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b

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Platelets 2001

Rome, Italy

November 23-24, 2001

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### Inflammation in acute coronary syndromes

ATTILIO MASERI

Institute of Cardiology, Catholic University of the Sacred Heart, Rome, Italy

Inflammation is becoming an intriguing focus of research as a possible pathogenetic component and therapeutic target in ischemic heart disease (IHD). The potential may develop from links between inflammation at least at three levels. First, the inflammatory response has been known for many years to play a major role in ischemia reperfusion injury and its reduction can limit myocardial damage. Second, inflammation is a very common feature of the chronic atherosclerotic process, as first described by Virchow and recently comprehensively reviewed by Ross.<sup>1</sup> Finally, inflammation, may be an acute pathogenetic component of instability in of about half of the patients with acute coronary syndromes, independently from the atherosclerotic and ischemic burdens.

Elevated values of circulating inflammatory markers, such as CRP, SAA, IL-6, IL-1ra are commonly found in acute coronary syndromes (ACS). Such elevations are associated with in-hospital and short-term adverse prognosis<sup>2</sup> and may reflect not only a high prevalence of myocardial necrosis, of ischemia-reperfusion damage and severe coronary atherosclerosis but also a primary inflammatory trigger of coronary instability. However, in patients with ACS the prevalence of a primary inflammatory pathogenetic component of coronary instability, as detectable by elevated CRP, varies considerably. Elevated CRP (above 3 mg/L) is found in less than 10% of normal individuals, in less than 20% of patients with chronic stable or variant angina but in over 65% of patients with unstable angina, Braunwald class IIIB and in over 90% of patients with acute infarction preceded by unstable angina, but in less than 50% of those in whom the infarction was totally unheralded (in samples taken before elevation of markers of necrosis).<sup>3</sup>

The absence of elevated CRP in over 30% of patients with severe unstable angina and in over

50% of those with acute myocardial infarction not preceded by unstable angina suggests that inflammation may not be the triggers of coronary instability in all patients and that its prevalence may vary in different ACS. Whether the long-term predictive value of CRP in normal subjects and in stable patients identifies all patients who will develop ACS or only those with elevated CRP is unknown.

The poor correlation between potential inflammatory agents and CRP levels may be at least partly explained by a variable individual response to inflammatory stimuli. The increase in CRP and IL-6 observed in response to the vascular trauma caused by coronary angioplasty or by uncomplicated cardiac catheterization and that observed after acute infarction was found to be linearly correlated to baseline CRP and IL-6 levels. This specific enhanced response observed *in vivo* may be related to monocyte responsiveness. Indeed, *in vitro*, IL-6 production by isolated monocytes from patients with unstable angina and elevated CRP and IL-6 is significantly greater than that produced by monocytes from patients with normal values.<sup>4</sup>

The very episodic nature and the commonly short duration of ACS, suggests that the inflammatory stimuli that cause the inflammatory process detectable systemically could be unrelated to the chronic inflammatory component of the atherosclerotic background. Its causes may be multiple, not necessarily the same in all patients, and their effect is likely to be modulated by the individual immunologic and inflammatory response.

Waxing, waning and persisting inflammatory stimuli would nicely fit the clinical pattern of waxing, waning and recurrent instability lasting some weeks, which is common in ACS. Recurring thrombotic stimuli also fit the common *post-mortem* finding of thrombi formed by separate layers of different age and composed of platelets which suggests that such thrombi developed as a result

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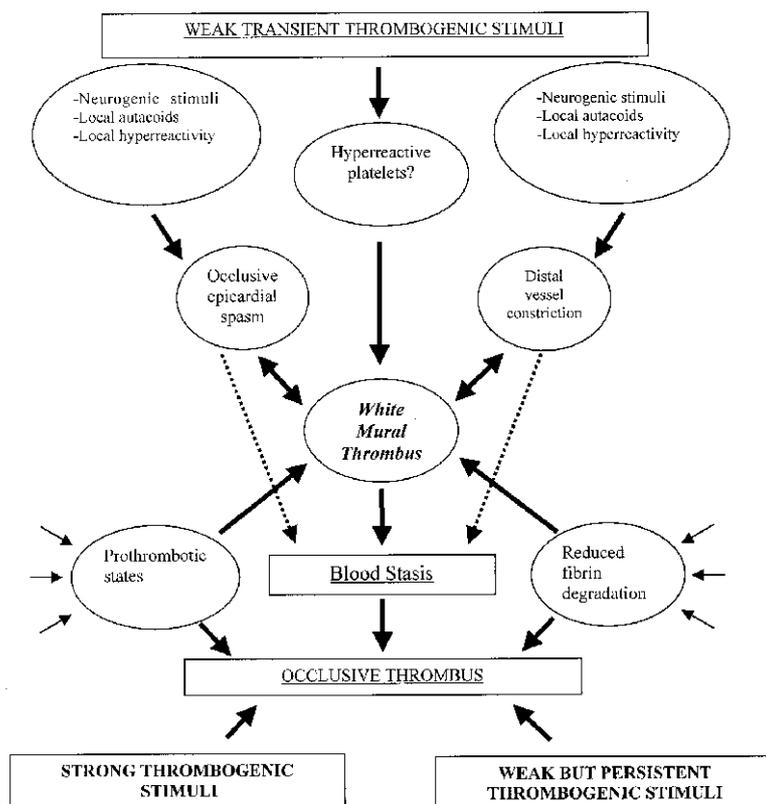


Figure 1. Vicious circles leading to the formation of an occlusive thrombus. An occlusive red thrombus can form rapidly within minutes at the site of highly thrombogenic injury (for example the rupture of a strongly thrombogenic plaque). An occlusive platelet thrombus can form gradually at the site of a weak, but very persistent thrombogenic stimuli (for example a persisting inflammatory process). A mural thrombus resulting from a plaque fissure or from a local inflammatory process may evolve into an occlusive thrombosis in the presence of prothrombotic states or of blood flow stasis induced by local or distal coronary constriction. The components of these vicious circles and their gain may have a variable importance and prevalence in different groups of patients. Prothrombotic states may result from any acquired or genetic alteration that leads to enhanced platelet reactivity and/or thrombin formation or to reduced fibrinolysis. (Modified from ref. #3).

of repeated, separate, weak thrombogenic stimuli persisting long enough to allow the progressive accumulation of platelets, but not strong enough to produce an occlusive red thrombus (Figure 1).

The inflammatory process detected in some patients with ACS may originate in the coronary arteries or somewhere else in the body; what causes the primary or secondary inflammatory involvement of the coronary arteries and whether the vulnerable coronary plaques are few or many and remain potentially vulnerable for weeks or months are matter of speculation.

In the complex pathogenic scenario outlined above, there are no grounds for generalizations. ACS are rare, occasional events even in patients with extensive coronary atherosclerosis and with prothrombotic states. Any single, common, putative trigger cannot explain such rarity. Thus ACS

are either the result of a very exceptional local event or of a very unusual coincidence of multiple, adverse local and possibly systemic events that may not have the same prevalence in different ethnic, geographical, age and sex groups.

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### Bioactive lipids and atherothrombosis

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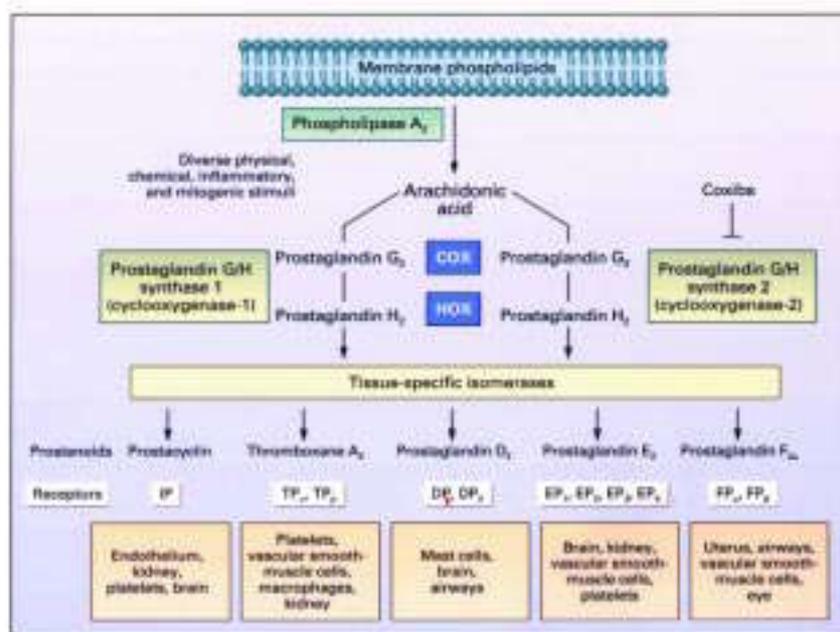
**A**rachidonic acid (AA) is subject to metabolism by prostaglandin (PG) G/H synthase (S), catalyzing the formation of PG endoperoxide intermediates from which PGs are formed by downstream synthases and isomerases (Figure 1). Increasingly, the role of AA itself<sup>1</sup> and PGs in cardiovascular biology is being informed by the exploitation of model systems and the emergence of pharmacologic probes for enzyme action<sup>2</sup> and receptor activation.<sup>3</sup> This review will highlight some of the more recent information in this field and its impact on cardiovascular therapeutics.

#### *Aspirin: still famous after all these years*

The molecular basis of the action of aspirin was resolved at the atomic level with the crystalization of PGG/H S by Loll Picot and Garavito.<sup>4</sup> Aspirin irreversibly acetylates a serine residue close to the catalytic site in the hydrophobic channel which leads into the core of the enzyme.<sup>5</sup> This differs from the pattern of inhibition afforded by typical non-steroidal anti-inflammatory drugs (NSAIDs) which are reversible, competitive inhibitors of PGG/H S. These properties discriminate particularly the action of aspirin from that of NSAIDs on the anucleate platelet. Even low doses of aspirin cumulate to inhibit platelet PGG/H S maximally and only synthesis of new platelets permits recovery of thromboxane (Tx) A<sub>2</sub> dependent platelet activation.<sup>6,7</sup> NSAIDs, by contrast, depress the capacity of platelets to generate TxA<sub>2</sub> into the functionally relevant range (> 95%<sup>8</sup>) for variable and generally incomplete segments of the dosing interval, thereby failing to afford cardioprotection.<sup>9</sup> Recently, we demonstrated that pre-dosing with the NSAID ibuprofen prevented access of aspirin to the active site of platelets in humans, disrupting the sustained pattern of platelet inhibition observed during chronic dosing with low dose aspirin. Interestingly, this interaction was not observed with diclo-

fenac, which is positioned differentially within the hydrophobic channel from ibuprofen.<sup>10</sup>

Aspirin has been shown to reduce the secondary incidence of myocardial infarction and stroke by about 25% in controlled clinical trials, which have recently been reviewed.<sup>11</sup> Its usefulness in the primary prevention of cardiovascular disease is much more arguable. For example, a review of 4 controlled trials of aspirin in primary prevention suggests that the small number of non-fatal myocardial infarctions which it prevents is almost precisely numerically offset by induction of upper gastrointestinal bleeds.<sup>12</sup> First, ticlopidine and then clopidogrel, P2Y<sub>12</sub> antagonists, were shown to have a marginally superior efficacy to aspirin in the secondary prevention of cardiovascular events. As P2Y<sub>12</sub> antagonists prevent aggregation of platelets by ADP,<sup>13</sup> it is perhaps unsurprising that evidence of the additive benefit of such agents to aspirin should emerge.<sup>14</sup> Similarly, the benefits derived from aspirin and dipyridamole in the secondary prevention of stroke and transient ischemic attack are roughly additive.<sup>15</sup> Dipyridamole has a range of actions which may have relevance to its clinical efficacy, including inhibition of adenosine transport and platelet phosphodiesterase. However, which of these actions is or are operative at the plasma concentrations attained by the newly reformulated preparation, which is clinically effective, remains to be seen. All of these drugs, aspirin, clopidogrel and dipyridamole are moderately effective and reasonably well tolerated. Indeed, they might merit consideration as a triple combination. Although multiple effects of aspirin beyond inhibition of platelet PGG/H S have been described,<sup>16</sup> it is unclear whether any have relevance at the concentrations of aspirin attained with chronic administration of doses in the range 75 mg-325 mg/day, which are cardioprotective. Indeed, the spectrum of aspirin efficacy is explicable solely in terms of its effects as a PGG/H S inhibitor.



#### Coxibs: beyond NSAIDs?

The recognition of a second form of PGG/H S, readily inducible by cytokines and growth factors, suggested that it was the primary source of PG formation in inflammation and cancer.<sup>2</sup> The tertiary structure of the two isoforms is remarkably similar to PGG/H S-1, but the hydrophobic channel of PGG/H S-2 is more accommodating to a wider range of substrates. It also has a side pocket which permits selectivity of drug action.<sup>5</sup> Interestingly, platelets only express PGG/H S-1, so selective inhibitors of the second isoform, such as the cyclooxygenase inhibitors (coxibs),<sup>2</sup> do not affect platelet function. For the same reason, combination therapy with a coxib does not prevent the platelet inhibitory effect of low dose aspirin.<sup>10</sup>

Several years ago we reported that coxibs depressed, in healthy individuals, the biosynthesis of prostacyclin (PGI<sub>2</sub>), the predominant PGG/H S product of vascular endothelium.<sup>17,18</sup> As PGI<sub>2</sub> inhibits platelet activation by all recognized agonists *in vitro* and is a vasodilator, we raised the possibility that this might confer a cardiovascular risk to the coxibs. This would be expected to be small, as other systems with similar biological properties - NO generation, for example - are thought to be unaffected by the coxibs. Furthermore, mice deficient in the PGI<sub>2</sub> receptor (the IP) do not clot spontaneously, but have an enhanced response to thrombotic stimuli.<sup>19</sup> Thus, one would anticipate that the clinical substrate would be relevant to this

mechanism and that the first signal might emerge as drug-related episodes in patients with thrombotic diatheses. Such events have been reported,<sup>20</sup> but are by their nature anecdotal. There is some evidence that coxibs enhance thrombosis in a canine model of vascular occlusion.<sup>21</sup> Aside from PGG/H S-2 being a rational drug target in inflammation, the development of selective inhibitors was also prompted by the prospect of enhancing the tolerability of NSAIDs. Thus, PGG/H S-1 is expressed constitutively in gastric epithelium, where it is thought to be the predominant source of cytoprotective PGs. Thus, the commonest adverse effect of NSAIDs, gastrointestinal (GI) intolerance, might be attributable to PGG/H S-1 inhibition by these isoform non-selective inhibitors.

This *COX-2 hypothesis* has been sustained by the results of the VIGOR trial, a comparison of GI outcomes in roughly 8,000 patients in whom rofecoxib was compared with the NSAID, naproxen.<sup>22</sup> The pre-specified primary GI endpoint was reduced very significantly, from roughly 4% to 2% by rofecoxib. Surprisingly, there was also a significant 4-fold divergence in the incidence of myocardial infarction between the groups and a trend was also evident in strokes and venous occlusive events. Aspirin was specifically excluded at entry to the trial. This minimized the number of participants at high risk of cardiovascular events, although some who should have been placed on aspirin were included (vide infra). While the total number of events was

small - less than 75 - and therefore the results may be due to chance, two mutually compatible mechanistic hypotheses have been advanced. The *Naproxen hypothesis* proposes that the comparator is distinct from other NSAIDs by virtue of a prolonged pharmacodynamic half life resulting in effective platelet inhibition throughout the dosing interval. Pharmacologic evidence consistent with this hypothesis has been presented.<sup>10</sup> Attempts to define the cardioprotective role of naproxen have been limited to overview analyses and retrospective case control comparisons. These indirect approaches have yielded conflicting results.<sup>23,24</sup> If the estimate of the size of the difference in cardiovascular event rates in VIGOR is valid, they are twice what might be expected from controlled trials of aspirin.

The *PGI<sub>2</sub> hypothesis* suggests that the depression of biosynthesis of this eicosanoid is of functional relevance. Recently, we have acquired data in mice indicating that deletion of the IP enhances both the platelet - as reflected by Tx biosynthesis - and vascular proliferative responses to catheter induced injury in the murine carotid. Deletion of the Tx receptor (TP), along with the IP, abrogated the consequences of PGI<sub>2</sub> deficiency (*Cheng Y and FitzGerald GA, personal communication*). How does this translate into the human condition? Firstly, aside from the species disparity, the experiment involves complete deletion of the IP, which may not model the ~70% depression of PGI<sub>2</sub> biosynthesis by the coxibs. Indeed, we have shown that whereas IP deletion accelerates atherogenesis in the mouse, depression of PGI-M excretion by ~70% with a PGG/H S inhibitor does not.<sup>25</sup> On the other hand, the recently reported divergence of the PGI synthase knockout (KO) from the IP KO (it has a more pronounced vascular and renal phenotype: Yokoyama C, *personal communication*) raises the possibility of a second PGI<sub>2</sub> receptor, perhaps PPAR. Furthermore, we have not modeled the coincidental deletion of PGE receptors which mediate similar responses in platelets and vascular cells as does the IP,<sup>26</sup> although coxibs are likely to inhibit PGE<sub>2</sub> biosynthesis along with PGI<sub>2</sub>.

The possibility that a cardiovascular hazard might emerge and render the coxibs a *zero sum game* has generated some alarm. However, the strength of the evidence in support of the *COX-2 hypothesis* is presently much more established than is its cardiovascular risk. Indeed, the interaction data described above<sup>10</sup> would favor rofecoxib over the NSAID ibuprofen, as combination therapy in those taking aspirin for cardioprotection. Meta-

analyses in individuals without risk factors for cardiovascular disease offer no indication of a hazard from coxib intake, as one might expect.<sup>24</sup> Indeed, very large trials in patients predisposed to thrombosis would probably be necessary to detect the magnitude of risk, based on the *PGI<sub>2</sub> hypothesis*. If such a risk does indeed exist, adjuvant therapy with aspirin may not be a straightforward solution. PGI<sub>2</sub> inhibits platelet aggregation by all known ligands, not just TxA<sub>2</sub>. Indeed, only half the individuals in VIGOR met the criteria for prophylactic aspirin.

#### *TP antagonists: born again*

Our studies in mice reintroduced consideration of the clinical efficacy of TP antagonists. Thus, we found that TP deletion or TP antagonism depressed the response to vascular injury. Indeed, this response was exaggerated in mice with directed vascular overexpression of the TP; here TP antagonists were also effective in depressing the response but at higher doses than in wild type littermates (*Cheng Y and FitzGerald GA, personal communication*). TP antagonists have been evaluated in controlled comparisons with aspirin in trials assessing the prevention of restenosis after coronary angioplasty.<sup>27,28</sup> In both cases, the hypothesis postulated that preserved ability to generate PGI<sub>2</sub> on the TP antagonist might lead to a better outcome. In the event, no difference was discernable. However, all patients were placed on aspirin initially, to prevent periprocedural myocardial infarction, prior to randomization to continued aspirin or the TP antagonist for the following 6 months. We now know from human studies that the aspirin regimen depressed the periprocedural increment in PGI<sub>2</sub> biosynthesis<sup>29</sup> and from the mouse that IP deletion enhances the proliferative response to angioplasty. Thus, defects of the trial design may have led to the premature conclusion that TP antagonists are just a more expensive version of aspirin.

It is also possible that the spectrum of efficacy of these compounds may be broader than previously thought. Thus, isoprostanes (iPs), free radical-catalyzed isomers of PGs may activate membrane receptors for PGs, including the TP.<sup>30,31</sup> In the case of the TP, it is possible that this may acquire functional importance in syndromes in which platelet activation and oxidant stress coincide. Thus, TP antagonists are more effective than an aspirin regimen, that completely abrogates Tx biosynthesis in limiting reocclusion following coronary thrombolysis in the dog.<sup>32</sup> This is a model in which increased TxA<sub>2</sub> biosynthesis and iP generation accompany the reperfusion phase. Similar biochemical abnormal-

ities characterize established human and murine atherosclerosis. Interestingly, PGG/H S-1 inhibition,<sup>25</sup> TP antagonism<sup>33</sup> and TP deletion (*Narumiya S, personal communication*) delay atherogenesis in the mouse. Recently, we have found that TP antagonism is more effective than PGG/H S inhibition in retarding murine atherogenesis (*Egan and FitzGerald GA, personal communication*) and that iP suppression with vitamin E adds to the efficacy of PGG/H S inhibition with indomethacin.<sup>34</sup> All of these results are consistent with the possibility that incidental, free radical catalyzed ligands, such as the iP, may activate the TP *in vivo*.

#### *Conclusion: variations on a theme*

Our understanding of the role of PGs and their receptors in the cardiovascular system is still quite naive. This situation is likely to improve as more models and pharmacologic probes emerge. We still have an incomplete picture of why we have two PGG/H S isoforms, never mind insight into the distinct roles of the two PGE S enzymes.<sup>35</sup> It is likely that more PG receptors will emerge. The recent identification of a second PGD receptor (DP2<sup>36</sup>), quite unrelated to the first, suggests a greater level of complexity than previously appreciated. Indeed, in the case of the TP, we have previously characterized two pharmacologic classes of receptor unexplained by the splice variants of the TP.<sup>37</sup> The biology of these subtypes, one mediating shape change, the other aggregation of platelets is reminiscent of the function of the two P2Y receptors in platelets, which are also quite unrelated in primary sequence.<sup>13</sup>

Finally, we have little information on how genetic variation in the proteins which synthesize<sup>38,39</sup> or respond to PGs<sup>40</sup> may modify drug response or disease expression. For example a polymorphism in the TP has been related to bronchoconstrictor response.<sup>40</sup> On the other hand, while some coxibs exhibit substantial interindividual differences in plasma concentration:response relationships<sup>17</sup> polymorphisms in that gene do not appear to be common or to influence drug response or product formation, at least *in vitro*.<sup>38</sup> Mutational analyses in zebrafish and, more recently in mice are likely to provide information in this area of research in the future.

#### *Acknowledgments*

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## Vascular Risk Stratification: Practical Problems

Chairmen: M. Chiariello, M. Volpe

### Hypercoagulability and athero- thrombosis: clinical suggestions and perspectives

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**H**ypercoagulability is an abnormal activation of the coagulation cascade in the absence of detectable thrombotic events.<sup>1</sup> The clinical impact of hypercoagulability first emerged in the 1980s, based on the results of a large prospective study, the Northwick Park Heart Study (NPHS).<sup>2</sup> In that study, subjects who died of coronary events, had had, at recruitment five years earlier, persistent abnormalities of some variables (fibrinogen, factor VII) suggestive of a hypercoagulable state. Other epidemiological studies have confirmed and extended the concept of hypercoagulability: similar to fibrinogen and factor VII, years before the development of unstable angina or acute myocardial infarction (AMI), other markers of hypercoagulability (Table 1), are often detectable in the absence of thrombotic events. These studies have also shown that the predictive power of some of these indices is at least as good as that of some established atherothrombotic risk factors (cigarette smoking, hypercholesterolemia, age, diabetes mellitus). In the following paragraphs, we shall examine these variables, focusing on their clinical impact and practical use.

#### *Fibrinogen and factor VII levels*

The NPHS first demonstrated that high plasma fibrinogen and factor VII levels, in apparently healthy subjects, increased the risk of a first ischemic event within 5 years by >80%.<sup>2</sup> Other studies have confirmed the association of high plasma fibrinogen levels with stroke and myocardial infarction and reinfarction, and have identified a series of factors affecting plasma levels of fibrinogen: in this respect, diet, age, male gender, pregnancy, menopause, oral contraceptives and inflammation enhance while physical activity, alcohol consumption, ticlopidine, or fibrates lower fibrinogen plasma levels.<sup>3</sup> Presently, high plasma fibrinogen is an independent cardiovascular risk factor and hyperfibrinogenemia dramatically increases the risk related to other risk factors.<sup>2-6</sup>

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#### *Abnormalities of fibrinolytic activity*

Prospective studies have shown that an abnormally low fibrinolytic activity (due to high levels of plasma PAI-1) increases the risk of ischemic events.<sup>7,8</sup> PAI-1 is a fast-acting inhibitor of tissue plasminogen activator (t-PA), the major proteolytic activator of fibrinolysis *in vivo*.<sup>9</sup> In a longitudinal cohort study of 109 randomly selected survivors of a first AMI before the age of 45,<sup>10</sup> high PAI activity was independently associated with myocardial reinfarction, and this association was as strong as that with dyslipoproteinemia, poor left ventricular performance, or multiple vessel-coronary artery disease. High PAI-1 levels are also associated with the risk of peripheral arterial disease and that of juvenile myocardial ischemia in patients with diabetes mellitus and/or hypertriglyceridemia.<sup>11</sup> A relationship between t-PA and PAI-1 levels, plasma triglycerides and BMI has been described.<sup>11</sup> In addition to triglycerides and BMI, plasma glucose levels, male gender and insulin-resistance contribute to raise whereas physical activity, weight loss, insulin or metformin contribute to lower plasma PAI-1 levels.

