants and anticoagulants does not seem to lead to an increased risk of bleeding. The number of minor bleeding complications per patient per year (4.3%) was not significantly different from the 6.2% reported in the ISCOAT study (Lancet 1996) or our own subsequent observations.

PERIPHERAL ARTERIAL DISEASE: IN VITRO INHIBITION OF LEUKOCYTE OXIDATIVE BURST BY (+)-CATECHIN AND ITS (+)-3-O-PROPIONYL- AND (-)-3-O-VALERYL-DERIVATIVES

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Introduction: Reactive oxygen species (ROS), produced in severe peripheral arterial disease (PAD) during oxidative burst of activated leukocytes, play a role in tissue damage. Therefore, molecules that counteract leukocyte ROS production could have a role in improving the prognosis of these patients. The present study was designed to in vitro evaluate the effects of (+)-catechin [(+)-C] and its esters, (+)-3-propionylcatechin [(+)-3-O-PC] and (-)-3-O-valerylcatechin [(-)-3-O-VC], on chemiluminescence (CL) activity of whole blood leukocytes (WBL) from PAD patients. *Methods.* Catechins were prepared as reported by Lambusta *et al.* patent (EP1088094, 2001) and diluted from 0.01 to 100 mM. Peripheral blood was collected from 5 healthy donors, 5 patients suffering from peripheral arterial disease in Fontaine stage II (PAD-II) and 4 from critical leg ischaemia (CLI). CL assays were performed according to De Sole protocol (1993), with and without PMA-stimulation of leukocytes. The responses were recorded as total counts x 90 min and expressed as mean $\pm$ SD. The statistical analysis was performed by ANOVA and values of $p<0.05$ were regarded as significant. Results and Discussion: In absence of drugs, CL counts from resting and PMA-stimulated cells of healthy controls were 4.88x106 $\pm$ 3.76x105 and $6.52\times10^7 \pm 4.21\times10^6$, respectively. Counts of PAD-II subjects did not differ from controls ($4.71\times10^6 \pm 3.41\times10^5$ and $5.92\times10^7 \pm 4.15\times10^6$, respectively), whereas counts of CLI patients were significantly higher ($8.73\times10^6 \pm 5.10\times10^5$ and $9.50\times10^7 \pm 5.11\times10^6$, respectively; $p<0.05$). On the whole, all catechin concentrations significantly inhibited either resting or PMA-stimulated CL emission. Esterified catechins were more active than (+)-C ($p<0.05$) on controls and on both patient groups; the inhibition rate of each catechin did not differ between the three subject categories in all experimental conditions. In conclusion, our results lead to take into account a potential use of these molecules in the field of vascular pathologies.

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ANTIPHOSPHOLIPID SYNDROME AND RECURRENT VENOUS THROMBOSIS: A CASE REPORT OF AN ELDERLY PATIENT

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Antiphospholipid syndrome (APS) is characterized by arterial and venous thromboses, recurrent abortions, thrombocytopenia (Hughes' syndrome) and circulating antiphospholipid antibodies, which can be demonstrated by coagulation tests (LAC), serological tests for syphilis (VDRL) and solid phase immunoenzymatic tests (aCL). APS can be associated with a systemic autoimmune disease, most frequently systemic lupus erythematosus (secondary APS) or not correlated with any recognizable autoimmune disease (primary APS). The diagnostic criteria include the above major clinical manifestations, other minor clinical symptoms (livedo reticularis, lower limb skin ulcers, valve disease, hemolytic anemia, renal disease, neurological disease) and abnormal laboratory findings. In order to make the diagnosis the clinical manifestations (major or minor) must be associated with positivity for any of the tests for aPL antibodies (lupus anticoagulant, anticardiolipin: IgG

>20 GPL - U/mL, IgM > 20 MPL - U/mL) confirmed on at least two occasions separated by a minimum of 6-8 weeks.[1-3] Positive tests have an uncertain significance in elderly subjects:[4] in fact, a high prevalence of anti-cardiolipin antibodies has been found in geriatric patients without any clinical manifestations.[5-7] For this reason the cut-off for aCL must be corrected for age, particularly in the very elderly.[8] *Case Report.* An 85-year old woman was referred to us with acute bronchitis and lower limb edema. She had a past history of COPD and vascular encephalopathy from chronic, multiple micro-infarcts. The patient's history revealed pregnancy-related nephritis at the age of 30 years, cholecystectomy because of gallstones and apppendicectomy at the age of 65, hypertension at the age of 70, and recurrent bronchitis in the last 10 years. In 1990 the patient was admitted to hospital because of poorly defined cerebro-meningeal hemorrhage. On that occasion a deep vein thrombosis of the leg was associated with transitory thrombocytopenia. The patient was receiving continuous treatment with losartan, phenobarbital, digitalis and furosemide and intermittent treatment with cortisone and aminophylline. During the admission the patient developed a fever despite antibiotic therapy and the lack of focal bronchopulmonary changes, skin eruptions on the face and lower limbs diagnosed as *eczematoid*, reddening of the oral cavity with microulcerations. The fever disappeared with nimesulide or cortisone. Laboratory tests showed marked and persistent rises in indices of inflammation (ESR, fibrinogen, C-reactive protein), increased LDH, β2microglobulin and D-dimer, hypergammaglobulinemia, increased CA-125. Despite antithrombotic prophylaxis with calcium heparin, the patient developed deep vein thrombosis of the popliteal and subpopliteal region of the left leg (site of the previous episode) with subsequent pulmonary micro-emboli confirmed by thoracic angiographic CT scans. The ECG showed sinus rhythm at all times. The signs and symptoms improved progressively under the influence of heparin infusion. Following the finding of a high titer (1:1280) of anti-ribosomal antibodies (fluorescence on Hep2 cells), although negative for antinuclear antibodies and anti-ENA antibodies (anti smooth muscle negative, anti-mitochondrial positive 1:20), anti-cardiolipin and lupus-type anticoagulant antibodies were investigated with the following results: p-dRVVT (dilute Russell viper venom time) 72 s (nv: 23-37), p-KCT (kaolin clotting time) 142 (nv: 65-140), ACL IgG >100 GPL/mL, IgM >100 MPL/mL. The patient was treated with prednisone e warfarin. *Conclusions.* The presence of major clinical criteria, associated with positive tests for dRVVT and ACL led us to make a diagnosis of antiphospholipid syndrome probably secondary to cutaneous lupus disease or, at least, a "lupus-like" disease. The patient's family did not give permission for a skin biopsy to be performed. The woman died a month later from congestive heart failure.

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