#### *Fibrinopeptide A*

Fibrinopeptide A (FpA), a 16 aminoacid-peptide derived from the cleavage of the fibrinogen  $\alpha$ -chain by thrombin, is a marker of fibrin formation. Patients with acute coronary syndromes (AMI and unstable angina) show raised levels of plasma FpA as well as of F1+2 prothrombin fragments.<sup>12</sup> The plasma levels of these two indices are comparable in patients with AMI and in those with unstable angina, making it conceivable that, rather than the characteristics of the thrombus, FpA and F1+2 peptides reflect systemic hypercoagulability. This in turn implies that factors other than hypercoagulability (e.g. the characteristics of the plaque and/or the severity of the stenosis) ultimately lead to occlusive (AMI) or subocclusive (unstable angina) thrombi.<sup>13</sup> Low concentrations of FpA are physio-

**Table 1 Predictive parameters of ischemic artery disease.**

Hypercoagulability parameters	Other predictive parameters	Indexes of organ damage
Fibrinogen	Homocysteine	LVH
Factor VII	Hematocrit/high blood viscosity	Albuminuria
PAI-1	Lp(a)	IMT
t-PA	Leukocyte count	Retinopathy
Fragment 1+2	APA	PVD
TAT		
FpA		
D-dimer		
vWF		

**Mutations:**

Fibrinogen B $\beta$  chain promoter  
 Factor VII (Arg353Gln)  
 PAI-1 (4G/5G)  
 Glycoprotein IIIa (T1565C)  
 Prothrombin (G20210A)  
 Factor V (G1691A, Factor V Leiden)

Abbreviations: vWF: von Willebrand factor; APA: antiphospholipid antibodies; LVH: left ventricular hypertrophy; IMT: intima-media thickness; TAT: thrombin-antithrombin complex; FpA: fibrinopeptide A; Lp(a): Lipoprotein (a); PVD: peripheral vascular disease.

logically present in the plasma, suggesting that a low-grade activation of the coagulation system (i.e. fibrin formation) is present in healthy subjects.<sup>14</sup> FpA may be measured by radioimmunologic or immunoenzymatic assays. However, the clinical impact of this marker is limited by artifacts (blood collection being a major source of variability) as well as to its brief half-life (3-5 minutes). In contrast, it may well be considered a marker of the coagulation process that immediately precedes blood sampling. The 24-hr urinary measurement of this peptide is reported to provide more reliable information.<sup>14</sup>

**F1+2 prothrombin fragment**

The F1+2 fragment (F1+2) is released from prothrombin converted to thrombin by prothrombinase. Its determination provides information concerning *in vivo* thrombin generation.<sup>15</sup> Thus, its pathophysiologic correlations and potential clinical implications are those previously discussed for FpA. As its half-life is about 90 min, it is a less transient index of activation of the coagulation system. Radioimmunoassays or immunoenzymatic methods measuring F1+2 fragment are now available.<sup>15</sup> However, F1+2 fragment values vary significantly according to the methods used. This makes very difficult comparisons of results from different labs.

**Thrombin-antithrombin complex (TAT)**

Thrombin-antithrombin complex (TAT) reflects thrombin formation and neutralization. This complex has a half-life of about 15 min. This has

allowed the development of specific radioimmunologic and immunoenzymatic assays. Increased TAT plasma levels have been reported in clinical settings with activation of the coagulation cascade, such as pulmonary embolism and deep venous thrombosis,<sup>16</sup> bacterial endotoxemia<sup>14</sup> and in some forms of leukemias.<sup>17</sup> As for other parameters, differing results may be obtained employing different methods. Moreover, the binding of thrombin to cellular receptors, with subsequent internalization, may markedly interfere with this measurement. Together with other abnormalities of coagulation parameters suggestive of a hypercoagulable state, a significant increase of plasma TAT has been reported, in the absence of thrombotic events, in subjects with homozygous homocystinuria.<sup>18</sup>

**D-dimer**

Over the last years, D-dimer, a cross-linked fibrin degradation product, has been widely studied as to the diagnosis of the venous thromboembolism. Presently, its determination is used to exclude a diagnostic suspicion of deep venous thrombosis. The role of this marker in the cardiovascular risk profile is poorly defined. Retrospective studies show correlations between D-dimer levels and AMI. However, D-dimer is known to increase following vascular damage. Thus, based on these studies, it is unclear whether high D-Dimer is consequence (*post-hoc*) or cause (*propter-hoc*) of ischemic events. On the other hand, prospective studies in healthy subjects show that – when compared to the risk of subjects with lower values – high D-dimer levels double the risk of AMI.<sup>19,20</sup> High D-dimer levels have also been reported to predict the risk of new events in subjects with CAD.<sup>20,21</sup> The laboratory evaluation of this marker is often difficult, as different methods and reagents are used for its measurement. Moreover, factors (e.g. age, prolonged immobilization, antithrombotic treatments) are known to affect its levels. In spite of this, D-dimer levels are often correlated with those of F1+2, TAT and FpA.<sup>22</sup>

**Polymorphic markers and ischemic events: clinical implications**

Over the last decade, a series of common molecular variations (polymorphisms) of proteins involved in hypercoagulable states have been identified. Interest in these genetic polymorphisms has been increased by the evidence that, *per se*, contribute to the interindividual variability (in some cases accounting for up to 30% of the total interindividual variability) of the encoded protein and that may further enhance the levels of individual plasma pro-

teins by conferring sensitivity to a certain environmental factors.<sup>23</sup> This is the case for the G→A polymorphism in exon 8 of the factor VII gene, responsible for an arginine to glutamine substitution at position 353. Subjects homozygotes for the arginine allele, show higher factor VII levels than homozygous for the glutamine allele, and an enhanced sensitivity to the raising ability of triglycerides on plasma Factor VII. Similarly polymorphisms within the fibrinogen  $\beta$  chain gene account for about 10% of interindividual variability of plasma fibrinogen and confer sensitivity to interleukin-6 (i.e. plasma fibrinogen increases due to inflammation and cigarette smoking).

At variance with the association with the plasma levels, the association between polymorphic markers and ischemic episodes is disputed. As a general rule, genetic tests are inadequate to replace functional methods for the measurement of the risk associated with the variables.<sup>23</sup> In a recent evaluation in young survivors of AMI, among the *candidate* polymorphic risk predictors, only the T1565C mutation of the platelet GP IIIa gene has been significantly associated to the risk of new ischemic episodes.<sup>24</sup>

#### *Markers of hypercoagulability: clinical relevance*

*Vis-à-vis* the evidence that much work is needed to identify reliable and simple laboratory markers of hypercoagulability, there is the central role of this state in coronary artery disease (CAD). The Thrombosis Prevention Trial (TPT),<sup>25</sup> is a recent English prospective study devoted to primary prevention of CAD in subjects at high cardiovascular risk. In this study, only non-fatal events were significantly reduced by chronic administration of 100 mg/d aspirin, whereas an important reduction of fatal events was achieved in patients on chronic low-dose warfarin (INR regimens  $\approx$  1.5). Thus, the increasing interest towards simple, cheap, reproducible tests to identify hypercoagulable states, implies that attention should be devoted to the following issues:

- As to predictivity, we have learned to rely on functional rather than on quantitative (or genetic) methods.
- The standardization of the methods that currently predict a cardiovascular risk is good for only few variables (e.g. in multiple measurements, the reproducibility of plasma fibrinogen assay is comparable to that of plasma cholesterol).
- *Established* risk factors, concomitant diseases,

physiological conditions and drugs, affect a variety of haemostatic parameters.

- Vascular injury due to venipuncture may cause artifacts in measuring some hemostatic variables (false positive).

Once defined the appropriate test(s) to evaluate a hypercoagulable states, the following issues should be taken into consideration to draw reliable clinical conclusions.

- The available prospective studies are population-based; the clinical impact has to be defined individually.
- A hypercoagulable state may contribute to estimate the individual risk over and above *established* risk factors.
- The combined evaluation of *established* risk factors and markers of hypercoagulability may help identify subjects at the highest atherothrombotic risk. As an example, the risk is highest when cigarette smoking, elevated LDL-cholesterol levels, or systolic hypertension coexist with high plasma fibrinogen levels. According to these data, when a hypercoagulable state coexists, the intensity of the intervention has to be maximal even in patients with a borderline hypertension or hypercholesterolemia, or an isolated systolic hypertension (i.e. single risk factors that currently do not require intensive treatments).
- In spite of their limited clinical impact, the data emerging from some *new* markers of hypercoagulability is providing important information as to the pathophysiology of acute coronary syndromes. For instance, patients with elevated FpA and F1+2 levels show higher mortality and poorer response to antithrombotic treatments.<sup>26,27</sup> Whether and the extent to what this implies a role of these variables as prognostic markers and predictors of adequacy of treatment needs to be defined in ad hoc studies.<sup>13</sup>
- A global evaluation based on the clinical picture is mandatory to draw correct diagnostic/therapeutic conclusions. An accurate clinical summary may help identify subjects (i.e. patients with ischemic events in the absence of traditional risk factors, young subjects with positive family history, patients with recurrent events or with failure of current antithrombotic strategies) in whom the evaluation of the markers of hypercoagulability is maximally useful. Markers with good reproducibility (fibrinogen, PAI, etc) should be chosen in this respect.
- Physicians should take record and remember

therapeutic/diagnostic data from patients whose outcome differs from that reasonable/predictable. Over the last years, this attitude helped to define the possibility, then confirmed and extended by epidemiological trials,<sup>28</sup> that established risk factors account for only 40% of future ischemic events. We believe that this same mental attitude will improve the clinical impact of markers of hypercoagulability, and define strategies of intervention *tailored* to the individual risk factor profile.

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**Carotid lesions as risk factor for  
ischemic heart disease**

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The increasing sophistication of ultrasound examination of carotid arteries consents evaluation of the whole arterial wall, detection of early lesions, and multiple examinations during follow-up. The technique is safe and non-invasive as well as less expensive.<sup>1</sup> Using high resolution B-Mode ultrasound examination, intima-media thickening (IMT) can be defined, according to Pignoli,<sup>2</sup> as an intima-media > 0.85 mm, while an asymptomatic carotid plaque (ACP) may be defined as an IMT > 1.3 mm as in the MIDAS,<sup>3</sup> PHYLLIS<sup>4</sup> and ELSA<sup>5</sup> studies or > 1.5 mm as in the VHAS Study.<sup>6</sup>

The measurement of IMT is reproducible, has a low variability and the analysis of images with a high-resolution video-recorder, possibly coupled with a computer-assisted system of analysis, may also analysis of the quality of the plaque.<sup>1</sup>

*Association of risk factors with IMT and/or asymptomatic carotid plaques*

It has been well demonstrated that IMT and asymptomatic carotid plaques are associated with several risk factors such as hypertension,<sup>8</sup> hypercholesterolemia or low HDL-cholesterol, smoking,<sup>8</sup> diabetes,<sup>9</sup> menopause,<sup>10</sup> ACE-gene polymorphism<sup>11</sup> and a history of premature death from CHD.<sup>12</sup>

Moreover, women with a high familial predisposition for CHD may be more vulnerable to cardiovascular effect from hostility and social support than high-risk men or men and women with low to medium risk.<sup>13</sup> Lipoprotein (a) and apolipoprotein (a) phenotypes have also recently been described to be associated with coronary and carotid atherosclerosis in men.<sup>14</sup>

In the GESCO-MURST-CIFTI-4 study<sup>8</sup> we considered 755 subjects, 371 females and 384 males, aged 18 to 85 years. In this study we found a significant increase of IMT (32.5% vs 14.7%) and ACP (10.75% vs 5.37%) in subjects with one or more

traditional risk factors compared with in subjects without risk factors. Moreover, the prevalence of IMT was significantly related to age, male gender, hypertension, non-insulin-dependent diabetes mellitus (NIDDM), and smoking while ACP was related to hypertension, NIDDM and hypercholesterolemia.<sup>8</sup> In another study we demonstrated that age and hypertension are the main predictors of extracranial cerebrovascular atherosclerosis after renal transplantation.<sup>15</sup>

*Relationship between IMT and/or ACP with fatal and non-fatal cardiovascular events*

Extracranial atherosclerosis is a common feature in patients with risk factors, and carotid lesions are a good predictor of cardiovascular events in the general population.<sup>16</sup>

Recently, Yamasaki *et al.* studied carotid IMT, predictors of its progression, and their relationship with incident CHD in Japanese patients with type 2 diabetes.<sup>17</sup>

They studied carotid IMT of 287 subjects with type 2 diabetes (mean age 61.6 years) without CHD or CVD at baseline, and followed them up for 3.1 years.<sup>17</sup> The annual progression of IMT was  $0.04 \pm 0.004$  mm/year.<sup>17</sup> Stepwise multivariate analysis demonstrated that independent risk factors for progression of IMT were the initial IMT ( $p < 0.001$ ), the average HbA1c level ( $p < 0.001$ ), and age ( $p < 0.001$ ).<sup>17</sup> Both the initial IMT and low HDL-c were identified as predictors of incident non-fatal CHD (angina pectoris or non fatal myocardial infarction, with a 3-year incidence of 10.1%).<sup>17</sup>

The prognostic value of IMT and/or ACP has also been confirmed by several population studies. In the Finnish Study, 1,288 Finnish males were studied and a linear increase of coronary risk related with common carotid and bifurcation lesions was demonstrated.<sup>18</sup>

In the Atherosclerotic Risk in the Communities

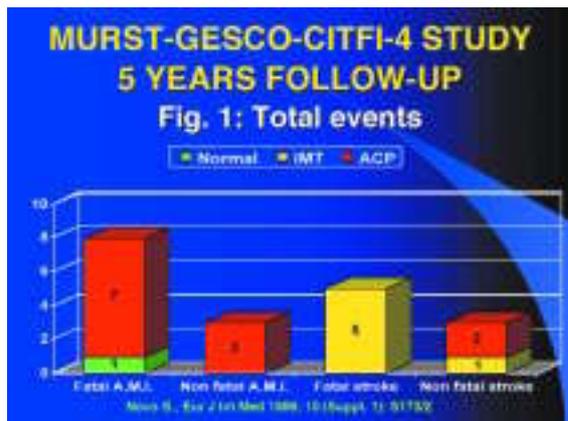


Figure 1.

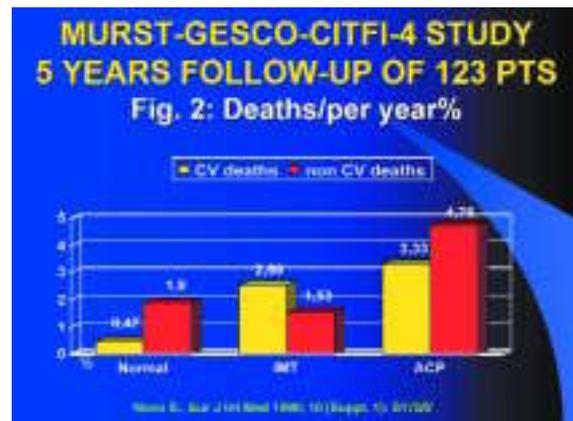


Figure 2.

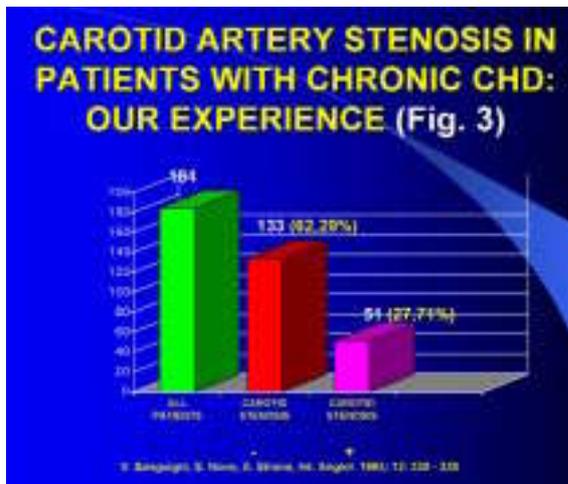


Figure 3.

**Table 1: ASYMPTOMATIC EXTRACORONARY ATHEROSCLEROSIS IN PATIENTS WITH CAD**

PARAMETERS	CAD (Case)	EXTRA CAD (Cases)	PERCENTAGE %
MONOVASCULAR CAD	82	10	12
BIVASCULAR CAD	107	23	21
TRIVASCULAR CAD	126	40	32
TOTAL CAD	315	73	23

Table 1.

(ARIC), the long-term follow-up of 15,800 subjects showed a significant association of IMT with cardiac and cerebrovascular events.<sup>19, 20</sup>

In the Rotterdam study a 2.7 year follow-up of 4,600 patients showed a positive relationship between IMT, AMI and stroke.<sup>21</sup>

The Cardiovascular Health Study demonstrated a significant and strong relationship between IMT and AMI and stroke, in a 6-year follow-up of 4,400 patients.<sup>22</sup>

In our experience, 123 patients in the GESCO-MURST-CITFI-4 study, were followed for five years. There were 42 with normal carotid arteries (Group I: mean age 56.84±3.96 years), 39 with IMT (Group II: mean age 63.18±13.21 years), and 42 with ACP (Group III: mean age 69.62±6.98 years).<sup>23</sup> The Group II patients were older than Group I patients ( $p=0.0037$ ) and Group III patients were significantly older than in Group II ones ( $p=0.007$ ).<sup>23</sup>

Patients were evaluated under the profile of traditional risk factors such as hypertension, NIDDM, hypercholesterolemia (> 200 mg%), hypertriglyceridemia (>170 mg%), smoking, obesity and family history of cardiovascular disease. IMT was defined as a thickening of more than 0.85 mm and ACP as a protrusion into the vascular lumen more than 1.5 mm. In the 5-year follow up, 21.42% of normal subjects showed a progression toward IMT, 28.57% a progression toward ACP and 11.9% died. In the group with IMT at baseline, 7.7% showed a partial regression toward normality, while 20.5% had a progression to ACP, 2.6% a non-fatal events and 20.5% died. In the group with ACP at baseline, 2.4% had a partial regression of ACP, 11.9% a non-fatal event while 45.2% died. Events were distributed thus: eight cases of fatal acute myocardial infarct (AMI), seven in patients with ACP and one

in a patient with normal carotid arteries, three non-fatal AMI in patients with ACP, five non-fatal strokes in patients with IMT, three non-fatal strokes were in patients with IMT and two in patients with ACP (Figure 1). Cardiovascular death was five times more common patients with IMT than in normal subjects (0.47 vs 2.56/year, respectively) and seven times more common in patients with ACP (0.47/years to 3.33/year) (Figure 2).

In the VHAS study<sup>6</sup> 486 patients with hypertension were treated with verapamil or clorthalidone for four years. In this study, fatal and non-fatal cardiovascular events were more frequent in patients with baseline plaques but a significant reduction was observed in the group treated with verapamil (19 versus 35,  $p < 0.01$ ).

Recently, Nicolaides *et al.* studied a very large cohort population formed of 13,221 low-risk, healthy, asymptomatic individuals in a 10-year follow-up study based on the morphology of carotid and femoral bifurcation, defined by B-mode ultrasonography.<sup>24</sup> Four classes were considered at inclusion: I with normal wall, II with IMT, III with non-stenosing plaque and IV with stenosing plaque. The incidence of cardiovascular events was the following: in class I 10/7989, in class II 81/930 (8.6%), in class III 239/611 (39.28%) and in class IV 381/470 subjects (81.06%).<sup>24</sup>

There were also 61 deaths of which 51 (5.5%) in class III or IV. So carotid stenosis account for 98.6% of cardiovascular events plus deaths in a 10-year follow-up of an apparently health population at low risk.<sup>24</sup>

### Conclusions

IMT and ACP are more frequent in males, increase with age and are influenced by the presence of risk factors, especially hypertension. The association of IMT or carotid plaque with coronary atherosclerosis and events has been clearly established. Our group,<sup>25</sup> studying 184 patients with ascertained CHD (previous AMI or effort angina) found 51 patients with both CHD and CAD (27.71%) (Figure 3), while Ciccone *et al.* in 315 patients with coronary stenosis studied with coronarangiography,<sup>26</sup> found extracoronary atherosclerosis in 23% of cases.<sup>26</sup> This percentage progressively increased from 12% in patients with monovascular disease, to 21% in patients with bivascular disease to 32% in patients with trivascular CHD (32%) (Table 1).

On the other hand, it is well established the concept that atherosclerosis is often a multifocal disease, being the expression of a unifying disease

that is atherothrombosis<sup>27</sup> and indeed multifocal atherothrombosis has been well demonstrated, in the CAPRIE study<sup>27</sup> and in the study by Aronow.<sup>28</sup> In the CAPRIE study, of 19,561 patients evaluated, 24.6% had peripheral arterial disease (PAD), 29.9% CHD, 19.2% PAD, 7.3% CVD plus CHD, 11.9% CHD plus PAD and 3.8% PAD plus CVD, while 3.3% of patients had all three areas involved.<sup>27</sup>

The study by Aronow<sup>28</sup> included 1886 patients aged > 62 years, of whom 8% had PAD, 9% CVD, and 21% CHD; however, 8% had CVD and CHD contemporaneously, 9% had CHD and PAD, 3% had PAD and CVD, and 5% had CVD, CHD and PAD.<sup>28</sup>

So, in conclusion, patients with IMT and or ACP have an important increase of total mortality as well as of cerebral and cardiac fatal and non-fatal events. So, asymptomatic carotid lesions must be considered an important risk factor for cardiovascular mortality and morbidity.

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## Vascular Risk Stratification: Practical Problems

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### Peripheral arterial disease as global vascular risk factor

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The risk factors for atherosclerotic disease of the legs are qualitatively no different from those in other districts, such as the coronary arteries and brain, hence it is no surprise that peripheral arterial disease (PAD) is very frequently associated with other cardiovascular diseases such as coronary artery disease (CAD) and cerebrovascular disease (CVD). When the first Framingham report on the incidence of intermittent claudication (IC) was published in 1970 its authors concluded that the increased risk of IC in CAD patients suggested *a common underlying basis for claudication and coronary artery disease*.<sup>1</sup>

A later report from the Framingham group<sup>2</sup> found electrocardiographic (ECG) abnormalities or clinical symptoms of CAD in 40-60% of subjects developing IC. Coronarographic findings in patients undergoing revascularization of the lower limbs indicate coronary involvement in as many as 90%.<sup>3</sup> From this viewpoint, therefore, PAD can be considered a marker of systemic atherosclerosis.

Some of the principal epidemiological studies reported the prevalence of cardiovascular diseases and it is now evident that not only patients with IC but also those with an abnormal ABI – which includes those with asymptomatic arterial disease – are more likely to have PAD plus atherosclerosis of other site(s).

The fact that atherosclerotic disease frequently occurs in more than one site helps to explain the natural history of PAD, which tends to cause limited local progression (worsening of claudication symptoms, need for amputation) but high levels of cardiovascular morbidity and mortality. This evident association between PAD and cardiovascular diseases raises the question of the prognostic implications for PAD patients, in terms of cardiovascular morbidity and mortality. As we mentioned, early observations already showed the apparently higher rate of cardiovascular mortality among patients with IC. Reunanen,<sup>4</sup> however,

questioned whether IC alone was a risk factor for increased cardiovascular mortality and, in fact, after correcting the figures for the presence of CAD, these authors found that IC no longer had any effect on mortality.

Later studies confirmed that IC found in response to a questionnaire was associated with a significant increase in the risk of cardiovascular mortality. In the Whitehall study,<sup>5</sup> in which 18,388 subjects were followed for up to 17 years, it was found that the risk of vascular mortality was three times higher for males with *probable* IC, independently of cardiovascular comorbidity. The more recent San Diego study,<sup>6</sup> with a ten-year follow-up, showed that patients with PAD diagnosed on the basis of the ABI had five times the normal risk of vascular death; excluding those who already had cardiovascular diseases at baseline, the ratio remained 4 for men and 5.7 for women. Still in the San Diego study<sup>6</sup> the prognosis for symptomatic PAD was worse.

In actual fact the role of ABI as a predictor of morbidity and mortality is now clear. Studies in the early 1990s show that it is an independent indicator of total and cardiovascular mortality.<sup>7</sup> We mentioned the San Diego study, which found a high risk of cardiovascular mortality among subjects with an abnormal ABI (<0.8).<sup>6</sup> Ogren *et al.*,<sup>8</sup> using multivariate analysis in a population investigated for carotid stenosis, ECG anomalies and presence of PAD, diagnosed on the basis of the ABI, found that after eight years of follow-up an ABI <0.9 was associated with a total mortality 2.4 times higher than normal, and a doubled risk of cardiovascular mortality.

The *Cardiovascular Health Study*<sup>9</sup> and the *Edinburgh Artery Study*,<sup>10</sup> using multivariate analysis on prospective observations of a large series (5,888 subjects in the Cardiovascular Health Study and 1,592 in the Edinburgh study), with adequate follow-up (six and five years, respectively), showed

that the risk of total and cardiovascular mortality was higher in patients with ABI <0.9, with a relative risk estimate between 1.5 and 1.8. The risk of death and non-fatal vascular events was higher in patients who had a low ABI together with risk factors such as diabetes or high blood cholesterol. The Cardiovascular Health Study established that every decrement of 0.1 in the ABI corresponded to a significant reduction in survival.<sup>11</sup> In the Italian ADEP study<sup>12</sup> a low ABI was one of the predictors of vascular events – fatal or non-fatal – in a population with IC.

While the strength of the ABI as a negative prognostic indicator seems clear, it also appears that subclinical abnormalities in the index imply a prognosis as negative as in symptomatic patients. Kornitzer in 1995<sup>13</sup> showed, after a ten-year follow-up, that an ABI <0.9 gave an odds ratio (OR) of 3.63 for cardiovascular mortality; among asymptomatic subjects aged 40-50 years LDL cholesterol had less weight as a prognostic indicator than the ABI (OR 1.69). In the Cardiovascular Health Study patients with subclinical vascular abnormalities, detected instrumentally and with the ABI, had a greater risk of developing disease than patients with no subclinical disorder.

Regardless of the presence of symptomatic PAD, which calls for local therapies, systematic screening for ABI among symptom-free subjects at cardiovascular risk is worth considering. A recent paper on the prevalence of PAD in primary care clinics is extremely interesting on this point.<sup>14</sup> The 6,979 patients screened were either elderly – 70 years or older – or aged between 50 and 69 years but smokers or diabetics. This selection was based on high-risk situations for PAD, as indicated by epidemiological findings. Patients were considered to have peripheral vascular disease if their ABI was lower than 0.90. The prevalence of PAD was 29% (1,865 patients); 44% of these only had PAD, with no atherosclerotic disease elsewhere. In 823 subjects PAD was diagnosed for the first time; 83% of the patients already diagnosed as having PAD knew about their disease, while only 49% of their doctors did. The authors conclude that under-diagnosis and doctors' lack of awareness of this frequent disease may be an obstacle to preventing cardiovascular ischemic events. This last point regards patients with PAD plus atherosclerotic disease in another district, such as the coronary arteries or cerebral circulation. Besides the data we have already considered, there is further evidence that such associations are frequent in the elderly, affecting up to 9% of men aged over 65 and 3%

of women. What is the prognosis for these patients with coronary or cerebral vascular pathologies and PAD in the legs? One might assume it is worse than when only one district is affected, and preliminary evidence does in fact confirm this. The relative risk of death at five years is significantly higher, around 1.7 in the BARI study<sup>15</sup> and around 1.25 in the CASS study.<sup>16</sup> This increase in risk in patients with coronary disease plus PAD seems to be comparable for symptomatic and asymptomatic forms.<sup>17</sup> If confirmed, this evidence might lead to more frequent screening for PAD – including asymptomatic forms – in patients with coronary and cerebrovascular disease.

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## The Overall Approach To Patients With Atherothrombosis

Chairmen: S. Coccheri, M. Mancini

### Efficacy and safety of aspirin in the long-term management of atherothrombosis

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The role of aspirin and other platelet-active drugs in the treatment and prevention of atherothrombosis has been reviewed by the Sixth ACCP Consensus Conference on Antithrombotic Therapy.<sup>1</sup> Moreover, additional information on the efficacy and safety of antiplatelet therapy is provided by the recent collaborative meta-analysis of 266 secondary prevention trials, prepared by the Antithrombotic Trialists' (ATT) Collaboration.<sup>2</sup> What follows is a brief update of these important documents.

#### *Balance of benefits and risks*

The absolute benefits of aspirin therapy substantially outweigh the absolute risks of major bleeding (particularly, gastrointestinal) complications in a variety of clinical settings characterized by moderate to high risk of occlusive vascular events (Table 1). However, in low-risk individuals the benefit/risk profile of such a preventive strategy is uncertain. Thus, a very small absolute benefit may be offset by exposure of very large numbers of healthy subjects to undue bleeding complications. The risk of upper gastrointestinal bleeding (UGIB) associated with medium-to-high doses of aspirin can be reduced to a relative risk of 2.0 vs non-users<sup>3</sup> by using the lowest effective dose of the drug (ie 75 to 160 mg daily). However, this risk can not be further reduced by other strategies since it is most likely related to the antiplatelet effect of aspirin, which is largely dose-independent for daily doses in excess of 30 mg.<sup>4</sup> Thus, recent studies have attempted to determine which groups of patients may derive particular benefit or experience harm from the use of low-dose aspirin for the primary prevention of ischemic heart disease.<sup>5,6</sup> Subgroup analysis of the Thrombosis Prevention Trial suggests that the benefit of low-dose aspirin may occur mainly in those with lower systolic blood pressures, although it is not clear even in these men that the benefit outweighs the

potential hazards.<sup>5</sup> A recently discontinued trial of low-dose aspirin in general practice failed to demonstrate a clearly favorable benefit/risk profile in men and women aged 50 years or older with one or more major cardiovascular risk factors.<sup>7</sup>

A meta-analysis of four primary prevention trials suggests that aspirin treatment is safe and worthwhile at coronary event risk equal to or greater than 1.5% per year.<sup>6</sup> The ATT Collaboration is currently conducting an overview of all randomised trials of aspirin vs placebo in low-risk subjects, based on individual patient data.

#### *Aspirin resistance*

The issue of aspirin *resistance* continues to be debated. This term has been used to describe a number of different phenomena, including the inability of aspirin to do the following: 1) to protect individuals from thrombotic complications; 2) to cause a prolongation of the bleeding time; or, 3) to produce an anticipated effect on one or more in vitro tests of platelet function.<sup>1</sup> Based on measurements of optical platelet aggregation in response to arachidonate and ADP, 5% and 24% of patients with stable cardiovascular disease who were receiving aspirin (325 mg/day for  $\geq 7$  days) were defined as *resistant* and *semiresponders*, respectively.<sup>8</sup> However, the lack of appropriate controls in this study (e.g., patients treated with another antiplatelet agent) precludes unequivocal interpretation of these findings. A recent report suggests that cyclo-oxygenase (COX)-2 expression in circulating platelets may contribute to this phenomenon.<sup>9</sup> Kawasaki *et al.* have suggested that aspirin *resistance* may be caused by an increased sensitivity of platelets to collagen.<sup>10</sup> Catella-Lawson *et al.*<sup>11</sup> have recently reported that prior administration of non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., ibuprofen) may interfere with the irreversible inactivation of platelet COX-1 by aspirin. This pharmacodynamic interac-

**Table 1. Benefit/risk ratio of antiplatelet prophylaxis with aspirin in different settings.**

Clinical setting	Benefit* Number of patients in whom a major vascular event is avoided per 1,000/year	Risk° Number of patients in whom a major GI bleeding event is caused per 1,000/year
Men at Low to High Cardiovascular Risk	1-2	1-2
Essential Hypertension	1-2	1-2
Chronic Stable Angina	10	1-2
Prior Myocardial Infarction	20	1-2
Unstable Angina	50	1-2

\*Benefits are calculated from randomized trial data reviewed in reference 1; °risks of upper GI bleeding are estimated from a background rate of 1 event per 1,000 per year in the general population of non-users and a relative risk of 2.0 to 3.0 associated with aspirin prophylaxis. Such an estimate assumes comparability of other risk factors for upper GI bleeding, such as age and concomitant use of NSAIDs, and may actually underestimate the absolute risk in an elderly population exposed to "primary" prevention. The absolute excess of major bleeding complications in the "primary" prevention trials reviewed in ref. #1 ranged between 0.3 and 1.7 per 1,000 patient-years. Reproduced from Patrono et al., Chest 2001 (ref. #1).

tion does not occur with rofecoxib, a highly selective COX-2 inhibitor.<sup>12</sup>

Thus, both the mechanism(s) and clinical relevance of aspirin *resistance* remain to be established. Until its true nature and prevalence are better defined, no test of platelet function is recommended to assess the antiplatelet effect of aspirin in the individual patient.<sup>1</sup>

#### Aspirin and reversible cyclo-oxygenase inhibitors

A variety of NSAIDs can inhibit thromboxane A<sub>2</sub> (TXA<sub>2</sub>)-dependent platelet function through competitive, reversible inhibition of platelet COX-1. In general, these drugs, when used at conventional analgesic dosage, inhibit reversibly platelet COX activity by 70 to 90%.<sup>1</sup> This level of inhibition may be insufficient to block platelet aggregation adequately *in vivo*, because of the very substantial biosynthetic capacity of human platelets to produce TXA<sub>2</sub>.<sup>13</sup> In fact, in a prospective population-based observational study of approximately 165,000 post-menopausal women, chronic use of non-aspirin NSAIDs was not associated with a protective effect against the risk of a first myocardial infarction (MI) (RR=1.32; 95% CI, 0.97-1.81).<sup>14</sup> Because non-aspirin NSAIDs have been inade-

quately investigated in terms of their potential cardiovascular effects, physicians prescribing these drugs to arthritic patients with prior vascular complications should not discontinue low-dose aspirin, even though concomitant administration of the two may amplify the risk of upper GI bleeding.<sup>1</sup>

The cardiovascular safety of selective COX-2 inhibitors (coxibs) in arthritic patients at low cardiovascular risk is currently being debated,<sup>12,15</sup> based on the recently reported results of two relatively large GI safety studies, VIGOR<sup>16</sup> and CLASS,<sup>17</sup> with short follow-up and inadequate statistical power to detect a realistic difference – one way or the other – in vascular end-points between coxibs and conventional NSAIDs. At least three possible explanations can be entertained in accounting for the statistically significant difference in MI between rofecoxib and naproxen (0.4% vs 0.1%), as reported by the VIGOR trial: 1) a cardioprotective effect of naproxen; but, there is no convincing evidence that conventional NSAIDs reduce the risk of MI at prescribed doses; moreover, it is unlikely that they inhibit platelet COX-1 by greater than 95% throughout the dosing interval; 2) a thrombogenic effect of coxibs; but, the size of the effect is not biologically plausible if due to incomplete inhibition of a single mediator of *thromboresistance*, i.e. prostacyclin (PGI<sub>2</sub>);<sup>18,19</sup> moreover, such an explanation is not substantiated by the CLASS results, though a smaller coxib effect cannot be excluded; 3) the play of chance; the apparent difference in VIGOR might represent an uneven distribution of a small number of events occurring over a short time frame in a low-risk population, as suggested by a recent meta-analysis of all rofecoxib trials.<sup>20</sup> An independent overview of all randomized comparisons between any coxib (celecoxib, rofecoxib, etoricoxib, valdecoxib and COX-189) and any non-selective NSAID appears to offer a feasible strategy to answer this question, and one that would not require a very large head-to-head randomized trial with vascular end-points.<sup>21</sup>

In conclusion, patients at moderate to high cardiovascular risk (e.g., those with chronic stable angina, prior MI or stroke/transient ischemic attacks) should be prescribed low-dose aspirin (75-100 mg daily) because its potential benefit clearly outweighs the risk of serious bleeding complications.<sup>1,2</sup> Should these patients require NSAID therapy, safety considerations as well as the lack of pharmacodynamic interactions with low-dose aspirin<sup>11</sup> would favor a specific COX-2 inhibitor over conventional NSAIDs. Patients at low cardiovascular risk (ie 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. In these patients, the absolute benefit to be derived from COX-1 sparing by specific COX-2 inhibition, in terms of reduced burden of serious GI complications *vis-à-vis* conventional NSAIDs, is likely to outweigh any potential harm to be derived from inhibition of COX-2-dependent PGI<sub>2</sub> biosynthesis.<sup>12</sup>

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## The Overall Approach To Patients With Atherothrombosis

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### Antioxidant therapy in vascular disease

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original paper

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#### *Free radicals and atherosclerosis*

In the last two decades a large body of evidence has been gathered in support of the hypothesis that oxidant stress, in particular the free radical-mediated oxidation of low density lipoprotein (LDL) (ox-LDL), plays a key role in atherogenesis.<sup>1-3</sup> Lipid peroxidation deriving from free radicals-mediated attack of polyunsaturated fatty acids and cholesterol vehicled in LDL generates a series of oxidation products which possess biological activity relevant to atherogenesis.<sup>4-7</sup> These products are responsible for oxidation of Apo B100, to be recognized by the scavenger receptor (SR), inhibit cholesterol de-loading from foam cells, are thrombogenic and cytotoxic, promote apoptosis, and lipid peroxidation.<sup>8,9</sup> Lipid peroxidation is a chain reaction and can be interrupted if free radical intermediates are intercepted by a hydrogen donor, a chain-breaking antioxidant. Antioxidants include  $\alpha$ -tocopherol, ubiquinol-10, and carotenoids which are present in the LDL particle.

The link between LDL oxidation and atherogenesis provided a rationale for testing the effectiveness of antioxidants on the development and progression of atherosclerotic disease. In this context, vitamin E, vitamin C and  $\beta$ -carotene have received the greatest attention, especially with reference to coronary artery disease.

#### *Antioxidants and atherosclerosis*

Vitamin E inhibits ox-LDL uptake by plaque resident foam cells *in vivo* in man.<sup>10</sup> The effectiveness of various antioxidant treatments was tested in several animal models of accelerated atherosclerosis. Inhibition of atherosclerotic lesion development was obtained with vitamin E in New Zealand hypercholesterolemic rabbits,<sup>11,12</sup> in Watanabe Heritable Hyperlipidemic (WHHL) rabbits,<sup>13</sup> and in Apo-E deficient mice.<sup>14</sup> The effectiveness of vitamin E supplementation on experimental athero-

sclerosis was also demonstrated in a rabbit model of restenosis after angioplasty.<sup>15</sup> Inhibition of atherosclerosis development was also obtained with synthetic antioxidants, including butylated hydroxytoluene (BHT), N,N'-diphenyl-phenylenediamine (DPPA), BO-653 and probucol.<sup>16</sup> On the other hand, probucol has been shown to enhance the development of atherosclerosis in LDL receptor-null mice and Apo E-deficient mice.<sup>16</sup>

Contrasting findings emerged from clinical trials with vitamin E. The ATBC study showed no changes in cardiovascular events during the follow-up.<sup>17</sup> The CHAOS study showed a significant reduction of cardiovascular events mainly dependent upon the reduction of non-fatal myocardial infarction.<sup>18</sup> In contrast, the GISSI-Prevenzione trial found that vitamin E did not affect the rate of non-fatal myocardial infarction, but did show a non-significant trend towards a reduction of cardiovascular death.<sup>19</sup> The HOPE study<sup>20</sup> did not achieve statistical significance for cardiovascular outcomes. In the SPACE trial, vitamin E supplementation significantly reduced the risk of a composite cardiovascular endpoint (consisting of fatal and non-fatal myocardial infarction, ischemic stroke, peripheral vascular disease, and unstable angina) and myocardial infarction.<sup>21</sup> In the Primary Prevention Project trial, the investigators did not recognize any effect produced by vitamin E on cardiovascular events.<sup>22</sup>

Contrasting findings have also been provided by arterial imaging studies of subjects treated with antioxidants. The ASAP study investigated the effect of vitamin E and vitamin C alone or in combination<sup>23</sup> on the progression of atherosclerosis, assessed by ultrasonographic evaluation of the common carotid mean intima-media thickness (IMT). The combination of vitamin E and C significantly slowed the progressive increase of IMT in men but not in women. The SECURE trial, a sub-

study of the HOPE trial, showed that vitamin E had a neutral effect on IMT.<sup>24</sup> The Multivitamins and Probuco trial evaluated the effect of probuconol, multivitamins (synthetic vitamin E, vitamin C, and  $\beta$ -carotene), or their combination on reducing the rate of angiographically assessed restenosis after balloon coronary angioplasty.<sup>25</sup> While probuconol significantly decreased the rate of restenosis, multivitamins did not have a significant effect.

#### Considerations on vitamin E supplementation

Supplemental antioxidants should improve plasma and tissue antioxidant levels and, in theory, reduce the oxidant stress. However, the natural and synthetic forms of vitamin E have different bioavailability. Synthetic vitamin E (all-*rac*-tocopherol or dl- $\alpha$ -tocopherol) contains equal amounts of eight stereoisomers of which only RRR- $\beta$ -tocopherol (d- $\beta$ -tocopherol) is present in nature. Thus, in synthetic preparations of vitamin E the RRR- $\alpha$ -isomer is only 12.5% of the total mixture. Plasma and tissue levels of vitamin E after supplementation are twice as high after intake of the natural form compared to after intake of the synthetic form.<sup>16</sup> In agreement, we have demonstrated that a 29% increase in vitamin E plasma concentration following all-*rac*- $\alpha$ -tocopherol supplementation is not sufficient to raise plasma antioxidant activity in healthy subjects.<sup>26</sup> Two mechanisms could account for this effect, the affinity for  $\alpha$ -tocopherol binding protein ( $\alpha$ -TBP), which regulates cellular uptake and intracellular translocation of  $\alpha$ -tocopherol, and hepatic catabolism of vitamin E to tetramethylhydroxychroman.  $\alpha$ -TBP has high ligand specificity for RRR- $\alpha$ -tocopherol and low affinity for non-natural stereoisomers,<sup>16</sup> compared with natural vitamin E, the synthetic form is preferentially metabolized to carboxyethylhydroxychroman.<sup>16</sup> Taken together, clinical trials with vitamin E are difficult to interpret and compare because of the different dosages and sources of supplementation used and the lack of relationship between daily dosage and plasma concentration of vitamin E.<sup>27</sup> In addition, bioavailability of vitamin E is strongly influenced by food intake, with very poor bioavailability if it is not taken during meals.<sup>28</sup> Thus, all trials that do not contain adequate information on bioavailability and related antioxidant activity of vitamin E should be very cautiously interpreted.

A recent study demonstrated that vitamin E supplementation had no effect on lipid peroxidation in healthy subjects despite a dose-dependent increase

of plasma vitamin E levels,<sup>29</sup> making a point of selecting patients with high oxidant stress as the targets for antioxidants. Measurement of lipid peroxidation markers, such as isoprostanes and oxysterols, represents a novel approach for studying oxidant stress *in vivo*. Both isoprostanes and oxysterols have been found in human atherosclerotic plaques and could, therefore, represent useful tools for assessing the relationship between oxidative stress and atherosclerosis progression and may be helpful for identifying of patients who could benefit from antioxidant treatment.

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## The Overall Approach To Patients With Atherothrombosis

Chairmen: S. Coccheri, M. Mancini

### The long-term use of blockers of the platelet ADP receptor in acute coronary syndromes

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Acute coronary syndromes comprise three different nosological entities: Q-wave acute myocardial infarction,<sup>1</sup> non-Q wave acute myocardial infarction<sup>2</sup> and unstable angina.<sup>3,4</sup> These entities have a largely common pathogenetic background, consisting of the fissuring or superficial erosion of an atherosclerotic plaque with subsequent platelet aggregation, the activation of coagulation, and thrombus formation.<sup>5</sup> The presence of a thrombus on a complicated atherosclerotic plaque is accompanied by high levels of systemic markers of hemostatic activation. Such markers are elevated not only in the acute phase, but for many months thereafter.<sup>6-8</sup> This observation is in strict agreement with the elevated levels of cardiovascular events characterizing the first months after the acute episode.<sup>9-15</sup> This has been recently confirmed in the Prospective Registry of Acute Ischaemic Syndromes in the UK (PRAIS-UK).<sup>16</sup> In this study, with a 6-month follow-up in patients with acute coronary syndromes without ST elevation, the incidence of new coronary events has been around 30%. Mechanisms responsible for the persistent activation of the hemostatic system are not yet fully elucidated. The hypothesis has been raised that ongoing platelet and coagulation activation are independent of the underlying atherosclerotic process, but rather are due to molecular alterations of the hemostatic mechanisms acting on circulating blood or at the level of the vessel wall.<sup>7</sup> It is also possible that the increased hemostatic activation may determine a kind of *vascular sensitization*, with subsequent increased responsiveness to relatively minor thrombogenic stimuli, thus responsible for new thrombotic events.<sup>17</sup> The above-mentioned observations and hypotheses are the rational background for the use of drugs with anticoagulant and antiplatelet action also in the period subsequent to the stabi-

lization of an acute event, and are the area with major recent advances in these last years.<sup>18</sup>

#### ADP receptor blockers

The efficacy of aspirin in secondary prevention of myocardial infarction and death is well known.<sup>19</sup> However, the clinical evidence of the resilient incidence of coronary mortality and morbidity despite the use of aspirin has suggested the need for more potent antiplatelet strategies. Many studies have been recently conducted with inhibitors of glycoprotein IIb-IIIa, the final common pathway of platelet aggregation. These have been proven effective in the setting of percutaneous coronary interventions.<sup>20</sup> However perplexities remain over their use in a purely medical setting.<sup>21</sup> Results in ST elevation myocardial infarction in conjunction with thrombolytics are still under discussion.<sup>22</sup> The use of these drugs will, however, always remain limited to the acute phase. The long-term use of oral GP IIb-IIIa inhibitors has, indeed, been largely disappointing.<sup>23-26</sup>

Thienopyridines (ticlopidine and clopidogrel) are – contrariwise – the most logical current approach to the long-term treatment of unstable coronary syndromes (and possibly all acute coronary syndromes). Thienopyridines inhibit one of the ADP receptors linked to decreased intraplatelet levels of cyclic AMP, thereby preventing ADP-mediated platelet activation. In the Clopidogrel versus Aspirin at Risk of Ischaemic Events (CAPRIE) study, a significant (albeit modest) reduction of thrombotic events was seen in patients with vascular atherosclerotic disease treated with clopidogrel vs aspirin. The benefit appeared particularly evident in the group of patients recruited with peripheral vascular disease. In any case, the drug has proven to be safe and well tolerated,<sup>27</sup> thus yielding the first real alternative to aspirin as an antiplatelet

agent supported by evidence of efficacy. Despite this, many investigators and physicians would not substitute aspirin for clopidogrel in most patients because of cost concerns. Since however clopidogrel and aspirin act with a different mechanism, an additive effect can be postulated. This has been shown in a number of small trials documenting the superior efficacy of aspirin plus a thienopyridine (ticlopidine mostly) in the prevention of stent restenosis. With this background, the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) study has been launched in unstable coronary syndromes.<sup>28</sup> In CURE, 12,562 patients who had presented within 24 hours after the onset of symptoms were randomly assigned to receive clopidogrel (300 mg immediately, followed by 75 mg once daily) (6,259 patients) or placebo (6,303 patients) 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 percent of the patients in the clopidogrel group and 11.4 percent of the patients in the placebo group (relative risk with clopidogrel as compared with placebo, 0.80; 95 % confidence interval, 0.72 to 0.90;  $p < 0.001$ ). The second primary outcome — the first primary outcome or refractory ischemia — occurred in 16.5 percent of the patients in the clopidogrel group and 18.8 percent of the patients in the placebo group (relative risk, 0.86,  $p < 0.001$ ). The percentages of patients with in-hospital refractory or severe ischemia, heart failure, and revascularization procedures were also significantly lower with clopidogrel. The benefit was remarkably consistent throughout many pre-specified subgroups, including, notably, people receiving a percutaneous coronary intervention after the randomization — despite having been nearly all assigned to an open-label thienopyridine for at least four weeks after the procedure.<sup>29</sup> There were significantly more patients with major bleeding in the clopidogrel group than in the placebo group (3.7 percent vs 2.7 percent; relative risk, 1.38;  $p = 0.001$ ), but there were not significantly more patients with episodes of life-threatening bleeding (2.1 percent vs 1.8 percent,  $p = 0.13$ ) or hemorrhagic strokes.

In conclusion, the antiplatelet agent clopidogrel has beneficial effects in patients with acute coronary syndromes without ST-segment elevation. However, the risk of major bleeding is increased among patients treated with clopidogrel.

The CURE was a landmark study in the long-term treatment of coronary patients. The results of the

CURE study have paved the road for new studies, currently planned or already running, in ST-elevation myocardial infarction, following angioplasty, to prevent stroke or in atrial fibrillation. In unstable coronary syndromes, in which the use of clopidogrel in addition to aspirin is likely to become standard practice soon, open questions remain about the optimal duration of therapy. Some concern remains about the risk of major bleeding. This increased risk is likely constant throughout the duration of clopidogrel plus aspirin administration, while the benefit is mostly a function of the decreased risk of thrombotic events, which clusters early on after the acute phase. The optimal window for the combined treatment could, therefore, undergo further refinement in the future.

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**Genetic polymorphisms of clotting factors and coronary heart disease**

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Significant progress has been made during the last thirty years in the acute treatment of coronary heart disease (CHD), but preventive cardiology still has much to accomplish. CHD has a complex multifactorial etiology in which the traditional environmental risk factors (age, dyslipidemia, diabetes, hypertension, smoking and many others) are now considered to be interconnected with genetic factors; indeed, clustering of CHD in families and genetic modulation of the blood levels of intermediate phenotypes (e.g. factors of the lipid, clotting, and blood pressure systems) have been observed.

Common variations (or polymorphisms) in many different genes could be casually linked to inter-individual differences in the risk of diseases, through quantitative variations of biochemical and physiological traits that span through the normal range of phenotypic variability.<sup>1</sup> Genetic variation, moreover, might combine with many different environmental exposures to determine an individual's level of resistance or susceptibility to develop a given disease. In the last 10 years, many genetic studies of CHD have focused on the identification and characterization of those genetic loci contributing to variation of the intermediate traits that are involved in the etiology of disease.

*Clotting/platelet factors and their genetic polymorphisms*

Genetic variability regulating the activity and/or the levels of related molecules in blood, also applies to coagulation factors; these are key proteins in the pathogenesis of thrombosis and some have been identified as risk factors for CHD.<sup>2</sup>

The coagulation process is a "cascade" of events that leads, through amplification of enzymatic reactions, to the formation of thrombin. The "cascade" is started by the prompt binding of circulating factor VII to tissue factor locally exposed or

produced by damaged endothelial cells and/or infiltrating macrophages, as occurs at the level of an atheromatous plaque. The factor VII-tissue factor complex activates factor X to factor Xa; the latter, in turn, in the presence of factor V continues the cascade, by activating prothrombin to thrombin, the specific enzyme that eventually converts fibrinogen into insoluble fibrin.

Common polymorphisms of coagulation factor VII gene, associated with relatively low blood levels of factor VII, are protective against myocardial infarction.<sup>3</sup> The presence of one of the protective alleles reduces the risk of familial and juvenile myocardial infarction in the Italian population by about 50%. The same polymorphisms have recently been shown to protect against myocardial infarction in Italian patients with a high degree of coronary atherosclerosis.<sup>4</sup>

In a study in young women, certain polymorphisms of the factor V gene increased the risk of myocardial infarction.<sup>5</sup> A rare genetic variant of fibrinogen  $\beta$ -chain, associated with high levels of circulating fibrinogen, increases the risk of familial myocardial infarction and peripheral arterial disease.<sup>6,7</sup>

More recently, factor XII, a clotting factor considered important in the traditionally defined "intrinsic" activation of clotting, has also been evaluated among emerging risk parameters in the Second Northwick Park Heart Study. Plasma FXIIa was increased in middle aged men at high risk of CHD and had a genetic determinant in a FXII gene polymorphism (the C46T variant).<sup>8</sup>

Contrasting results on protective or risk-producing associations have been found in different clinical settings and populations and when using different study designs,<sup>9,10</sup> thus raising the question of whether different environmental exposure to the risk could contribute to the variable expression of a genetic risk.

At variance to the rare "strong" genetic mutations, polymorphisms make only a relatively small contribution to the overall risk of developing a multifactorial disease. Moreover, the contribution of polymorphisms to the risk of disease is often limited to specific subgroups.<sup>11,12</sup> In the case of CHD, the global risk might result from a complex interaction between environmental and genetic factors. Indeed, rather than influencing basal levels of proteins, the presence of polymorphisms may increase or decrease the susceptibility to disease by modulating the response to environmental factors, such as diet, smoking, physical activity, and exposure to infectious agents. As an example, the presence of the Q353 allele of coagulation factor VII gene modulates the risk for CHD mainly by decreasing the detrimental effect of smoking.<sup>13</sup> Like air-bags in a car, "protective" factors appear not to be necessary if the driving style is safe and no accident occurs. In contrast, they show their life-saving effect in the case of hazardous driving leading to a crash.

In an opposite direction, factor V genetic polymorphisms over-express the detrimental action of major cardiovascular risk factors, such as smoking, hypertension, diabetes mellitus or obesity.<sup>5</sup>

Similarly, the B2 allele of the BclI polymorphism of fibrinogen has been shown to increase the risk of cardiovascular disease related to seropositivity for *Helicobacter pylori*.<sup>14</sup> The latter infection increases fibrinogen levels and, consequently, the risk of myocardial infarction. The presence of the B2 polymorphism has an additive effect on both fibrinogen levels and the risk of myocardial infarction in patients seropositive for *Helicobacter pylori*. In contrast, in the absence of *Helicobacter pylori* infection, B2 polymorphism seems to have little, if any, effect on the risk of CHD.

Furthermore, evidence could be presented that genetic polymorphisms potentiate the effect of environmental factors on quantitative variations of biochemical and physiologic traits. Factor VII polymorphisms appear to influence the positive correlation of plasma factor VII with triglyceride levels in carriers of the R allele-variants but not of the Q allele-variants.<sup>15</sup> These findings suggest that subjects carrying the Q allele-variant of factor VII are protected from the activation of factor VII in response to dietary fat intake. Thus, the risk attributable to diet could differ in populations with different prevalences of the Q allele.

As already mentioned, the influence of genetic polymorphisms on the risk of CHD may be particularly expressed in some subgroups of patients and not in others. This is highlighted, as an example, by

our recent experience with the polymorphism PIA1/PIA2 of the gene encoding platelet glycoprotein IIIa, a component of the platelet receptor IIb IIIa, a major mediator of platelet aggregation responses. The common polymorphism PIA1 /PIA2 has been variably associated with vascular disease. To clarify its role in coronary risk, a meta-analysis of published data was conducted. A total of 34 studies on coronary artery disease (CAD), and 6 on re-stenosis after revascularization were identified, for a total of 9,095 cases and 12,508 controls. In CAD, the overall odds ratio for carriers of the PIA2 allele was 1.10 (95% CI: 1.03 to 1.18), and it was 1.21 (95% CI: 1.05 to 1.38) in subjects younger than 60. The overall odds ratio was 1.31 (95% CI: 1.10 to 1.56) after revascularization procedures. Therefore, the association of PIA2 status with overall cardiovascular disease in the general population was significant but weak; a higher risk has been identified in less heterogeneous subgroups, such as younger cohorts and the subset of re-stenosis with stents.<sup>12</sup>

### Conclusions

The study of genetics will modify our approach to common diseases since it opens up the possibility of defining risk categories that could benefit from individualized strategies of prevention and therapy. Thanks to genetic developments we can now understand some of heterogeneity in the expression of clinical phenotypes and in the variability of individual responses to environmental risk factors such as diet, smoking or dysmetabolic conditions. Following the examples derived from the coagulation system, efforts should be made to consider risk factors for cardiovascular disease always in a context of gene-environment interactions.

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**Platelets and oxygen radicals:  
mechanisms of functional modulation**

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**A**ntioxidant therapy, which has been used in large clinical trials, is becoming an attractive treatment in preventing cardiovascular complications secondary to atherosclerotic processes. The rationale for such a therapy is based on the assumption that oxidant stress plays a pivotal role in the progress of atherosclerotic lesions.

The oxygen radicals (ROS), whose action is reduced by antioxidant substances can be produced within the vascular system by myocytes, endothelial cells and mainly by neutrophils.<sup>1</sup>

The first study on platelets demonstrating that it is possible to detect superoxide radicals in such cells was published in 1977.<sup>2</sup> Furthermore, in this paper, Marcus *et al.* demonstrated that the quantity of superoxide generated by platelets was constant and did not increase after aggregation by agents such as collagen and thrombin.<sup>2</sup> Only later, was it demonstrated that platelets are able to produce hydrogen peroxide after stimulation with zymosan or latex particles,<sup>3</sup> with arachidonic acid, calcium ionophore A23187, phorbol-myristate-acetate (PMA) and thrombin<sup>4</sup> or with collagen.<sup>5</sup>

The enzymatic pathway able to generate oxygen radicals in human platelets is not completely clear. Of the various potential sources of oxygen radical generation in platelets the involvement of NADH/NADPH oxidase seems to be the most important for two reasons: 1) the enzymatic activity of NAD(P)H-cytochrome C reductase is present in platelets;<sup>4</sup> 2) its inhibitor, diphenylene iodonium (DPI), suppressed platelet aggregation induced by ADP and thrombin<sup>6</sup> and by anoxia-reoxygenation.<sup>7</sup> Moreover, it has recently been demonstrated that platelets express p22phox and p67phox proteins, components of NADH/NADPH oxidase.<sup>8</sup> As these authors had also seen that O<sub>2</sub><sup>-</sup> production by the calcium ionophore A23187 and by 12-tetradecanoyl-phorbol-13-acetate (TPA) is inhibited by DPI, they suggested that the p22phox-based

NADH/NADPH oxidase system is one of the important sources of ROS in platelets.

In vascular cells another potential source of oxygen radical generation is xanthine oxidase.<sup>1</sup> Although there is no evidence of any activation of this enzyme by platelet agonists, and despite the absence of evidence that platelet activity is modified by specific inhibitors of xanthine oxidase, it was reported that the platelet activity of xanthine oxidase is elevated in unstable angina, thus demonstrating that this system could also operate in platelets.<sup>9</sup>

The principal mechanisms that regulate NADH/NADPH oxidase in platelets seem to depend on arachidonic acid metabolism, as was first observed in macrophages;<sup>10</sup> in fact, collagen and N-ethyl-maleimide (NEM) use arachidonic acid metabolism in inducing production of O<sub>2</sub><sup>-</sup>.<sup>11,12</sup> It was suggested that protein kinase C activation may also have a role in mediating O<sub>2</sub><sup>-</sup> generation, but it has recently been demonstrated that this activation is not essential.<sup>8</sup>

Several pieces of evidence lead us to assume that oxygen radical formation is important for platelet activation: in fact, reducing the cytosolic concentration of hydrogen peroxide by means of catalase, the aggregation induced by collagen was inhibited;<sup>5</sup> moreover, diphenylene iodonium, an NADPH-oxidase inhibitor that prevents the generation of oxygen-derived free radicals, reduced thrombin- and ADP-induced platelet aggregation,<sup>6</sup> and superoxide dismutase (SOD) triggered the aggregation of platelets exposed to subthreshold concentrations of arachidonic acid and collagen.<sup>13</sup> The production of oxygen radicals seemed to be important during the initial phase of platelet activation induced by different agonists.<sup>14,15</sup> Confirming that oxygen radicals can activate early events in platelet activation, it was shown that the production of oxygen radicals through a mechanism

of anoxia-reoxygenation can induce a sharp aggregation of platelets.<sup>7</sup>

Many data suggest that oxygen free radicals play a more important role in platelet aggregation induced by collagen than by other agonists.<sup>5,13,15,16</sup>

Collagen has been identified as the main thrombogenic macromolecule present in the extracellular matrix underlying the subendothelium<sup>17</sup> as it provides an important site for platelet adhesion.<sup>18</sup> Besides supporting adhesion, collagen also induces platelet activation, thromboxane A<sub>2</sub> production and granule secretion, leading to platelet aggregation and subsequently to thrombus formation. All of these mechanisms seem to be amplified by the production of oxygen radicals. In fact catalase, a scavenger of H<sub>2</sub>O<sub>2</sub>, reduces all the biochemical pathways induced in platelets by collagen, either treated with aspirin or not.<sup>16</sup> It has not been checked yet whether this mechanism accounts for superoxide anion-dependent amplification of platelet activation. However, inhibition of protein tyrosine phosphatases induced by oxygen radicals, demonstrated by several authors, would account for the difference seen between platelet activation by collagen and by other agonists. In fact, collagen signal transduction occurs through a tyrosine kinase-dependent activation of phospholipase C $\gamma$ 2,<sup>19,20</sup> an enzyme that is inhibited by protein tyrosine phosphatases. Moreover, catalase had no effect on platelet activation by G protein-coupled agonists,<sup>16</sup> such as ADP and thrombin, that activate platelets by a mechanism different from that of tyrosine kinase activation.<sup>21,22</sup> The inhibition of protein phosphatases by oxygen radicals might also be the mechanism responsible for platelet activation induced by anoxia-reoxygenation which is dependent on arachidonic acid production after activation of phospholipase A<sub>2</sub>,<sup>7</sup> a MAP kinase dependent process.<sup>23,24</sup> This mechanism has already been demonstrated in other cell lines.<sup>25,26</sup> Therefore, it seems that one of the main biochemical pathways activated by oxygen radicals is arachidonic acid production but, as reported above, there is also evidence that arachidonic acid produces oxygen radicals. For this reason it is possible to hypothesize that oxygen radicals have their own cycle of auto-amplification, similar to the other well-known amplification cycles operating in platelets.

The fact that oxygen radicals have a role in platelet activation has also been indirectly assessed in an *ex vivo* study using antioxidants. Antioxidant molecules can interfere with platelet function mainly by inhibiting collagen-induced platelet activation.<sup>27</sup> The most used antioxidant molecule in *ex*

*vivo* studies is vitamin E.

Studies in humans investigated the capacity of vitamin E to interfere with platelet function. Davi *et al.*<sup>28</sup> showed that, at a daily dosage of 100-600 mg, vitamin E significantly decreased the urinary excretion of 11-dehydro-thromboxane B<sub>2</sub>, a marker of *in vivo* platelet activation. Similar results were obtained in healthy volunteers in whom vitamin E administered at a daily dosage of 600 mg significantly inhibited collagen-induced platelet activation and collagen-induced platelet H<sub>2</sub>O<sub>2</sub> production.<sup>29</sup> Moreover, an oral supplementation of 400-600 IU/ die of vitamin E induces an antiadhesive effect in platelets.<sup>30,31</sup>

Vitamin E has the same effect as catalase in that it reduces H<sub>2</sub>O<sub>2</sub> production by activated platelets.<sup>29</sup> Another site of action proposed for vitamin E is protein kinase C (PKC),<sup>32</sup> but this is difficult to evaluate given that the vitamin E concentration used in *ex vivo* study cannot easily be reached in human plasma; moreover, vitamin E has most effect on collagen-induced platelet response, which seems to be linked to its antioxidant property rather than to PKC inhibition for two main reasons; 1) vitamin E reduces platelet adhesion to collagen, a process which does not require PKC activation;<sup>19</sup> 2) vitamin E reduces both phospholipase C activation and calcium mobilization induced by collagen,<sup>29</sup> two biochemical events which precede PKC activation.

For all of these reasons it was suggested that a combination of aspirin with vitamin E could be useful for the treatment of atherosclerosis progression in patients with cardiovascular disease. However, interventional trials with vitamin E provided conflicting results. In fact, of the three trials with vitamin E plus aspirin, only the CHAOS study<sup>33</sup> demonstrated a beneficial effect of such a combination, whereas the GISSI<sup>34</sup> and the HOPE<sup>35</sup> studies did not confirm this effect. Unfortunately, neither the GISSI study nor the HOPE study provided information on the plasma levels of vitamin E achieved after oral supplementation, whereas the CHAOS study showed plasma values of vitamin E close to 50  $\mu$ M. This is a very important point as it has been demonstrated that vitamin E absorption is extremely difficult and that this difficulty is increased when the synthetic form of the vitamin is used.

To confirm the importance of a natural way of vitamin E assumption, there are studies which take into consideration particular lifestyles, especially those concerning Mediterranean diet.

In 1990 it was reported that the incidence of myocardial infarction in French people, despite them having the same percentage of smokers and the

same dietary fat content as Americans, is one third less than in the USA;<sup>36</sup> this phenomenon has been called the *French Paradox*. Further epidemiological studies have stressed that there is an inverse relationship between moderate consumption of wine and the incidence of cardiovascular diseases.<sup>37,38</sup>

It was later demonstrated in an *ex vivo* study that moderate consumption of red wine reduced platelet aggregation in whole blood, while white wine did not have any effect.<sup>39</sup> Moreover, it was demonstrated that collagen-induced platelet aggregation could be inhibited by drinking 750 mL grape juice for 10 days, whereas orange juice or grape-fruit juice had no effect.<sup>40</sup>

The ability of red wine and grape juice to reduce the incidence of coronary heart diseases seems to be due to the presence of polyphenols, in which red wine is richer than white wine. In the past, different studies have shown that some polyphenols present in red wine, particularly the flavonoids, inhibit platelet aggregation induced by different agonists; such a reduction seems to be more evident in collagen-activated platelets. Yet, in all these studies the lowest concentrations of polyphenols that were able to inhibit platelet activation were higher than those that can be found in the plasma after moderate consumption of wine.<sup>41</sup>

However, it was recently demonstrated that two flavonoids present in red wine, catechin and quercetin, synergistically inhibit *in vitro* collagen platelet activation, at concentrations that can be found in the plasma after a moderate consumption of red wine.<sup>42</sup>

In conclusion, the *in vivo* studies seem to indicate that of flavonoids, present in the Mediterranean diet, have a role in preventing cardiovascular disease, while the utility of supplementation with antioxidant molecules such as vitamin E deserves further studies. However, the *in vitro* studies clearly demonstrated that oxygen radical production is an important step for platelet activation, although the exact mechanisms regulating such activity and the way they are produced require further investigation.

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**Inhibition of cholesterol biosynthesis as a new antithrombotic strategy**

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Recent studies have identified the role of lipids and lipoproteins in relation with the initiation and progression of atherosclerotic disease. On the other hand, epidemiological studies have shown the cholesterol levels in plasma are an independent risk factor for coronary disease. At present data are available, which indicate that aggressive hypolipidemic strategies, aimed at reducing plasma low density cholesterol and triglyceride levels and/or at increasing the levels of high density lipoprotein (HDL) cholesterol result in the reduction in the incidence of cardiovascular events.

Lipids and lipoproteins are known to affect a variety of functional aspects of blood cells and of cells of the vascular wall, which the formation of atheroma as well as the development of a proinflammatory-prothrombotic phenotype. Elevated levels of cholesterol in plasma influence functional and biochemical features of vascular endothelium with resulting impairment of its vasodilatory properties. In addition, elevated levels of cholesterol in plasma may induce platelet hyperreactivity as well as they may increase the biosynthesis of proaggregatory mediators by platelets.

Moreover cholesterol accumulation in monocytes/macrophages increases the expression of tissue factor (TF), which in turn contributes to vessel wall thrombogenicity. Indeed, elevated levels of the prothrombin fragment 1+2, an *in vivo* marker of thrombin generation have been found in plasma of patients with type IIa hypercholesterolemia. At present a variety of drugs with hypocholesterolemic activity are available. In particular, inhibitors of HMGCoA reductase enzyme have

been recently proven to be effective in reducing plasma cholesterol levels, even if at a different extent according to the compound considered. Moreover, these drugs have been shown to reduce the incidence of cardiovascular events both in primary and secondary prevention studies. It has been proposed that these drugs may have direct antithrombotic effects. They reduce platelet adhesion to vascular endothelium, platelet aggregation, urinary excretion of thromboxane metabolites and the expression of tissue factor by monocytes/macrophages. In addition it has been shown, at least *in vitro*, that statins ameliorate the fibrinolytic potential through the increase in the secretion of tissue type plasminogen activator (tPA) and the concomitant decrease in the secretion of its putative inhibitor, PAI-1.

Overall these activities may be dependent on the capacity of statins to reduce plasma cholesterol levels. On the other hand it has been proposed that statins may directly influence several aspects involved in the formation of thrombi with mechanism, which are not dependent on their effects on cholesterol biosynthesis. In particular the capacity of these drugs to interfere with the biosynthesis of isoprenoids, which are involved in complex biochemical pathways relevant in cell functions, is presently under investigation.

## Planning Antithrombotic Therapy For Patients With Atherothrombosis

Chairmen: G. Gensini, F. Crea

### Antiaggregant therapy and/or anticoagulant therapy in the cerebrovascular patient

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In Western countries stroke is the most common life threatening neurologic disease, the third leading cause of death after heart disease and cancer, and the leading cause of adult disability. The accepted pathogenic mechanism of cerebral ischemia are cardiac embolism, atherothrombosis of precerebral and cerebral arteries, nonatherosclerotic vasculopathies, and hematologic disorders. Vasospasm and hemodynamic factors may also be involved. The optimal therapy for stroke prevention is guided by the specific pathogenesis.

#### Anticoagulants

Atrial fibrillation is a common arrhythmia, affecting 2-5% of the general population over the age of 60. It can be found in about 15% of all stroke patients and in about 2-8% of patients with previous transient ischemic attacks (TIA). Incidence of ischemic stroke in patients with nonvalvular atrial fibrillation, ranges between 2-5% per year. Following an initial stroke, the stroke recurrence rate varies in different studies between 2-15% in the first year, and is about 5% yearly, thereafter. Other high risk sources of cardiogenic embolism include mitral stenosis, mechanical prosthetic valves, recent myocardial infarction, left ventricular mural thrombi, atrial mixoma, dilated cardiomyopathies, infective endocarditis, marantic endocarditis, patent foramen ovale, atrial septal aneurysm, aortic arch atheroma, and mitral valvular strands. Nevertheless, the cause of 30% to 40% of all ischemic strokes remains undetermined and cardiac mechanisms are suspected to account for a substantial percentage of all cryptogenic strokes.

Five randomised trials evaluated the role of anticoagulation for the primary prevention of stroke in patients with nonvalvular atrial fibrillation.<sup>1-5</sup> In four trials (AFASAK, SPAF, CAFA, and SPINAF),<sup>1-4</sup> moderate oral anticoagulation was compared with placebo whereas in a fifth trial (BAATAF)<sup>5</sup> oral anti-

coagulants were compared with aspirin. All trials were stopped early because anticoagulant treatment was clearly indicated in terms of a net difference in the primary outcome measures. Warfarin may reduce the risk of stroke by about two-thirds with an acceptable risk of bleeding.<sup>6</sup> The annual rate of total bleedings was less than 2% in patients treated with oral anticoagulants.

In two primary prevention trials (AFASAK; SPAF)<sup>1,2</sup> aspirin, at the dose of 75 and 325 mg/day, and placebo were compared in patients with chronic atrial fibrillation. A statistically significant risk reduction was found in about 25% of patients treated with aspirin. The risk of stroke or systemic emboli was reduced from 6.3 to 3.6% per year. The relative efficacy and safety of aspirin versus warfarin remained unresolved. SPAF-II trial compared warfarin, with an INR from 2.0 to 4.5 with aspirin 325 mg/day for the primary prevention of ischemic stroke and systemic embolism. The reduction in absolute rates of primary events by warfarin compared with aspirin were 2% and 4% per year, respectively.

The EAFT7 evaluated the role of anticoagulants (INR 2.5-4.0) in patients with TIA or minor stroke. In those patients the annual rate of any outcome event was 17% in patients given placebo and 8% in patients treated with oral anticoagulants. A 47% reduction of the risk (95% CI: 21-64%) was obtained. The annual rate of new strokes alone was reduced from 12% with placebo to 4% with oral anticoagulation. The annual risk of major bleedings was 2.8% with anticoagulation and 0.7% with placebo.

Antiplatelet therapy may represent an alternative for secondary prevention of stroke and other serious vascular events in patients with atrial fibrillation. The comparison of aspirin and placebo treated patients in the combined groups (anticoagulation eligible versus ineligible) showed an

event rate of 19% in the placebo group and of 15% in the aspirin treated patients. Moreover, the high stroke rate in the EAFT was consistent with the observation from other randomised trials, that prior stroke or TIA was an important predictor of increased stroke risk in patients with atrial fibrillation.<sup>7</sup>

No data from large, well designed, randomised trials were available to adequately assess the efficacy of oral anticoagulants for secondary prevention of noncardioembolic stroke. The only large randomised trial currently available<sup>8</sup> compared high intensity oral anticoagulation (INR 3.0 to 4.0) with aspirin (30 mg/day). This study was stopped prematurely because of an excess of major bleeding complications in the anticoagulant group. For this reason the comparative efficacy of anticoagulation versus aspirin for prevention of cerebral ischemia could not be determined. In summary, there is enough evidence that oral anticoagulant therapy is highly effective for primary and secondary prevention of stroke in patients with atrial fibrillation.

Most of the major bleeding complications occur with an INR of 5.0 or above.<sup>9,10</sup> Actual and targeted INRs varied between 1.5 and 4.5 in the different trials. The optimal intensity of anticoagulant therapy is 2.0 to 3.0 in patients with non-rheumatic atrial fibrillation and 2.5 to 3.5 in patients with mechanical heart valves.<sup>9,10</sup>

#### *Antiplatelet drugs*

Antiplatelet agents have gained a definite role in the secondary prevention of atherothrombotic TIA and stroke. In the late seventies, both the American and the Canadian Studies,<sup>11,12</sup> showed the clinical efficacy of high dose aspirin in patients with previous atherothrombotic TIA or minor stroke. The same therapeutic approach provided weak or no result in asymptomatic patients with carotid bruits, embolic heart disease, or peripheral arterial disease.<sup>13</sup>

The most effective dose of aspirin has been widely debated. After the early administration of 1300 mg daily, doses between 30 and 300 mg daily were tested, mainly to avoid gastrointestinal toxicity, and to inhibit platelet thromboxane A<sub>2</sub> more than the endothelial prostacyclin synthesis.<sup>11,12,14</sup> The UK-TIA Aspirin Trial<sup>14</sup> compared the efficacy of 1200 mg versus 300 mg aspirin daily or placebo, in preventing completed stroke, myocardial infarction, and death in patients with previous TIA or minor stroke. The incidence of new vascular events was reduced by 15% in the group taking aspirin

1200 or 300 mg daily with respect to the placebo group. Completed stroke and cerebral death were only 7% less in treated patients. Both the 1200 and 300 mg daily aspirin treatments provided the same results, except for a lower gastrointestinal toxicity of the lower dose. The Dutch TIA Trial Study Group<sup>15</sup> compared the efficacy of aspirin 30 mg with 283 mg daily. The lower dose was no less effective in the prevention of vascular events than the higher dose and had fewer adverse effects. The Swedish Aspirin Low-Dose Trial (SALT)<sup>16</sup> showed a 18% reduction in the risk of primary outcome events such as stroke or death, and a reduction of 16-20% in the risk of secondary outcome events such as stroke, two or more TIAs within a week, or myocardial infarction, in patients treated with aspirin 75 mg daily with respect to placebo.

The ASA and Carotid Endarterectomy trial (ACE)<sup>17</sup> compared low (81 mg/day or 325 mg/day) and high (650 mg/day) or 1300 mg/day aspirin doses in patients undergoing carotid endarterectomy. The ACE results lend further direct support to the premise that low dose aspirin was at least as effective as the high dose.

Ticlopidine was the drug of choice in patients with aspirin intolerance. The Canadian American Ticlopidine Study (CATS)<sup>18</sup> compared ticlopidine 500 mg daily with placebo, showing a 30% reduction in the risk of stroke, myocardial infarction, and vascular death in the treated group. The Ticlopidine Aspirin Stroke Study (TASS)<sup>19</sup> evaluated the efficacy of ticlopidine 500 mg daily with aspirin 1300 mg daily in patients with TIA or minor stroke. Ticlopidine was slightly more effective than aspirin, with a 21% reduction of the risk of fatal and non-fatal stroke, although adverse events such as neutropenia and diarrhea were more frequent.

Clopidogrel is a thienopyridine derivative as ticlopidine. Its antithrombotic effects were evaluated in the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Event (CAPRIE)<sup>20</sup> study. CAPRIE was a randomised, blinded, multicenter trial designed to assess the relative efficacy of clopidogrel (75 mg/day) and aspirin (325 mg/day) in reducing the risk of the composite outcome of ischemic stroke, myocardial infarction or vascular death, and to determine their relative safety. The CAPRIE study indicated that clopidogrel was more effective than aspirin in reducing the combined risk of ischemic stroke, myocardial infarction or vascular death in patients with atherosclerotic vascular disease. The beneficial effects of clopidogrel for the combined vascular end-point seem to be comparable to the effects of ticlopidine, without

the negative adverse effects profile.

The European Stroke Prevention Study-II (ESPS-II),<sup>21</sup> studied patients who had experienced either an ischemic stroke or TIA in a multicenter, randomised, blinded, factorial, placebo-controlled study with four treatment groups. The four twice-daily treatments were as follows: aspirin 25 mg; extended-release dipyridamole, 200 mg; aspirin 25 mg plus extended-release dipyridamole, 200 mg; and placebo. The study showed that both extended-release dipyridamole and aspirin had an independent and statistically significant effect in reducing the risk of stroke recurrence, and the combination of extended-release dipyridamole plus aspirin was additive and produced highly significant benefits for stroke prevention. ESPS-II has been criticised because of ethical concerns over the use of placebo and because of disqualification of information on more than 400 patients.

A formal overview of the literature on long-term secondary prevention of vascular disease by antiplatelet treatment<sup>22,23</sup> in patients with a history of TIA, atherothrombotic stroke, unstable angina, or myocardial infarction, showed that those treatments might reduce vascular mortality by 17%, non-fatal myocardial infarction by 34%, non-fatal stroke by 25%, and the composite of the above end-points by 25%. The efficacy was independent from age, sex, previous TIA, stroke, myocardial infarction, and peripheral arterial disease, hypertension, diabetes, TIA of the brain or of the eye, whereas low-risk patients (asymptomatic) had less pronounced benefits.

The cost of treatment to prevent stroke should ideally be less than the cost (direct and indirect) of the stroke prevented. Aspirin and anticoagulants for patients with an embolic source in the heart seem to be cost effective, while at present, the widespread use of clopidogrel in TIA and stroke patients does not seem the same. More robust data about the cost and the effectiveness of the combination of antiplatelet agents are awaited from trial in progress.

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## Planning Antithrombotic Therapy For Patients With Atherothrombosis

Chairmen: G. Gensini, F. Crea

### Clopidogrel and the CURE results

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original paper



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The treatment of acute coronary syndromes (ACS) without ST elevation has been completely revolutioned in the last few years. Until just a few years ago, the only medications used in this condition were aspirin and heparin, but now several new antithrombotic drugs have been shown to be affective 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 in patients with ACS.

However, the Gp IIb/IIIa blockers are generally given intravenously and can therefore only be used as acute in-hospital treatment. 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 experience death or myocardial infarction (MI) by 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 in the long-term chronic treatment of ACS, specifically the oral GP IIb/IIIa blockers.

However, these new promising 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 agonist at the platelet receptor.

Clopidogrel has been shown 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 been tested by the CURE trial and, if shown effective, could be much more likely accepted into clinical practice. The CURE trial evaluated the efficacy and safety of the antiplatelet agent clopidogrel when given with aspirin in patients with acute coronary syndromes without ST elevation. Twelve thousand five hundred and sixty-two patients who had 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, nonfatal 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 percentages of patients with in-hospital refractory or severe ischemia, heart failure, and revascularization procedures were also significantly lower with clopidogrel. 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.

#### Conclusions

The antiplatelet agent clopidogrel has beneficial effects in patients with acute coronary syndromes without ST-segment elevation in addition to aspirin. However, the risk of major bleeding is increased among patients treated with clopidogrel.

## Planning Antithrombotic Therapy For Patients With Atherothrombosis

Chairmen: G. Gensini, F. Crea

### Thrombolytic therapy, angioplasty or something else: which is the therapeutic paradigm in patients with acute myocardial infarction?

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The treatment of acute myocardial infarction (AMI) has substantially changed during the past 2 decades. Two of the major advances in the treatment of AMI have been a) the improvement in coronary care unit, which provides continuous monitoring of the patient, and b) the introduction of reperfusion therapy. Several consecutive randomized clinical trials with drugs such as aspirin, beta-blockers, thrombolytics, and ace inhibitors or primary intervention (angioplasty) have shown positive results. It is therefore not surprising to see a decrease of 71% in 30-day mortality from 1975 to 1995.<sup>1</sup> The greatest contribution to the decrease in acute mortality was achieved by the use of aspirin, which increased markedly from around 5% in 1975 to 75% in the early 1990s. Thrombolysis and primary angioplasty had widespread since 1985 and have had substantial effects on reducing mortality.

The superiority of primary angioplasty over thrombolytic therapy for treatment of ST elevation AMI in terms of hospital mortality,<sup>2-5</sup> has been shown in several randomized trials and meta-analysis. Two large registries, representative of practice in a community setting, however failed to confirm a significant benefit for primary angioplasty over thrombolytic therapy, *although numeric trend existed* (6.4 % mortality for primary angioplasty versus 11.3% for thrombolysis).<sup>6-8</sup>

Since the advent of reperfusion therapy for acute ST elevation myocardial infarction, the *open artery hypothesis* suggested that benefit is achieved from early reopening of the occluded coronary artery, which limits the size of infarction, reduces the degree of left ventricular dysfunction, and improves survival.<sup>9</sup> The greatest benefit occurs with the shortest time to obtain complete reper-

fusion, meaning the Thrombolysis in Myocardial Infarction (TIMI) flow grade 3. Almost 75% of patients treated by primary angioplasty attain TIMI 3 flow 100 min after admission to hospital,<sup>10</sup> whereas only 50-55% of patients treated with alteplase have TIMI-3 flow at 90 min and this proportion does not substantially improve over the subsequent hours.<sup>11</sup> The addition of a full dose of a GP IIb/IIIa receptor antagonist, such as abciximab, eptifibatide, or tirofiban to half-dose alteplase<sup>12</sup> or to half-dose reteplase<sup>13</sup> resulted in nearly 80% of patients achieving complete reperfusion at 90 min without a substantial increase in side effects. After that numerous studies confirmed the benefit of a patent infarct related artery, more careful examination of the degree of reperfusion was performed using the Thrombolysis in Myocardial Infarction (TIMI) flow grading system devised in the TIMI 1 trial.<sup>14</sup> When differentiating apparently normal TIMI grade 3 flow from more delayed TIMI grade 2 flow in patent arteries, greater myocardial salvage and improved survival were observed in patients who achieved TIMI grade 3 flow.<sup>15</sup> The analysis by Stone *et al.*<sup>16</sup> sheds more light on the importance of TIMI 3 flow. After combining data from >2,500 patients in the 4 Primary Angioplasty in Myocardial Infarction (PAMI) trials, they compared patients who achieved TIMI grade 3 flow spontaneously on the angiogram before primary percutaneous coronary intervention (PCI), who comprised 16% of the population, with those who had TIMI 0 to 2 flow. Those patients with spontaneous TIMI grade 3 flow had improved left ventricular function, lower rates of congestive heart failure, and lower mortality. In addition, they observed that procedural success was higher in patients with baseline TIMI 3 flow.

The restoration of TIMI-3 flow after procedure was also a powerful predictor of survival: 6 month mortality was 22% with final TIMI 0/1 flow, 6% with TIMI-2 flow, and 2% with final TIMI-3 flow.<sup>16</sup>

The strategy of early pharmacological treatment might also include drug administration in the ambulance car. The strategy of facilitated PCI for ST elevation myocardial infarction provides that the pharmacological treatment (e.g., glycoprotein IIb/IIIa inhibitors and thrombolytic drugs) is initiated while patients are being transported to the cardiac catheterization laboratory for primary PCI. This strategy is designed to achieve the earliest possible reperfusion, while maintaining the benefits of primary PCI - complete reperfusion, relief of coronary obstruction, and prevention of arterial remodeling with primary stenting. These benefits have also translated into lower rates of death, reinfarction, and stroke (especially intracranial hemorrhage) when compared with thrombolysis.<sup>5</sup>

Primary PCI is especially effective when combined with glycoprotein IIb/IIIa inhibition.<sup>17</sup> Multiple studies have reported a 25%-30% rate of reocclusion after successful thrombolytic therapy.<sup>18-21</sup> In contrast, the incidence of reocclusion after balloon angioplasty appears to be significantly lower (ranging from 5% to 16%), and even lower after stent implantation (<6%).<sup>22</sup> The American College of Cardiology (ACC) and American Heart Association (AHA) recommend that primary angioplasty should be used as an alternative to thrombolytic therapy only if performed in a timely fashion in high-volume centers by physician skilled in the procedure and supported by experienced personnel.<sup>23,24</sup> In fact, data from National Registry of Myocardial Infarction 2 and 3 showed that patients with AMI treated at hospital with high or intermediate volumes of angioplasty had lower mortality with primary angioplasty than with thrombolysis (4.0 vs. 5.6%,  $p < 0.001$ ). In contrast, patients with AMI treated at hospital with a low angioplasty volume had equivalent mortality outcomes with primary angioplasty or thrombolysis (6.2 vs. 5.9%  $p = 0.58$ ).<sup>25</sup> There was a significantly lower rate of non fatal stroke with primary PCI versus thrombolysis, even at these low-volume hospitals (0.4% versus 1.1% for high versus low volume;  $p < 0.001$ ).

The strategy of initial thrombolysis followed by immediate PCI was initially less encouraging.<sup>26-29</sup> However, a recent re-evaluation of such a strategy in the Plasminogen activator Angioplasty Compatibility Trial (PACT) found an improvement in early TIMI 3 flow with half-dose t-PA administered before PCI.<sup>30</sup> Four trials (TIMI 14, SPEED, ADMIRAL,

RAPPORT) showed advantage of using IIb/IIIa inhibitors in the Emergency Department before PCI to improve TIMI myocardial perfusion grade 3 (which is an important predictor of mortality). Recently, in the GUSTO-V and ASSENT -3 trials, the combined therapy with half-dose thrombolytic plus IIb/IIIa inhibitors has demonstrated the highest effectiveness for improving early reperfusion. However, this benefit is not translated in a reduction in early mortality, and, furthermore, it was obtained at the cost of a higher rate of thrombocytopenia, major bleeding complications, and blood transfusion.<sup>31,32</sup>

Compared with thrombolysis and primary balloon angioplasty, primary PCI with stent implantation is associated with a significant reduction in the combined end point of death, reinfarction, stroke or repeat target vessel revascularization (TVR) for ischemia at six months, as well as in recurrent unstable ischemia. The median length of initial hospitalization was also reduced in the stent group, suggesting that the costs associated with primary stenting may be offset by reductions in the need for subsequent care. Stent implantation in the infarct-related artery (IRA) is feasible, safe and a useful adjunct to primary PCI. Stent implantation leads to a large intraluminal gain: this may produce a more rapid and complete restoration of flow in the IRA (that are important determinants of outcome). Five randomized trials (FRESCO, GRAMI, PAMI, STENTIM-2, ZWOLLE) have shown that primary stenting provides clinical benefits beyond those of primary PTCA.<sup>33-37</sup> The best angiographic results in patients with acute myocardial infarction have been achieved with early glycoprotein IIb/IIIa inhibitor administration in combination to stent implantation. This strategy demonstrated to improve myocardial salvage and a better clinical outcome.<sup>17,38,39</sup> Other percutaneous strategies include transluminal extraction atherectomy (TEC), RESCUE, X-Sizer, AngioJet, and intravascular ultrasonic thrombolysis. These strategies allow us to removing the thrombus from the IRA. The TEC device macerates atherosclerotic plaque and coronary thrombi by the interaction of a rotating cutter and a continuous vacuum suction. Intravascular ultrasonic thrombolysis (Acolysis System, Angiosonic, Morrisville, NC, USA) applies low frequency ultrasound for a rapid mechanical thrombolysis. The initial results of ultrasonic thrombolysis in 15 patients with acute anterior MI showed successful reperfusion (TIMI-3 flow) in 87% with a low in-hospital complication rate.<sup>40</sup>

What about something else? In the current era,

primary PCI appears to be the reperfusion strategy of choice for the treatment of AMI. In particular, a combination of half-dose fibrinolytic agent with glycoprotein IIb/IIIa blocker before angioplasty seems to be helpful. This approach is being investigated in the CADILLAC-2 and FINESSE trials. Pending these trials, we believe that the primary pitfall is to minimize the delay in initiating reperfusion therapy: this should include the triple therapy (i.e., the association of a reduced-dose of lytic with IIb/IIIa inhibitors and the eventual primary PCI). Such a therapy can theoretically provide both the greatest early infarct-related artery patency at 30-60 minutes, and a greater 60-90 minute with the additive benefits of the approach of facilitated PCI.

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## Planning Antithrombotic Therapy For Patients With Atherothrombosis

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### Glycoprotein IIb/IIIa antagonists in acute coronary syndromes

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Coronary thrombosis plays a major role in the pathogenesis of several acute coronary syndromes, including unstable angina, acute myocardial infarction, coronary thrombolysis and coronary angioplasty or stenting. Direct coronary angiography has demonstrated the presence of thrombus in patients with unstable angina, consistent with a role for thrombosis in this disease. Moreover, genetic variants in several thrombotic genes occur at a higher frequency in patients with acute myocardial infarction. These include variants in prothrombin, factor V, factor VII and fibrinogen. Moreover, several environmental risk factors for acute coronary syndromes promote thrombosis, including smoking and hyperlipidaemia. It is this propensity for thrombosis that distinguishes human disease from animal models of atherosclerosis, such as the apolipoprotein-a knockout mouse, where thrombosis is rarely detected.

Platelets play a key role in the initiation of thrombosis of arteries and there is strong evidence that they are also important in acute coronary syndromes. Patients with acute coronary syndromes have evidence of increased platelet activity demonstrated using a variety of techniques including the excretion of thromboxane metabolites and the expression of granular proteins on the platelet surface. As with genes involved in thrombosis, variants in genes involved in platelet activation and aggregation have been linked to acute coronary syndromes. For example, the PLA2 variant of glycoprotein (GP) IIb/IIIa has been linked to acute myocardial infarction, particularly in young patients and in smokers, independent of other gene variants. A variant in the receptor for collagen, a platelet activator has also been linked to coronary thrombosis, as have variants GPIIb, the receptor for von Willebrand factor.

#### *Platelets and thrombosis*

Platelets adhere to sites of vascular injury through a number of receptors interacting with extracellular matrix proteins, including vWF, vitronectin, collagen and fibrinogen. Adhesion is mediated by corresponding receptors, GPIIb/IIIa, GP $\alpha$ v $\beta$ 3, GP $\alpha$ 2 $\beta$ 1 and GPIIb, and GP $\alpha$ 2 $\beta$ 3 (GPIIb/IIIa), respectively. The interaction between GPIIb/IIIa and vWf is shear dependent in that it is stimulated under high shear conditions, as exist in the stenosed coronary artery. These interactions are not passive, as they require activation of the receptor and ligand binding triggers platelet activation, so called 'outside-in' signalling. 'Outside-in' signalling results in the reorganisation of the cytoskeleton, the phosphorylation of kinases and the release of platelet agonists, such as thromboxane and ADP that act to amplify the adhesion-triggered platelet activation. Platelet activation results in the expression of further activated adhesion receptors that bind additional platelets. The resulting platelet aggregate acts as a nidus for the assembly of coagulant proteins and the initiation of thrombosis.

#### *Regulating platelet function: agonists and inhibitors*

Platelets release several products that stimulate platelet activity, largely through G-protein coupled receptors on the platelet surface membrane. Thromboxane, the major product of cyclooxygenase in platelets and a target for aspirin, acts through two receptors, TP $\alpha$  and  $\beta$ , to induce activation of protein kinase C, a rise in intracellular calcium and inhibition of adenylate cyclase. ADP acts through at least three GPCRs linked through distinct signalling systems to induce platelet activation, one of which (PTY12) is the target for ADP antagonists, ticlopidine and clopidogrel. Platelets

also release GAS-6, a recently identified platelet protein that appears to act as a platelet agonist. Thus disruption of the gene for this protein in mice results in enhanced platelet activity.

Platelets are also activated by thrombin, derived by the action of prothrombinase on prothrombin at the platelet surface. Several platelet receptors for thrombin have been identified, including a series of protease activated receptors (PAR), GPCRs that are in effect substrates for the enzyme. Cleavage of the extracellular aminoterminal of PAR1 and 4, exposes epitopes that act as the agonists for the receptor. Thrombin also binds to GPIb, which may serve to bind thrombin in proximity to PARs and in this way contribute to the platelet effects of thrombin. Many other agonists for platelets have been identified (epinephrine, prostaglandin E<sub>2</sub>, 8-iso-PGF<sub>2α</sub>), but their role *in vivo* is less clear.

#### *Platelet inhibition*

In addition to platelet agonists, there are endogenous platelet inhibitors that serve to regulate platelet activity. Prostacyclin, generated from arachidonic acid by cyclooxygenase in endothelial cells, activates guanylate cyclase through a Gi-linked GPCR IP, increasing platelet c'AMP, which in turn triggers protein kinase A activity. Nitric oxide, also derived from endothelial, triggers guanylate cyclase and the generation of platelet c'GMP, which acts in tandem with c'AMP to suppress platelet activity. A major target of this pathway is VASP, which limits the activation of the integrin glycoprotein IIb/IIIa. Nitric oxide is metabolized to peroxynitrite by the peroxidase of cyclooxygenase, an activity that is not inhibited by aspirin. Recently, antiplatelet activity has been attributed to GPV, a component of the of the vWf receptor, in that mice deficient in GPV show enhanced platelet function.

#### *GPIIb/IIIa: a multifunctional adhesion receptor*

GPIIb/IIIa is one of a family of proteins called integrins, as they integrated the intracellular and extracellular environments of the cell. These receptors are composed of two subunits ( $\alpha$  and  $\beta$ ) that are products of distinct genes and that form a heterodimeric complex on the cell surface. The complex, amongst other functions, provides binding sites for adhesion proteins and acts to bind cells to each other or to the extracellular matrix. GPIIb/IIIa is only expressed on platelets and on megakaryocytes, with 50-80,000 complexes on the platelet surface. The primary ligand for the receptor is fibrinogen, although other adhesion proteins can

bind, including vitronectin, a major component of extracellular matrix. Under resting conditions, GPIIb/IIIa only interacts with fibrinogen that is bound to surfaces. However, upon platelet activation, the receptor alters its conformation and expresses a binding site for soluble fibrinogen. The change in conformation is mediated by phosphorylation of the cytoplasmic tail of the  $\beta$  subunit, although other components of the receptor regulate the expression of the ligand binding site. The change in conformation is dramatic, with evidence of 'shuffling' of disulfide bonds in the cysteine rich  $\beta$  subunit. Indeed, the receptor expresses an isomerase activity, one that is capable of breaking and remaking disulfide bonds. Following activation and subsequent ligand binding, the receptor undergoes additional conformational changes that may play a role in 'outside-in' signalling. This signalling is linked through a series of kinases to subsequent events, such as reorganisation of the cytoskeleton and secretion that are absolutely required for platelet aggregation. Thus, platelet aggregation is not simply a result of fibrinogen acting as a 'glue' between adjacent platelets.

#### *GPIIb/IIIa antagonists: basic mechanisms*

Antiplatelet therapies have largely been directed at preventing platelet activation (aspirin for thromboxane, clopidigrel for ADP). Their effectiveness reflects the fact that thromboxane and ADP mediate the platelet aggregation response, at least in part, to most platelet agonists. However, the approach is limited, as potent agonists are not dependent on thromboxane or ADP for their effect. Platelet aggregation can be completely prevented by antagonists of GPIIb/IIIa, small molecules that mimic the receptor recognition sequences in the ligand or antibodies to the receptor. These compounds bind to the receptor in both the inactive and activated states and block the binding of the natural ligand, either directly or allosterically, that is by changing the conformation of the receptor. As a consequence, platelet aggregation to all agonists is prevented, although activation (the upstream signalling induced by agonists) is not prevented. In experimental models, GPIIb/IIIa antagonists are highly effective in preventing thrombosis, an effect that is enhanced by concomitant administration of a thrombin inhibitor, such as hirudin and by inhibitors of thromboxane.

#### *GPIIb/IIIa antagonists: clinical application in acute coronary syndromes*

GPIIb/IIIa antagonists have been studied through large-scale clinical trials in patients with a acute

coronary syndromes, including unstable angina and myocardial infarction, and in patients undergoing coronary intervention. The overall effect is a reduction in recurrent events, broadly including death, myocardial infarction or the need for urgent revascularisation. The benefit varies and is arguably less than expected given the effectiveness of GPIIb/IIIa antagonists in suppressing platelet aggregation *ex vivo* and the dramatic increase in bleeding time in patients on these drugs. Recent studies with oral GPIIb/IIIa antagonists have shown an increased risk of events, in particular cardiac death raising the possibility that in some patients the drugs trigger thrombosis. Drug-induced 'outside-in' signalling through the GPIIb/IIIa receptor provides a potential mechanism for the outcome in trials of oral agents, and may be limiting the benefit of intravenous agents, at least in some patients. Evidence for a 'partial agonist' effect of GPIIb/IIIa antagonists has been based on both *in vitro* and *ex vivo* experiments, showing that GPIIb/IIIa antagonists may partially activate platelets, particularly evident at lower concentrations of the drug. Moreover, small ligand antagonists induce an active conformation of the receptor. However, other studies have failed to show any platelet activation or any evidence that fibrinogen binds to platelets as the GPIIb/IIIa antagonist dissociates from the receptor (Tables 1 and 2).

Population heterogeneity may explain the variability in the response to GPIIb/IIIa antagonists. Genetic analysis within the OPUS-TIMI-16 trial of the oral GPIIb/IIIa antagonist orbofiban, in patients with acute coronary syndromes showed an association between the PLA2 variant of GPIIIa and outcomes. Moreover, the variant was linked to a poor response to the drug, both in terms of recurrent myocardial infarction and even the risk of bleeding. Thus, while the risk of bleeding was increased in non-carriers, in a dose-dependent manner, PLA2 carriers showed no increase in bleeding events. Such a mechanism may explain the variability in the response to intravenous agents also, although the issue has not been addressed.

The response to GPIIb/IIIa antagonists may also be influenced by clinical factors, in particular the underlying risk of subsequent events. In some trials of intravenous agents in acute coronary syndromes, patients with elevated plasma troponin showed a greater benefit. (The finding suggests that the reported induction of apoptosis of isolated rat neonatal cardiomyocytes by GPIIb/IIIa antagonists, is of doubtful clinical relevance.) Concomitant therapy may also influence response.

**Table 1. GPIIb/IIIa antagonists in Percutaneous Coronary Intervention(PCI).**

Study (year)	Subjects (number)	Treatment Arms	Duration (months)	Event rate*	p value <sup>o</sup>
EPIC (1994)	2,099	Placebo	1	12.8%	NS
		Abciximab bolus only		11.5%	
		Abciximab bolus + infusion		8.3%	
IMPACT II (1997)	4,010	Placebo	6	11.4%	0.063
		Eptifibatide (low dose)		9.2%	
		Eptifibatide (high dose)		9.9%	
RESTORE (1997)	2,139	Placebo Tirofiban	1	12.2% 10.3%	0.16
CAPTURE (1997)	1,265	Placebo Abciximab	1	15.9% 11.3%	0.002
EPILOG (1997)	2,792	Placebo + standard heparin Abciximab + low dose heparin Abciximab + standard heparin	1	11.7% 5.2% 5.4%	<0.001
EPISTENT (1998)	2,399	Stent + placebo PTCA+ abciximab Stent + abciximab	1	10.8% 6.9% 5.3%	0.007 <0.001
ESPRIT (2000)	2,064	Placebo Eptifibatide (double bolus)	1	10.4% 6.8%	0.0034
TARGET (2001)	5,308	Tirofiban Abciximab	1	7.6% 6.0%	0.038
ADMIRAL (2001)	300	Primary stenting + placebo Primary stenting + abciximab	1	14.6% 6.0%	0.01

\*Events refer to all deaths, MI and urgent revascularisation in most cases (please consult original trial for full details). <sup>o</sup>p value for most significant result included.

**Table 2. GPIIb/IIIa antagonists in acute coronary syndromes (ACS).**

Study (year)	Subjects (number)	Treatment Arms	Duration	Event rate**	p value <sup>o</sup>
PARAGON 1998	2,282	Placebo + heparin	1	11.7%	0.67
		Lamifiban (low dose)		10.6%	
		Lamifiban (high dose)		12.0%	
PRISM 1998	3,232	Heparin	1	17.1%	0.34
		Tirofiban		15.9%	
PRISM-PLUS 1998	1,915	Heparin	6	17.9%	0.004
		Tirofiban		Stopped	
		Tirofiban + heparin		12.9%	
PURSUIT 1998	10,948	Placebo Eptifibatide bolus + infusion	1	15.7% 14.2%	0.04
GUSTO IV ACS 2001	7,800	Placebo	1	8.0%	NS
		Abciximab (24hr)		8.2%	
		Abciximab (48hr)		9.1%	

\*Events refer to all deaths, MI and urgent revascularisation in most cases (please consult original trial for full details). <sup>o</sup>p value for most significant result included.

Greater benefit is seen in patients undergoing coronary intervention than those receiving medical therapy alone, and the response is optimal in patients receiving aspirin and heparin. There may also be differences between GPIIb/IIIa antagonists. For example, abciximab dissociates slowly from the receptor and can be detected for up to 15 days on the platelet surface, where other agents dissociate rapidly, reflecting their plasma half-lives. In addition, abciximab has a broader spectrum of pharmacological effect as it also binds to the integrin  $\alpha$ V $\beta$ 3, the receptor for vitronectin.  $\alpha$ V $\beta$ 3 is a mediator of smooth muscle and endothelial cell proliferation, potentially through an interaction with vascular endothelial growth factor receptor(s). There are also a small number of  $\alpha$ V $\beta$ 3 receptors on platelets (500/platelet), although their role in platelet activation and aggregation is unknown. Interaction with  $\alpha$ V $\beta$ 3 may explain the greater clinical benefit of abciximab in patients undergoing coronary intervention.

#### Conclusion

GPIIb/IIIa antagonists are effective in acute coronary syndromes, although the effect is limited. This may reflect the heterogeneity in the clinical background or in the response to different agents. Understanding these heterogeneous factors may help target GPIIb/IIIa antagonists to those most likely to benefit.

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## Venous And Arterial Thromboembolism: From Heparin To The New Antithrombotics

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### Home treatment and secondary prevention of deep vein thrombosis

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#### *General characteristics of treatment*

The keystone of treatment of deep vein thrombosis (DVT) of the lower limbs is anticoagulant drugs. Correct use of these drugs blocks extension of the thrombus and reduces the risks of pulmonary embolism and recurrent venous thromboses in the weeks following the initial episode.<sup>1-6</sup> Over the last decade low molecular weight heparin has successfully joined the traditional treatment of standard heparin. Low molecular weight heparins have some advantages over the classical heparin treatment and in certain situations allow home treatment of selected patients.

The low molecular weight heparins most tested for this indication are nadroparin (450 U/kg/die), enoxaparin (200 U/kg/die), logiparin (175 U/kg/die) and dalteparin (200 U/kg/die) in one or two administrations per day. These doses produce a marked and persistent antithrombotic effect, without excessively prolonging the activated partial thromboplastin time (APTT). The cumulative incidence of recurrent thrombotic and/or embolic events in the early phase of treatment and during the subsequent three months lies between 1.5 and 5%.

The results of some meta-analyses have recently become available. These meta-analyses were intended to evaluate prospective studies comparing secondary prophylaxis with low molecular weight heparin and standard heparin.<sup>7</sup> The results are extremely important. They demonstrate that the low molecular weight heparins are at least as effective and safe as standard heparin in achieving the treatment objectives in DVT. Surprisingly, since it was completely unexpected, there was a statistically significant reduction in long-term mortality in patients treated with the low molecular weight heparins. This decrease was to a large extent caused by a reduction in mortality associated with neoplastic disease progression. It should be stated immediately that a post-mortem exam-

ination was not carried out in many of the patients with cancer who died. It is theoretically significant that the low molecular weight heparins caused a decrease in thrombo-embolic events which are frequently the cause of death in patient with advanced cancer. It cannot, however, be excluded that these agents do in some way influence the evolution of malignant neoplastic diseases, particularly in the advanced metastatic stages.<sup>7</sup>

#### *Home treatment of DVT*

The results of two large-scale, randomized prospective studies were reported in the 1990s.<sup>8,9</sup> Overall a thousand symptomatic outpatients with DVT associated or not with pulmonary embolism were randomized to one or other of the following two strategies: hospital treatment with standard continuous intravenous heparin treatment or domiciliary treatment (partial or complete) with a low molecular weight heparin (nadroparin in one study, enoxaparin in the other) administered subcutaneously at doses adjusted according to body weight. Treatment with coumarins was started simultaneously with the heparin in both treatment groups. The patients were followed up for six months from the date of entry into the study. These two studies demonstrated the feasibility and safety of home treatment of uncomplicated cases of DVT. The frequency of serious bleeds and recurrent thrombo-embolic events was similarly low in both treatment groups (Table 1). In detail, there were no deaths within the first fortnight in any patient treated at home. The domiciliary management was welcomed by the patients and caused, in both studies, a substantial reduction in costs, even when the analysis took into consideration the de facto costs assumed by the patients and their families.

Following the publication of the results of these clinical trials, other prospective cohort studies

added persuasive support to the concept that domiciliary treatment of DVT with low molecular weight heparins is feasible and safe,<sup>10-13</sup> such that this indication is now accepted and authorized by the Italian health authorities. A considerable percentage of patients with acute DVT can nowadays receive an effective, safe and satisfying treatment with low molecular weight heparins. It should, however, be immediately stated that there still remain clear indications for hospital admission. These indications are DVT in alcoholic patients, or patients with poor compliance, patients with other conditions which require hospital admission or in whom the suspicion of an occult malignancy is high, patients with clinical signs and symptoms of pulmonary embolism (that is, with manifest symptoms, while the scintigraphic finding of asymptomatic pulmonary embolism, extremely common in patients with a proximal DVT, has no value and is not, therefore, a contraindication to home treatment), patients at high risk of bleeding because of anticoagulant treatment, patients who live a long way away from the hospital, elderly patients living alone and any patient in whom it is not possible to monitor the oral anticoagulant treatment. However, even in these groups of patients the availability of the new classes of drugs means that the time spent in hospital can be reduced, thus producing a notable saving in terms of health care costs. In any case it is essential to adopt a flexible strategy, adapted from patient to patient and aimed at limiting admission duration and thus costs, which can be simply early discharge after a normal admission, outpatient treatment, or full home treatment.

There are some aspects of home treatment which should be clarified. First and foremost, it is not an excuse for not carrying out instrumental diagnostic strategies which are essential in all cases of DVT in order to avoid unnecessary treatment; secondly, the search for the cause of the DVT (occult malignancy, thrombophilic state, etc.) must not be neglected; and finally it implies that the establishment and maintenance of the oral anticoagulant therapy, which must be associated with the heparin therapy, are managed directly by community structures which are presently ill-prepared for this task. Home treatment of DVT requires an additional organizational force compared with the usual, standard care. The patient is instructed on the methods and significance of the treatment which he or she is called in part to self-manage (without creating excessive anxiety), must have preferential access to medical advice and always be able to contact medical or paramedical staff.

**Table 1. Main results of the two multicenter studies evaluating home treatment of DVT with low molecular weight heparins.**

	Koopman et al. <sup>8</sup>		Levine et al. <sup>9</sup>	
	LMWH (n=202)	UFH (n=198)	LMWH (n=247)	UFH (n=253)
Recurrent DVT/PE	14 (6.9%)	17 (8.6%)	13 (5.3%)	17 (6.7%)
Major bleeding	1 (0.5%)	4 (2.0%)	5 (2.0%)	3 (1.2%)
Death	14 (6.9%)	16 (8.1%)	11 (4.4%)	17 (6.7%)

The fear that patients at home might become ambulant too early or at any rate at the wrong time is cancelled by the persuasive demonstration that early ambulation - in correctly anticoagulated patients without clinical symptoms of pulmonary embolism - is not only not dangerous, but indeed is desirable.<sup>14,15</sup> This also applies to cases in which the echoDoppler shows a so-called *free-floating* thrombus within the lumen of the vein. There have been several authoritative demonstrations that this finding has no significant value.<sup>16,17</sup> On the other hand, in all the clinical studies carried out so far which have demonstrated the safety of domiciliary treatment, the patients were invited to start walking early, at a time compatible with the symptoms in the lower limb.

Patients treated at home with a low molecular weight heparin are recommended to have at least one platelet count done after 5-7 days: the risk of thrombocytopenia, although decidedly lower with these drugs than with standard heparin, is not completely eliminated.

#### *Secondary prevention of deep vein thrombosis: the available strategies*

Secondary prevention means the therapeutic strategy followed by a patient immediately after an episode of DVT in order to prevent subsequent episodes. The classic studies from McMaster University showed that if the acute phase of the treatment of DVT is not followed by correct anticoagulation for at least 12 weeks patients are at a high risk of a recurrent event.<sup>18</sup>

The therapeutic regimes which have been shown to be of equivalent efficacy are the following:

Subcutaneous calcium heparin at individualized doses, capable that is of prolonging the APTT, tested in the first 3-4 days, by about 1.5 times the control value (usually 20,000 U/die in two administrations given subcutaneously). This method of pre-

vention, not commonly used because of the need for prolonged parenteral administration, is the only one practical in pregnant women (because of the high risk of fetal damage associated with the use of warfarin) and in patients for whom it is difficult, for whatever reason, to perform regular blood tests: in fact, once the appropriate, personalized dose of heparin has been established for each patient during the hospital admission, subsequent laboratory monitoring is not necessary.

Conventional doses of oral anticoagulants, capable of prolonging the prothrombin time (PT), expressed as the International Normalized Ratio - INR - to between 3 and 4.5. This method of anticoagulation, once widely used (because it was transferred from experience in arterial disorders, and particularly in patients with artificial heart valves) has now been practically abandoned and substituted by the lower dose oral anticoagulation described in the following paragraph. Despite this, some indications remain for the use of these conventional doses in selected conditions, for example protection against recurrent thromboembolism in patients with primary or secondary antiphospholipid antibody syndrome.<sup>19</sup> Another indication is in patients, most of whom who have malignant neoplastic diseases, who have recurrent venous thromboembolic episodes despite anticoagulation at *prudent* doses.

Less intense doses of oral anticoagulants, capable of prolonging the PT, expressed as INR, to between 2 and 3. This therapeutic scheme is much more preferable than the preceding regime because of the lower risk of hemorrhagic complications for the same level of protection against a recurrent thromboembolism. Precisely because of the low risk of bleeding complications, this regime is associated with a high quality of life.

Some observations, aimed at testing the efficacy and safety of low molecular weight heparins for this indication, suggest that such drugs could be used with at least comparable results to those achieved with standard heparin, and with a lower risk of bleeding than that associated with oral anticoagulants.<sup>20-24</sup>

#### *Duration of secondary prevention*

As has already been mentioned, the duration of secondary prevention is conventionally fixed at 12 weeks after the thromboembolic episode on the basis of research by Hull *et al.*<sup>18</sup> This choice received important confirmation from two prospective studies which randomized a large series of patients to anticoagulation for 4 weeks or 3 months after the

acute DVT, unequivocally demonstrating the advantage of the longer period of anticoagulation.<sup>25,26</sup> A more recently published study, carried out with an excellent methodology on a large population of patients with a recent venous thrombosis or pulmonary embolism, indicated the unequivocal advantages in terms of risk-benefit of a regime of anticoagulation continued for six months in comparison to one used for six weeks.<sup>27</sup> Nevertheless, given that the control population had been treated with a shorter anticoagulation regime than that commonly recommended, we do not consider that it possible to conclude that all patients with recent venous thromboembolic disease must receive secondary prevention for a period exceeding 12 weeks. In confirmation of this, an extremely recent publication does not demonstrate any advantage from continuing dicoumarol therapy for 6 months compared with the normal administration for 3 months.<sup>28</sup>

There have been numerous reports in the past on the benefits of periods of anticoagulation shorter than 12 weeks. We encourage maximum care in interpreting these data because they arise from questionable methodological analyses: in fact they were drawn from uncontrolled or retrospective observations or small series of patients. The recent publication by Schulman *et al.*, just referred to, refutes the efficacy of an indiscriminant period of anticoagulation of only six weeks in patients with proximal thrombosis.<sup>27</sup> A reasonable exception could be made for patients with isolated distal thrombosis in the absence of permanent thromboembolic risk factors.<sup>28</sup>

There is sufficient clinical and experimental evidence to recommend that anticoagulant treatment be continued for longer than 12 weeks in some patients, such as those in whom the risk factors implicated in the first episode remain. If, for example, a patient remains immobilized for more than 3 months, objectively there is no sense in interrupting the secondary prevention at 12 weeks! If the cause cannot be removed (for example, an advanced stage of cancer, or a lupus collagen disease) the anticoagulant treatment should be continued for the whole of the patient's life.

One problem which raises conflicting opinions is the most appropriate duration of anticoagulation in subjects with hereditary thrombophilia. One recent prospective, cohort study showed that carriers of factor V Leiden and the G20210A variant of prothrombin had a significantly higher risk of recurrent thromboembolism than did patients who were not carriers.<sup>29</sup> Despite the lack of data demon-

strating the risk-benefit ratio of continuing treatment, long-term oral anticoagulation might be proposed for carriers of AT, protein C and protein S deficiencies; homozygotes for factor V Leiden or prothrombin variant or double heterozygotes for the aforementioned anomalies; and in all cases of clinically severe venous thromboembolic episodes and/or those of *spontaneous* onset (that is in the absence of the common acquired risk situations: malignancy, surgery, trauma, etc.).

As far as concerns the duration of anticoagulation in patients with thrombosis and proven antiphospholipid antibodies, a recent study, although with a retrospective, uncontrolled design, suggested the wisdom of continuing anticoagulation indefinitely at a high dose (INR > 3.0) in all cases resulting positive for antiphospholipid antibodies.<sup>19</sup> These high doses were claimed to have been more effective than more prudent doses (INR < 3.0) and treatment with aspirin. Consensus on this matter has not, however, been reached. The opinion that treatment should be continued indefinitely only in patient with central venous thromboses and in those with apparently spontaneous venous thromboses is widespread and that high doses should be reserved to those patients in whom more prudent doses have shown to be ineffective. Finally, the demonstration of various types of fibrinolytic abnormalities in patients with a previous venous thrombosis does not in any way legitimize prolonged anticoagulant therapy.

The results of our recent experience, devoted to the long-term follow-up of patients with DVT suggest that patients with apparently spontaneous venous thrombosis (a minority of the patients were carriers of risk factors such as occult malignancy or hereditary anomalies, but more frequently did not have any of the currently known risk factors) had a considerably higher risk of recurrent thromboembolism than patients with thrombosis secondary to removable causes.<sup>30</sup> A recent publication showed that two years of anticoagulation were significantly more effective in these patients than three months although the advantage in terms of protection from recurrent thromboembolism was partially counterbalanced by the increased incidence of major bleeding episodes.<sup>31</sup> Furthermore, there is no evidence that prolonged anticoagulation eliminates the risk of recurrence once the anticoagulation has been stopped. A very recent multicenter, Italian study which compared three months of anticoagulation with one year in this type of patient, clearly demonstrated that at the end of the two-year follow-up, the incidence of

recurrent thromboembolism was identical in the two groups of patients.<sup>32</sup> Future studies should investigate whether different strategies from those used so far (different drugs or less intense regimens of anticoagulation) could be more beneficial than the ones so far tested in terms of risk-benefit in patients with idiopathic venous thromboses.

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## Venous And Arterial Thromboembolism: From Heparin To The New Antithrombotics

Chairmen: P.P. Gazzaniga, G.G. Nenci

### Heparins: their established role in acute coronary syndromes and perspectives in atrial fibrillation

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The use of anticoagulants in cardiology has steadily increased over the last decade. The reasons explaining why this treatment is so largely accepted are basically three: 1. the average strong expression of thrombin on vascular surfaces; 2. the unfavourable shear conditions affecting many if not all major cardiovascular diseases; 3. the huge scientific evidence substantiating the clinical practice.<sup>1</sup>

Thrombin generation and fibrin formation take place continuously in normal individuals, to a minimal extent (yet increasing with age).<sup>2</sup> After vascular disease onset, the activation of the coagulation system runs in parallel with inflammatory processes and culminates with the occurrence of acute coronary syndromes. In other cardiovascular pathologies the dominant factor determining the occurrence of thrombosis is blood rheology, with anticoagulants being particularly effective at low shear rates, as it typically occurs in the left atrium of patients with atrial fibrillation.

Traditionally heparins in cardiology have been used for the short-term treatment of established-ongoing thrombosis, but the recent availability of low molecular weight heparins opens new ways toward medium to long-term treatments, where prevention may become the main issue.

#### Acute coronary syndromes

The broad definition *acute coronary syndromes* encompasses different clinical situations: ST-segment elevation myocardial infarction, non-ST-segment elevation myocardial infarction and unstable angina. The two latter conditions are usually put together in most major clinical trials because pathogenesis is thought to be similar; however it is clear that the short and long-term prognosis in patients with non-ST-segment elevation myocardial infarction (being diagnosed as either CK/CK-

MB or troponin positivity) is definitely worse than that affecting patients without signs of ongoing myocardial damage.

#### ST-segment elevation myocardial infarction

Unfractionated heparin in the absence of aspirin has been shown to be effective in the reduction of major outcomes in acute myocardial infarction (Table 1). From the meta-analysis however, it is clear that heparin treatment in conjunction with aspirin offers a tiny, yet measurable effect at the expense of a similar increase in the odds of developing major haemorrhage. In the context of coronary thrombolysis, intravenous unfractionated heparin in conjunction with alteplase does not improve coronary artery patency at 90 minutes,<sup>4</sup> but it does at 18 and 81 hours,<sup>1</sup> patency being related to the achieved activated partial thromboplastin time (aPTT). There are no data on the effects of intravenous unfractionated heparin with alteplase on clinical outcomes; however the 1% lower mortality observed in the GUSTO-I study, favouring alteplase (against streptokinase) was obtained combining alteplase with intravenous heparin.<sup>5</sup> Among patients receiving streptokinase intravenous heparin was not superior to subcutaneous heparin in terms of mortality, reinfarction, bleeding, infarct-related patency and coronary reocclusion.

Unfractionated heparin has many drawbacks. The drug has a narrow therapeutic index, is difficult to titrate because of complex pharmacokinetics and pharmacodynamics and requires extensive laboratory monitoring. Thus, even the adoption of well acknowledged infusion nomograms results in suboptimal aPTT values in most cases, particularly after thrombolysis, when the effects of both drugs on the coagulation system are present.

Recently there has been a renewed interest in

**Table 1. Effects of unfractionated heparin in acute myocardial infarction: a meta-analysis.<sup>3</sup>**

	Risk reduction for 1000 treated patients			
	No aspirin	P	Aspirin	P
Death	- 35	0.002	- 5	0.03
Reinfarction	- 15	0.08	- 3	0.04
Stroke	- 10	0.01	- 1	0.01
Pulmonary embolism	- 19	0.001	- 3	0.0001
Major bleed	+ 10	0.01	+ 3	0.001

**Table 2. ASSENT-3 major outcomes. Tenecteplase thrombolysis plus low molecular weight (LMWH) versus unfractionated (UF) heparin.<sup>9</sup>**

	LMWH %	UF %	RR	95 % CI	p	Benefit*
1° efficacy end-point	11.4	15.4	0.74	0.63-0.87	0.0002	-40
1° efficacy-safety end-point	13.7	17.0	0.81	0.70-0.93	0.0037	-33

CI: confidence intervals; RR: relative risk; \* for 1000 treated patients.

the issue of heparin treatment in conjunction with thrombolysis, following the observation that low molecular weight heparin (enoxaparin), along with alteplase, may decrease the 1-week coronary reocclusion (TIMI-3 to TIMI 0-1) from 9.1% to 3.1% ( $p=0.12$ ) compared to unfractionated heparin in the HART-II study<sup>6</sup> and dalteparin may decrease ischemic episodes, compared to placebo after streptokinase (16% vs 38%,  $p=0.04$ ) in the BIOMACS-II study.<sup>7</sup> In the ASSENT-PLUS study 439 patients were treated within 6 hours from symptoms onset with either dalteparin or unfractionated heparin in conjunction with alteplase.<sup>8</sup> Dalteparin improved the TIMI-3 flow at 4-7 days (69% vs 62%,  $p=0.16$ ), the angiographic appearance of thrombus (19% vs 27%,  $p=0.054$ ), the occurrence of TIMI 2/3 flow plus no thrombus (72% vs 58%,  $p=0.004$ ) and reinfarction rate within 7 days (1.4% vs 5.4%,  $p=0.02$ ).

More recently clinical outcomes have been addressed by the ASSENT-3 study.<sup>9</sup> In this study 4078 patients with acute myocardial infarction within 6 hours from onset of symptoms were treated with enoxaparin (intravenous bolus plus subcutaneous maintenance) or intravenous unfractionated heparin along with tenecteplase. The primary efficacy end-point of the study (30-day composite of mortality, in-hospital reinfarction or in-hospital refractory ischemia) and the primary efficacy plus

safety end-point (above end-points plus in-hospital intracranial haemorrhage or in-hospital major bleeding) were reduced by low molecular weight heparin (Table 2). Among individual components of the primary end-points, death was reduced from 6.0% to 5.4% ( $p = \text{NS}$ ), in-hospital reinfarction from 4.2% to 2.7% ( $p=0.0009$  including the abciximab arm), in-hospital refractory ischemia from 6.5% to 4.6% ( $p<0.0001$ ); there was a slight increase in major bleeding (from 2.2% to 3.0%,  $p=0.0005$  including the abciximab arm), but not in intracranial haemorrhage (0.9% in both groups). Other positive outcomes linked to low molecular weight heparin therapy include the reduction in invasive cardiac procedures (35.3% to 32.5% ( $p=0.06$ )) and in urgent percutaneous coronary intervention (14.4% to 11.9%,  $p<0.0001$ ). The benefit seems to apply to almost all subgroups.

After the ASSENT-3 study a new standard for the treatment of acute myocardial infarction is established: the bolus injection of both a thrombolytic and an antithrombin agent makes the reperfusion strategy *easy to do* and feasible everywhere in the field.

Things are rapidly moving and presently the concept of a *pure* blockade of factor Xa by a synthetic pentasaccharide (ORG 31540/SR90107A) is being developed. The first study with this agent (PEN-TALYSE) was performed in 316 patients with ST-elevation of less than 6 hours and showed an interesting reduction in 5-7 days coronary reocclusion (TIMI-3 to TIMI 0-1) from 7.0% (unfractionated heparin) to 0.9% (pentasaccharide) ( $p=0.065$ ).<sup>9</sup>

#### *Unstable angina and non-Q-wave myocardial infarction*

The advent of low molecular weight heparins has definitely upgraded antithrombin therapy in patients with unstable angina and non-Q-wave myocardial infarction. Several large-scale clinical trials have definitely shown that low molecular weight heparin by either dalteparin, enoxaparin or nadroparin is better than placebo and at least equivalent to intravenous unfractionated heparin in preventing the progression to death/myocardial infarction. For one of them (enoxaparin) the possibility has been shown by the meta-analysis to reduce the occurrence of death and myocardial infarction compared to unfractionated heparin.<sup>11,12</sup> Although this end-point was not achieved separately by both ESSENCE and TIMI-11B trials, the efficacy on the triple end-point death/myocardial infarction/urgent revascularization was observed in each trial.

**Table 3. Meta-analysis of heparins in unstable angina/non-Q-wave myocardial infarction.**<sup>11,12</sup>

	Unfractionated/ low molecular weight heparin/ enoxaparin	Control/placebo/ unfractionated heparin	Odds ratio	95% CI
Unfractionated heparin vs placebo/control D/MI	7.9 %	10.4 %	0.67	0.45-0.99
Low molecular weight heparin vs placebo D/MI	1.6 %	5.2 %	0.34	0.20-0.58
Low molecular weight heparin vs unfractionated heparin D/MI	2.2 %	2.3 %	0.88	0.69-1.12
Enoxaparin vs unfractionated heparin (essence-timi 11B) D/MI	7.1 %	8.6 %	0.82	0.69-0.97
D/MI/UR	15.6 %	18.8 %	0.80	0.71-0.91

CI: confidence intervals; D: death; MI: myocardial infarction; UR: urgent revascularization.

Thus, not only low molecular weight heparin offers an easy way to anticoagulate patients with acute coronary syndromes, allowing for subcutaneous administration of fixed doses to be given without laboratory monitoring, but also the efficacy in terms of reduction of refractory symptoms and, with it, of urgent revascularization makes the treatment attractive and potentially self-paying. An excess in major bleeding complications has not been observed after low molecular weight heparin administration, also including those patients requiring early coronary angiography and angioplasty. However, an increase in chest-tube blood drainage and blood transfusion after coronary artery by-pass surgery (but no excess in life-threatening hemorrhage or need for chest re-opening) has been observed when surgery is undertaken less than 12 hours after low molecular weight heparin.<sup>13</sup> Thus, if allowed by clinical circumstances, surgery should be post-poned at least 12 hours after low molecular weight heparin injection.

Recent registry studies suggest that low molecular weight heparins may be safely substituted for unfractionated heparin in patients receiving glycoprotein IIB-IIIa blockers,<sup>14</sup> apparently decreasing the bleeding risk. However this issue is still open to question and actually addressed by on-going clinical trials.

A direct comparison between enoxaparin and the pentasaccharide ORG 31540/SR90107A in more than 1000 patients with no-ST-elevation is currently ongoing (PENTUA).

At present the meta-analysis does not support the concept of long-term treatment with low molecular weight heparin in patients with unstable angina/non-Q wave myocardial infarction, an idea arising from the observation that coronary events continue to occur for months after the onset of the dis-

ease. However the issue is still not resolved: patient selection, the dosage of low molecular weight heparin, concomitant medical and mechanical treatments might all affect the results. In a post-hoc analysis of the FRISC-II study the extended dalteparin treatment significantly reduced mortality at three months in patients with minor troponin T elevation (3.9% to 2.1%,  $p=0.045$ ), or ST-segment depression (4.8% to 2.4%,  $p=0.04$ ) and in those with both troponin elevation and ST-segment depression (6.0% to 2.2%,  $p=0.009$ ).<sup>15</sup>

#### Atrial fibrillation

Patients with atrial fibrillation are a rapidly growing population which carries the risk of potentially fatal or devastating thromboembolic complications. The risk of thromboembolism increases with age, in presence of high blood pressure, left ventricular systolic or diastolic dysfunction, prior thromboembolism, valvular heart disease, diabetes mellitus. Risk is also increased early after the onset of atrial fibrillation and at the time of cardioversion (either electrical or pharmaceutical).

Usually anticoagulation for atrial fibrillation is based on warfarin, with heparin covering the peri-operative periods and in general as a bridge to full oral anticoagulation. However warfarin treatment is frequently boring both to the patient and the physician and entails the risk of major bleeding with a tiny, yet distinct, risk of intracranial haemorrhage. Particularly in the first two months after warfarin initiation large fluctuations in the INR values are frequently observed, exposing patients at the risk of both thromboembolism and bleeding. In the recently published ACUTE study<sup>16</sup> the rate of major bleeding after 8 weeks of warfarin treatment in patients undergoing traditional cardioversion was 1.5% (an

annualized rate of 9.0%/year); in those patients requiring only a 4-weeks warfarin treatment after a strategy of immediate cardioversion following a negative transesophageal echocardiogram, the annualized major bleeding risk was 9.6%/year.

Not only the quality of anticoagulation affects the risk of bleeding, but probably the efficacy of cardioversion as well. Recent observations suggest that the time to first therapeutic INR may be as long as 2 weeks and the time to a 3-weeks therapeutic INR may be on average 6 weeks.<sup>17</sup> The prolongation of the time spent before cardioversion might, in turn, affect the efficacy of the manoeuvre.

Recently it has been shown that low molecular weight heparin may actually promote the dissolution of left atrial thrombi at transesophageal echocardiography and small studies have been conducted using low molecular weight heparin as a unique means to anticoagulate patients undergoing cardioversion. Whether or not low molecular weight heparin will partially or totally replace warfarin in the management of pericardioversion embolic risk will be determined by ongoing trials.

### Conclusions

Low molecular weight heparin is now strongly recommended by both the European Society of Cardiology and the American College of Cardiology in the treatment of unstable/angina and non-Q-wave myocardial infarction (level of evidence A).

The management of atrial fibrillation requiring cardioversion with low molecular weight heparin is attractive and presently addressed by ongoing studies.

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## **Venous And Arterial Thromboembolism: From Heparin To The New Antithrombotics**

*Chairmen: P.P. Gazzaniga, G.G. Nenci*

### **New pharmacologic strategies for arterial and venous thromboembolism**

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Unfractionated heparin, coumarins, and aspirin, are effective antithrombotic agents, but they have a number of limitations. Unfractionated heparin and coumarins have a narrow therapeutic window and a highly variable dose-response relationship and thus they require close laboratory monitoring. Aspirin fails to block platelet activation by agonists other than thromboxane A<sub>2</sub>. Two new classes of agents, low molecular weight heparins and the glycoprotein IIb/IIIa antagonists, have shown clear benefits over traditional antithrombotic agents in several clinical indications. A further improvement is expected from selective thrombin and factor Xa inhibitors, modulators of the protein C pathway, and inhibitors of the factor VIIa/tissue factor pathway.

Selective thrombin inhibitors can be classified as direct and indirect (with respect to whether they require or not a plasma cofactor). Among the direct inhibitors the polypeptides hirudin, hirulog and the low molecular weight non-peptide inhibitors argatroban, efegatran and inogatran are the most extensively evaluated while melagatran has been shown to be effective when given orally. Dermatan sulphate, a selective indirect thrombin inhibitor, that acts through the heparin cofactor II, has been shown to be effective in the prevention

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of venous thromboembolism and in the management of patients with heparin-induced thrombocytopenia.

Selective inhibitors of factor Xa inhibit this enzyme through an antithrombin-independent pathway. These inhibitors include tick anticoagulant peptide (TAP) and antistasin which are available in recombinant forms, and a number of non-peptide compounds. However, the most promising selective inhibitor of factor Xa is a synthetic analog pentasaccharide, the high affinity antithrombin-binding sequence of heparin.

Strategies to enhance the protein C anticoagulant pathway include activated protein C concentrates, and recombinant soluble thrombomodulin.

The inhibitors of the factor VIIa/tissue factor pathway include recombinant tissue factor inhibitor (TFPI), inhibitors of activated factor VII (FVIIa), and a nematode anticoagulant protein designated as NAPc2.

## Venous And Arterial Thromboembolism: From Heparin To The New Antithrombotics

Chairmen: P.P. Gazzaniga, G.G. Nenci

### Overview of the clinical results of pentasaccharide in major orthopedic surgery

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The development of potent and safe antithrombotic agents still represents an important challenge.<sup>1</sup> The pentasaccharide fondaparinux is a new synthetic antithrombotic molecule which selectively inhibits factor Xa by binding to antithrombin.<sup>2-6</sup> In addition, fondaparinux has a favorable pharmacokinetic profile allowing a once-daily subcutaneous administration.<sup>7</sup> The antithrombotic efficacy and safety of fondaparinux have been investigated in various thrombotic disorders<sup>8,9</sup> and recently in a large global clinical program performed in patients undergoing major orthopedic surgery. We report here a meta-analysis of the data obtained in four phase III clinical trials which were designed with the same comparative drug, endpoints and Adjudication Committee.<sup>10-13</sup>

#### Design and Methods

##### *Study design and patient population*

The respective efficacy and safety of fondaparinux and enoxaparin in the prevention of venous thromboembolism following major orthopedic surgery were studied in four multicenter, randomized, parallel-group, double-blind clinical trials.<sup>10-13</sup> Patients were considered for inclusion if they were scheduled for the type of surgery studied (Table 1). The main reasons for exclusion are presented in the Table 2.

##### *Medications and Dosing Schedule*

Patients were randomly assigned to receive subcutaneously either fondaparinux (Arixtra®, Sanofi-Synthelabo and NV Organon) or enoxaparin (Clexane®/Klexane®/Lovenox®, Aventis Pharma) in a double-blind manner. Fondaparinux was administered at the dose of 2.5 mg, once daily. The first injection was to be performed six hours (four to eight hours) post-operatively, and the second injection at least twelve hours after the first one but no more than 24 hours after surgical closure.

Enoxaparin was to be administered according to one of the standard North-American regimens in two studies, i.e., twice-daily, at a dose of 30 mg started twelve to twenty-four hours post-operatively<sup>11,12</sup> and in the other two studies, enoxaparin was administered according to the other approved regimen, i.e., once daily at a dose of 40 mg started 12 hours before surgery.<sup>10,13</sup>

##### *Outcome measures*

The primary efficacy outcome was venous thromboembolism, defined as deep-vein thrombosis, pulmonary embolism, or both, up to day 11. Secondary efficacy outcomes included total, proximal and distal only deep-vein thrombosis and pulmonary embolism (fatal and non-fatal) up to day 11, and pulmonary embolism (fatal and non-fatal) up to day 49. Patients were systematically examined for deep-vein thrombosis by mandatory ascending bilateral contrast venography of the legs between days 5 and 11, but no more than two days after the last study drug injection, or earlier if thrombosis was clinically suspected. Symptomatic pulmonary embolism was confirmed by high-probability lung scanning, pulmonary angiography, or helical computed tomography, or, in the event of death, at autopsy. The primary safety outcome included fatal bleeding, bleeding that was retroperitoneal, intracranial, intraspinal or involved any other critical organ, bleeding leading to reoperation, and overt bleeding with a bleeding index of two or more. The bleeding index was calculated as follows: [number of units of packed red blood cells or whole blood transfused] plus [(pre-bleeding) minus (post-bleeding) hemoglobin values, in grams per deciliter]. Secondary safety outcomes were death, other bleeding, and any other adverse events. Efficacy outcomes, bleeding and death were adjudicated by a central independent committee whose members were unaware of the patients' treatment assignment.

**Table 1. Inclusion criteria of the four phase III clinical trials.**

Study	Type of surgery
EPHESUS & PENTATHLON 2000 <sup>10,11</sup>	Primary elective total hip replacement surgery or revision of at least one component of a previously implanted total hip prosthesis
PENTAMAKS <sup>12</sup>	Elective major knee surgery, i.e. surgery requiring resection of the distal end of the femur or proximal end of the tibia, or revision of at least one component of a previously implanted total knee prosthesis
PENTHIFRA <sup>13</sup>	Standard surgery for fracture of the upper third of the femur, including femoral head and neck (if surgery was planned within 48 hours after admission)

### Statistical analysis

Odds reductions with 95% confidence intervals for each study and each type of surgery were calculated for the primary efficacy endpoint. Before pooling all the efficacy data, the homogeneity between the four studies was tested (Zelen's exact test). The common odds reduction was estimated with a two-sided 95% confidence interval using a stratified exact approach. Safety parameters were analyzed by summing the numbers of events observed in each study.

### Results

#### Study populations and patient characteristics

A total of 7,344 patients were randomized between November 1998 and January 2000 in 375 centers distributed worldwide. The safety analysis was performed in 7,237 (98.5%) patients since 107 patients, equally distributed between the two treatment groups did not receive any study drug. The primary efficacy analysis was made in 5,385 (73.3%) patients since in 1,852 patients, equally distributed between the two treatment groups, appropriate surgery was not performed, or venography could not be performed or was not evaluable by day 11. The treatment groups in all four studies were well balanced for baseline characteristics and for treatment compliance. Finally, follow-up was continued to day 49 for all but 70 patients (36 in the fondaparinux group and 34 in the enoxaparin group) and its duration was comparable between the two groups.

#### Incidence of venous thromboembolism

The results show superior efficacy of fondaparinux over enoxaparin in preventing venous thromboembolism (Table 3) with a risk reduction of 55.2% in favor of fondaparinux ( $p=10^{-17}$ , Figure 1). Importantly, the superior efficacy of fondaparinux over enoxaparin was also found for proximal deep-

vein thrombosis with a risk reduction of 57.4% (95% confidence interval: 35.6% to 72.3%), (Table 3). The incidences of fatal and non-fatal pulmonary embolism up to day 49 were low (<1%) and did not differ between the two groups.

#### Bleeding episodes and death

The superior efficacy of fondaparinux over enoxaparin was achieved without any increase in

**Table 2. Exclusion criteria of the four phase III clinical trials.**

• Multiple trauma affecting more than one organ system, or if >24 hours had elapsed between the causative trauma and hospital admission in the hip fracture surgery (PENTHIFRA <sup>11</sup> )
• Planned bilateral joint surgery during the same procedure or within two weeks after inclusion (EPHESUS, PENTATHLON 2000 and PENTAMAKS, <sup>8,10</sup> )
• Women of childbearing age if pregnant or not using effective contraception
• Active bleeding
• Acute bacterial endocarditis
• Documented congenital or acquired bleeding disorder
• Current ulceration or angiodysplastic gastrointestinal disease
• Hemorrhagic stroke or brain, spinal or ophthalmological surgery within the previous three months
• Planned indwelling intrathecal or epidural catheter during the study treatment period, unusual difficulty in achieving epidural or spinal anesthesia (e.g. more than two attempts)
• Hypersensitivity to heparin, LMWH, porcine products or iodinated contrast medium
• Contraindication to anticoagulant therapy
• Serum creatinine concentration above 2 mg/dL in a well hydrated patient
• Platelet count below $100 \times 10^9/L$
• Need for anticoagulant therapy
• Current addictive disorders

**Table 3. Incidence of efficacy and safety outcomes up to day 11.**

	Fondaparinux n/N (%)	Enoxaparin n/N (%)
<b>Efficacy Outcomes</b>		
Venous thromboembolism (primary outcome)	182/2862 (6.8)	371/2703 (13.7)
Any deep-vein thrombosis	174/2677 (6.5)	363/2698 (13.5)
Any proximal deep-vein thrombosis	35/2756 (1.3)	81/2775 (2.9)
Distal deep-vein thrombosis only	141/2704 (5.2)	293/2709 (10.8)
Non-fatal pulmonary embolism	9/3603 (0.2)	7/3608 (0.2)
Fatal pulmonary embolism	2/3603 (0.1)	3/3608 (0.1)
<b>Safety Outcomes</b>		
Fatal bleeding	0/3616 (0.0)	1/3621 (0.0)
Bleeding in critical organ	0/3616 (0.0)	1/3621 (0.0)
Bleeding leading to re-operation	12/3616 (0.3)	8/3621 (0.2)
Bleeding with bleeding index >2*	84/3616 (2.3)	53/3621 (1.5)
Wound infection	37/3616 (1.0)	29/3621 (0.8)
Complications at surgical site leading to prolonged hospitalization or re-hospitalization	52/3616 (1.4)	52/3621 (1.4)
Any death	15/3616 (0.4)	21/3621 (0.6)

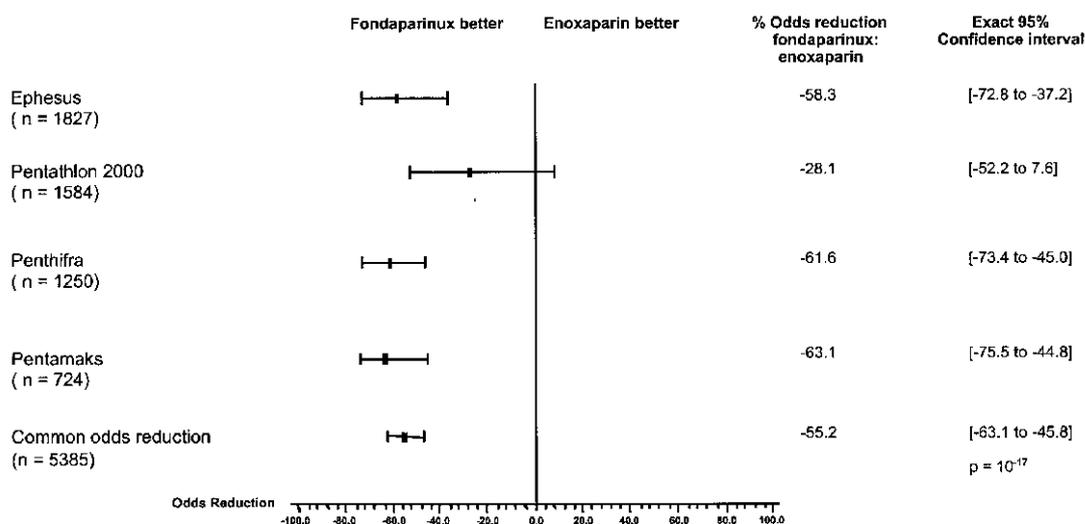
*n* is the number of patients with events and *N* is the total number of patients assessed for this event. \*The bleeding index was calculated as follows: [number of units of packed red blood cells or whole blood transfused] + [(pre-bleeding) - (post-bleeding) hemoglobin (g/dL) values].

the risk of clinically important bleeding which was low in both groups (Table 3). The number of deaths from any cause and the number of any other adverse events did not differ between the two treatment groups (Table 3).

## Discussion

The present meta-analysis of four phase III clinical trials performed in orthopedic surgery demonstrates that 2.5 mg of fondaparinux, a new synthetic factor Xa inhibitor, administered once daily, starting six hours post-operatively, significantly reduces the rate of venous thromboembolism as compared with approved enoxaparin regimens. Importantly, the incidence of proximal deep-vein thromboses which are more prone to embolize was particularly decreased by fondaparinux with a reduction in risk of 57.4% compared with enoxaparin. The incidence of clinical pulmonary embolism was low and less than 1% in both treatment groups.

This efficacy was not associated with an increased bleeding risk. The incidence of clinically relevant bleeding events associated with the fon-



**Figure 1. Incidence of venous thromboembolism up to day 11 (primary efficacy). Odds reduction per study and estimated common odds reduction (percentages and exact 95 percent confidence intervals) with fondaparinux relative to enoxaparin. Homogeneity test for elective hip replacement studies:  $p=0.068$ ; homogeneity test for all studies:  $p=0.058$ ; common odds ratio test:  $p=10^{-17}$ .**

daparinux administration was low and comparable to that observed with enoxaparin (Table 3).

In conclusion, this meta-analysis shows that 2.5 mg of fondaparinux, once daily, starting six hours post-operatively appears to be the new reference antithrombotic strategy in the prophylaxis of venous thromboembolism in orthopedic surgery, being both very effective and safe.

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## Venous And Arterial Thromboembolism: From Heparin To The New Antithrombotics

Chairmen: P.P. Gazzaniga, G.G. Nenci

### New antithrombotic agents in the management of venous thromboembolism

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**A**nticoagulants inhibit thrombin generation and fibrin formation. Heparin and warfarin, the established antithrombotic agents, show efficacy, but have some limitations. Unfractionated heparin has limitations in terms of pharmacokinetic and biophysical properties and in terms of safety. Coumarins require careful laboratory monitoring because of concerns about safety.

The development of new antithrombotic agents has been stimulated by clinical needs and by advances in biotechnology that have made it possible to produce drugs that target specific steps in thrombogenesis. In particular, low-molecular-weight heparin and direct inhibitors of thrombin have been evaluated clinically. Orally active direct inhibitors of thrombin and factor Xa may replace coumarins in the near future. Unfractionated heparin and the coumarins have two major limitations: a narrow therapeutic window and a highly variable dose-response relation. Consequently, the use of these drugs can be complicated by serious bleeding and their anticoagulant effects must be monitored closely by laboratory tests. The unpredictable anticoagulant effect of heparin reflects its propensity to bind non-specifically to plasma proteins and proteins released from activated platelets and endothelial cells. The concentrations of these heparin-binding proteins are often increased (to variable degrees) in patients with thromboembolic disease which limits the amount of heparin available to interact with antithrombin. The erratic anticoagulant effect of coumarins is less well understood, but the likely explanations are: variability in the affinity of warfarin for its hepatic receptor, changes in vitamin K content of diet, fluctuations in bioavailability, concomitant use of interacting drugs, inappropriate

dosage adjustment, and poor compliance. Another limitation of unfractionated heparin is its inability to inactivate thrombin bound to fibrin and factor Xa bound to activated platelets trapped within the thrombus. This is an important limitation because these thrombus-associated clotting enzymes may cause thrombus growth during heparin treatment and reactivate the thrombotic process once treatment is stopped. There is no convincing clinical evidence that newer anticoagulants have a wider therapeutic window than heparin or coumarins. However, low molecular weight heparins and some direct inhibitors of thrombin have a more predictable anticoagulant effect than unfractionated heparin. Clinical studies have clearly demonstrated that twice-daily subcutaneous injections of low molecular weight heparin are as well tolerated and effective as unfractionated heparin in the treatment of deep vein thrombosis. As a consequence, this simplified approach is now used routinely in many countries. Home treatment for venous thromboembolic disease is also becoming a common practice. Further simplification has been suggested by the demonstration that a fixed once-daily dose of low molecular weight heparin is as effective and safe as the same drug administered by a twice-daily regimen, or as intravenous unfractionated heparin. The role of new anticoagulants is not yet fully known. New antithrombotics and those currently in development include agents acting through a variety of mechanisms, but most aim at inhibiting only one specific coagulation step. It is anticipated that higher selectivity will allow better control of anticoagulation therapy (Figure 1).

Tissue factor pathway inhibitors act at an early point in the coagulation cascade to inhibit the tis-

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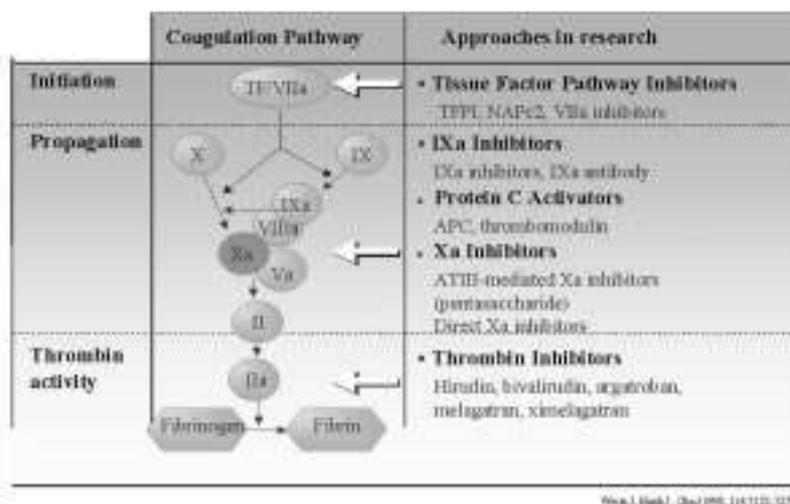


Figure 1

sue factor/factor VIIa complex. This counters the initiation of the coagulation cascade, but may have less impact on its amplification. Direct thrombin inhibitors, such as hirudin, act directly on thrombin to block its activity as well as the feedback mechanisms linked to thrombin. Hirudin is more effective than heparin in unstable angina and non-Q-wave infarction, apparently more effective than unfractionated or low molecular weight heparin in the prevention of venous thrombosis after major orthopedic surgery, and is effective in patients with heparin-induced thrombocytopenia. However, hirudin causes more bleeding than heparin.

Other agents, including the IXa inhibitors, protein C activators and selective factor Xa inhibitors, such as pentasaccharide, inhibit the generation of thrombin.

Pentasaccharide is the first of a new class of synthetic antithrombotics: the selective inhibitors of factor Xa. It is the most advanced competitor of low molecular weight heparins, which are the reference drugs in prophylaxis and treatment of venous thromboembolism. It is thus interesting to compare the mechanism of action and pharmacokinetics of these two classes of drugs. Low-molecular-weight heparins are obtained from unfractionated heparin preparations of animal extractive origin, and are multi-targeted drugs inhibiting through angiotensin III (ATIII), mainly factors IIa, IXa and Xa. Pentasaccharide is entirely obtained by

chemical synthesis. Pentasaccharide molecules have a high affinity for angiotensin III (ATIII) and have a specific binding site within the ATIII molecule. This binding results in selective inhibition of factor Xa via ATIII. Factor Xa plays a central role in the cascade and its inhibition results in strong inhibition of thrombin formation and clot growth. A linear relationship exists between the pentasaccharide dose and inhibition of thrombin generation, through both the extrinsic and the intrinsic pathways. Phase I studies conducted in volunteers with Pentasaccharide showed essentially linear kinetics both in young and elderly subjects. Bioavailability of pentasaccharide is complete when given via the subcutaneous route, with a rapid onset of action and low variability of effect. The half-life of pentasaccharide is longer than that of low molecular weight heparins: 13–21 hours and about 4 hours respectively. Once or twice daily subcutaneous injections of low molecular weight heparins are used prophylactically in Europe and North America respectively, while a once-daily administration of pentasaccharide ensures a complete 24-hour antithrombotic effect. According to these results, a once-daily dose of Pentasaccharide for all patients was chosen for Phase II and III studies in prophylaxis of venous thromboembolism in major orthopedic surgery, in order to demonstrate an improved benefit/risk ratio in comparison to low molecular weight heparins.

## Best Abstracts

## TERAPIA DOMICILIARE DELLA TROMBOSI VENOSA PROFONDA: CHI RICOVERIAMO?

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La TD della TVP è una pratica clinica ormai largamente diffusa in molti centri specializzati. I criteri di esclusione dalla TD, mutuati dai principali studi, sono generici e la loro applicazione varia da centro a centro. Abbiamo analizzato retrospettivamente le caratteristiche cliniche di 100 pazienti consecutivi con TVP trattati presso il Centro Trombosi dell'Ospedale di Circolo di Varese e abbiamo confrontato i pazienti in TD e quelli ricoverati. I criteri di esclusione alla TD da noi applicati sono: scarsa affidabilità (per l'assunzione della terapia e per l'adesione alle visite di controllo), elevato rischio emorragico, altre patologie richiedenti il ricovero. Dei nostri pazienti (92 TVP arto inferiore e 8 arto superiore, 12 con concomitante EP sintomatica), 72 sono stati interamente trattati a domicilio (età media 61.2, range 27-91) e 28 ricoverati (età media 68.8, range 26-88). Quasi la metà dei ricoveri sono stati motivati da patologie neoplastiche (12 casi, 43%): 6 pazienti con neoplasia metastatizzata nota e condizioni cliniche scadenti; 2 pazienti con neoplasia nota in apparente remissione e sospetto di recidiva (confermato); 4 pazienti con anamnesi negativa e sospetto clinico di tumore (confermato in 2 ed escluso negli altri 2). I sospetti clinici erano legati al riscontro di trombosi bilaterale (2), trombosi femoro-iliaca senza interessamento della poplitea (2), anemia di nuovo riscontro (1) e lesione polmonare sospetta alla radiografia del torace (1). Altre cause di ricovero sono state: prevista impossibilità di gestione domiciliare (10) per condizioni scadenti associate a patologie concomitanti (5) o scarsa affidabilità (5), elevato rischio emorragico (2), sintomatologia importante (4). Sono rimaste idiopatiche 12 TVP (43%). Tra i pazienti in TD, erano idiopatiche 41 TVP (57%). Due pazienti sono stati studiati ambulatorialmente per sospetta neoplasia senza esito. Avevano una neoplasia nota 12 pazienti (16.5%), tutti molto favorevoli alla TD. Dei 72 pazienti in TD, 3 sono stati persi al follow-up a 3 mesi. Tra i rimanenti si sono verificate 1 recidiva di TVP (1.5%) (in paziente non neoplastico), 1 emorragia maggiore (macroematuria in neoplasia vescicale nota) (1.5%), 3 minori (4%) e 3 decessi (4%), tutti per la neoplasia nota. Tra i ricoverati si sono verificate 2 recidive di TVP (7%), 3 emorragie minori (11%) e 5 morti (18%), di cui una per embolia polmonare, tutte in pazienti neoplastici. La TD eseguita con regolari controlli ambulatoriali è sicura ed efficace ed è ben accettata dai pazienti. Occorre affinare dei criteri per indicare il ricovero nel sospetto di neoplasia. Nella nostra realtà questo determina degenze di circa 14 giorni.

## FREQUENZA DELLA TROMBOFILIA EREDITARIA NEL PAZIENTE ONCOLOGICO: RISULTATI PRELIMINARI

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Il tromboembolismo venoso è una comune complicanza nel paziente neoplastico. La fisiopatologia della trombogenesi associata alla neoplasia è in larga misura determinata da una condizione di trombofilia acquisita. Scarsamente conosciuta è l'associazione fra ipercoagulabilità neoplastica e trombofilia ereditaria. *Scopo del lavoro.* Esaminare nel paziente oncologico la frequenza di alcuni difetti ereditari della coagulazione associati con il tromboembolismo venoso, quali il difetto di antitrombina III, proteina S e proteina C, la mutazione C1691A del fattore V (fattore V Leiden), la variante G20210A della protrombina ed il polimorfismo C677T dell'enzima 5-10 metilentetraidrolato-reduttasi (MTHFR). *Metodi.* Cinquantapazienti consecutivi (età media 68 anni), affetti da tumori solidi dell'apparato digerente e della pelvi, e 50 soggetti di controllo, comparabili per età, sono stati arruolati nello studio. L'attività dell'antitrombina III e della proteina C sono state determinate con metodo cromogenico e l'attività della proteina S con test coagulativo. Il fattore V Leiden ed il polimorfismo C677T della MTHFR sono stati determinati con la polymerase chain reaction (PCR) e l'analisi di restrizione e la variante G20210A della protrombina con la tecnica di allele-specific PCR. *Risultati.* Nei pazienti neoplastici la frequenza delle alterazioni congenite della coagulazione era la seguente: difetto di proteina S 6%, difetto di proteina C 2%; individui portatori dell'allele A del Fattore V Leiden 2% (GA genotipo), dell'allele A della variante G20210A della protrombina 10% (GA genotipo) e dell'allele T del polimorfismo C677T della MTHFR 74% (44% CT e 30% TT genotipo). Non sono stati osservati casi con difetto di antitrombina III. Nel gruppo di controllo la frequenza delle alterazioni congenite della coagulazione era la seguente: difetto di proteina S 2%; individui portatori dell'allele A del fattore V Leiden 2% (GA genotipo), dell'allele A della variante G20210A della protrombina 4% (GA genotipo) e dell'allele T del polimorfismo C677T della MTHFR 74% (48% CT e 26% TT genotipo). Non sono stati osservati casi con difetto di antitrombina III e di proteina C. La frequenza dei diversi fattori di rischio trombofilico non era statisticamente differente fra i due gruppi. *Conclusioni.* Nel nostro gruppo di pazienti oncologici la frequenza della trombofilia ereditaria non differisce da quella dei soggetti di controllo. Ulteriori studi sono in corso per confermare questi risultati e comprendere la loro rilevanza clinica.

## MARCATORI EMOSTATICI E INFIAMMATORI IN RAPPORTO AL RISCHIO A 1 ANNO DI MORTE VASCOLARE O NUOVI EVENTI VASCOLARI NON FATALI IN PAZIENTI CON PRIMO EPISODIO DI ICTUS ISCHEMICO

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**Introduzione.** Vi sono dati sperimentali che suggeriscono che infiammazione e trombosi siano strettamente correlate nelle malattie vascolari. È stata proposta la misurazione dei marcatori di infiammazione o trombosi come metodo per migliorare la previsione del rischio nei pazienti con eventi cardiovascolari. Utilizzando le informazioni ottenute durante il periodo di reclutamento 1998-1999 contenute nella Banca Dati dell'Ictus di Villa Pini, abbiamo valutato il valore dei livelli di D-dimero, proteina C reattiva, fibrinogeno, e altri indicatori del rischio come predittivi di un end-point definito come l'insorgenza di morte vascolare o di nuovi eventi cardiaci o cerebrali non fatali, in qualunque ordine, nel corso del follow-up di un anno. **Metodi.** Sono stati analizzati, entro le prime 24 ore dall'insorgenza dell'infarto, i livelli di proteina C reattiva, fibrinogeno, e D-dimero di 231 pazienti inclusi nella banca dati prospettica della nostra Istituzione. Le curve di sopravvivenza cumulativa libera da eventi vascolari in rapporto al livello di ciascuno di questi indicatori sono state determinate con il metodo di Kaplan-Meier, con l'uso di log-rank test. Gli aggiustamenti per il possibile effetto confondente di età, fattori di rischio vascolare, tipo e gravità dell'ictus sono stati effettuati con il multivariate Cox proportional hazards model. **Risultati.** Nel corso del follow-up, l'end-point è stato osservato nel 21% dei 62 pazienti con livelli di D-dimero inferiori a 312 µg/L, rispetto al 31% dei 114 pazienti con livelli compresi tra 312 e 1327 µg/L e il 38% dei 55 pazienti con livelli superiori a 1327 µg/L ( $p=0,1234$ , log-rank test e  $p=0,0528$ ,  $\chi^2$ -test per la tendenza, rispettivamente). L'incidenza degli eventi era del 12% nei 52 pazienti con livelli di proteina C reattiva inferiori a 5 mg/L, del 25% nei 129 con livelli compresi tra 5 e 33 mg/L, e infine del 62% nei 50 con livelli superiori a 33 mg/L ( $p<0,0001$  e  $p<0,0001$ , rispettivamente). L'incidenza degli eventi è risultata pari al 18% nei 73 pazienti con livelli di fibrinogeno inferiori a 3,78 g/L, al 29% nei 112 con livelli compresi tra 3,78 e 6,17 g/L, e infine del 50% nei 46 pazienti con livelli superiori a 6,17 g/L ( $p=0,0012$  e  $p=0,0006$ , rispettivamente). All'analisi multivariata è risultato che solo l'elevazione dei livelli di proteina C reattiva (HR:1,98, 95%CI:1,21-3,24;  $p=0,0062$ ) era un predittivo indipendente del rischio di eventi a un anno, diversamente dall'elevazione dei livelli di fibrinogeno e D-dimero. **Conclusioni.** L'elevazione dei livelli di proteina C reattiva, ma non di fibrinogeno e D-dimero, dopo ictus ischemico è fortemente legata al rischio a un anno di nuovi eventi vascolari fatali e non.

## OMOCISTEINEMIA E PNEUMOPATIA CRONICA OSTRUTTIVA

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**Introduzione.** Da studi sperimentali ed epidemiologici è emersa l'esistenza di una correlazione tra broncopneumopatia cronica ostruttiva (BPCO) e rischio di malattie coronariche. Gli autori hanno ipotizzato che l'iperomocisteinemia, un fat-

to di rischio indipendente per le malattie cardiovascolari, possa giocare un ruolo rilevante in questo processo. **Metodi.** Sono stati misurati i livelli plasmatici di omocisteina totale in 16 pazienti con esacerbazione acuta di BPCO (11 uomini e 5 donne, di età pari a  $70,94\pm 7,78$  anni), in 17 pazienti con BPCO stabile (15 uomini e 2 donne, di età pari a  $70,39\pm 6,83$  anni) e in 16 soggetti sani (11 uomini e 5 donne, di età pari a  $70,27\pm 6,62$  anni). Sono stati esclusi i pazienti con neoplasie, i fumatori, i pazienti con diabete mellito, malattie cardiovascolari e insufficienza renale. Il sangue venoso è stato prelevato a digiuno e i prelievi sono stati raccolti in vacutainer contenenti EDTA. I campioni sono stati raccolti in ghiaccio, e il plasma è stato separato entro 15 minuti e congelato a  $-80^\circ\text{C}$  per le successive analisi. I livelli di omocisteina sono stati determinati con HPLC. **Risultati.** Vedi Tabella 1 alla fine del testo. I livelli plasmatici di omocisteina totale sono apparsi significativamente più elevati nei pazienti con esacerbazione acuta di BPCO rispetto ai controlli ( $p=0,005$ ) e nei pazienti con BPCO stabile rispetto ai controlli ( $p=0,015$ ). Non sono state osservate correlazioni significative tra i pazienti con esacerbazione acuta di BPCO e quelli con BPCO stabile. Tredici pazienti con esacerbazione acuta di BPCO sono stati trattati con teofillina, un farmaco che potrebbe interferire con il metabolismo dell'omocisteina; il confronto tra i livelli plasmatici di omocisteina totale di questi pazienti ( $15,66\pm 5,18$ ) e quelli dei 17 pazienti con BPCO stabile che non assumevano teofillina ( $17,5\pm 9,93$ ) non ha evidenziato differenze significative ( $p=0,552$ ). **Conclusioni.** Il nostro studio dimostra, per la prima volta, che i pazienti con BPCO presentano livelli plasmatici elevati di omocisteina, che potrebbe fungere da fattore di rischio per l'aterosclerosi prematura osservata in questi soggetti. Rimangono da approfondire i meccanismi del rapporto tra iperomocisteinemia e BPCO.

Table 1.

	Pazienti con esacerbazione acuta di BPCO	Pazienti con BPCO stabile	Controlli
N	16	17	16
Hcy (mmol/L)±SD	15.49±5.08	17.49±9.50	11.19±2.53
PaO <sub>2</sub> (mmHg)±SD	59.7±9.0	74.3±10.5	88±7.5
PaCO <sub>2</sub> (mmHg)±SD	48.8±8.2	43±5.3	40±4.9
pH±SD	7.35±3.3	7.39±3.5	7.4±1.5
FEV1 (litri)±SD	1.18±0.44	1.55±0.93	2.71±0.53
FVC (litri)±SD	2.15±0.7	2.76±1.3	3.01±0.42
Trattamento con teofillina	13	—	—

## EFFETTO DELL'ACIDO ACETILSALICILICO (ASA) SU TRE TEST DI FUNZIONALITÀ PIASTRINICA

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Scopo del nostro lavoro è valutare l'efficacia antiaggregante piastrinica dell'aspirina attraverso tre diversi metodi di laboratorio su due gruppi (A) e (B) di 30 pazienti in terapia rispettivamente con 100 mg/die e 300 mg/die di ASA da almeno una settimana e un gruppo di controllo C (20 soggetti sani). L'età dei pazienti studiati era tra 50 e 77 anni, valori di ematocrito e di conta piastrinica nella norma. In tutti i soggetti sono stati determinati: a) il tempo di emorragia con Surgicutt, stru-

mento automatico che pratica un'incisione cutanea standardizzata; b) l'aggregazione piastrinica con aggregometro PACK-4 (Ditta Helena Laboratories), con ADP a concentrazione finale di 3 mM, e acido arachidonico (AA), a concentrazione finale di 500 mg/mL; c) il tempo di emorragia in vitro su strumento PFA 100 (Dade Behring). Il principio di misurazione di questo strumento si basa sul modello *in vitro* del vaso sanguigno lesionato: il sangue citratato è aspirato attraverso un capillare ed una membrana ricoperta di collagene e ADP o Epinefrina; sull'apertura della membrana si forma un trombo, fino a determinare l'occlusione, con un tempo di chiusura (TC) che costituisce un indice di funzionalità piastrinica. I valori di riferimento dei tre test per la normalità sono: tempo di emorragia 2-8 minuti; aggregazione max% ADP 30-75; AA% 72-92; tempo di chiusura su collagene/epinefrina < 160 secondi, su collagene/ADP < 120 secondi. I risultati ottenuti permettono di rilevare che: 1) sia nel gruppo A che nel gruppo B i tempi di emorragia identificano solo parzialmente i pazienti in terapia rispetto al gruppo di controllo; 2) L'aggregazione piastrinica identifica i due gruppi in terapia rispetto al gruppo di controllo ( $p < 0,001$ ), senza differenze significative ( $p = 0,83$ ) per quanto riguarda l'attività aggregante da AA, che è inibita; l'attività aggregante da ADP risulta ridotta maggiormente nel gruppo B rispetto al gruppo A ( $p < 0,015$ ). 3) L'effetto dell'aspirina sul tempo di chiusura su collagene/epinefrina è incostante, sia per i fattori già riportati dalla letteratura (tempi della fase preanalitica, livello plasmatico del fattore von Willebrand), sia per la buona sensibilità dello strumento (95%), ma la più modesta specificità (80%). La differente sensibilità all'aspirina sul prolungamento del TC di cartucce collagene-epinefrina, rispetto all'inalterato TC di cartucce collagene/ADP, è in grado di differenziare l'effetto farmacologico dalle turbe congenite dell'emostasi primaria. La minore risposta per l'ADP rispetto agli altri metodi è legata alla differente tipologia del campione (sangue intero e plasma).

#### MODIFICAZIONI EMOREOLOGICHE E MICROCIRCOLAZIONE DURANTE IL TRATTAMENTO CON ANTIAGGREGANTI PIASTRINICI E CON ANTICOAGULANTI ORALI IN PAZIENTI AFFETTI DA VASCULOPATIE CRONICHE

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Scopo del nostro studio è stato quello di valutare l'influenza di alcuni farmaci comunemente usati nella pratica clinica sull'assetto emoreologico e microcircolatorio di pazienti con vasculopatie ischemiche croniche. Abbiamo esaminato 48 pazienti (età media  $72 \pm 14$ ), di cui 30 in terapia antiaggregante piastrinica: 18, 10F e 8M, assumevano ticlopidina 250 mg/die, 12, 4F e 8M, assumevano acido acetilsalicilico 150 mg/die. Gli altri 18 pazienti (9F e 9M) erano in terapia anticoagulante orale con warfarin (I.N.R. tra 2 e 3.5). In tutti i pazienti abbiamo valutato: la viscosità ematica (Reometro piatto-cono Carri-Med a  $10 \text{ s}^{-1}$  di shear-rate), l'esame emocromo con il Coulter-Count, il fibrinogeno plasmatico con il metodo coagulativo, la morfologia eritrocitaria con il metodo di Zipursky-Forconi, la VCAM-1, molecola di adesività vasocellulare (metodica Elisa), indice di disfunzione endoteliale, attivata dai radicali liberi e dallo stress ossidativo. Questi parametri sono stati valutati prima di iniziare la terapia, dopo 7 giorni e dopo 3 mesi. I risultati ottenuti mostrano nei pazienti in terapia con Ticlopidina una riduzione della viscosità in maniera progressiva e statisticamente significativa e del fibrinogeno plasmatico (da  $7.56 \pm 0.89$  a  $7.29 \pm 1.16$  a  $6.62 \pm 0.88$  cPs; da  $468 \pm 130$  a  $448 \pm 120$  e a  $369 \pm 113$  mg %). La morfo-

logia eritrocitaria mostra una prevalenza dei discociti sulle forme a scodella (E.M.I., indice morfologico eritrocitario, da 0.61 a 0.72), il valore medio della VCAM-1 si riduce senza significatività statistica ( $26.8 \pm 8.9$ ;  $24.5 \pm 7.6$ ;  $22.45 \pm 8.6$  ng/ml). Il gruppo in terapia con acido acetilsalicilico mostra un valore medio di viscosità che va da  $7.52 \pm 1.94$  che poi si riduce a  $7.13 \pm 1.36$  e risale a  $7.85 \pm 1.62$  cPs, il fibrinogeno subisce un lieve aumento dopo 3 mesi ( $468 \pm 117$ ,  $459 \pm 120$ ,  $488 \pm 110$  mg%). La morfologia delle emazie mostra sempre una prevalenza di discociti rispetto alle forme a scodella in tutte le misurazioni; il valore della VCAM-1 presenta una lieve ma costante riduzione sia a breve che a lungo termine ( $24.45 \pm 9.3$ ;  $22.53 \pm 7.9$ ;  $21.78 \pm 8.8$ ). Nei pazienti in terapia con warfarin, la viscosità ematica si riduce significativamente dopo 7 giorni ( $7.81 \pm 1.44$ ;  $7.54 \pm 1.57$ ) per poi risalire a  $7.63 \pm 1.27$  cPs; il fibrinogeno plasmatico passa da  $417 \pm 104$  a  $366 \pm 112$  e a  $347 \pm 58$  mg%, quest'ultimo valore è statisticamente significativo. La morfologia eritrocitaria mostra, in condizioni basali, una prevalenza delle forme discocitiche rispetto alle forme a scodella, queste si riducono dopo 7 giorni e poi risalgono (E.M.I.: 0.66, 0.92, 0.72). Il valore della VCAM-1 non subisce sostanziali modificazioni nelle varie misurazioni. I nostri risultati mettono in evidenza che la ticlopidina ed il warfarin presentano un effetto positivo sulla viscosità, infatti riducono i livelli di fibrinogeno plasmatico, fattore molto importante sia della viscosità e che del sistema coagulazione-fibrinolisi. I farmaci antiaggreganti piastrinici dimostrano inoltre un modesto effetto favorevole anche sulla VCAM-1, molecola di adesività vasocellulare prodotta dall'endotelio ed espressione di disfunzione endoteliale; queste osservazioni, che completeremo anche con la valutazione dei metaboliti dell'ossido nitrico, dimostrano che l'azione di farmaci attivi sull'aggregazione piastrinica possiede effetto favorevole anche sulla disfunzione endoteliale che è la condizione patologica alla base della progressione della malattia aterosclerotica.

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