Thrombosis

trends in hematology oncology

haematologica 2002; 87:757-770

http://www.haematologica.ws/2002_07/757.htm

Perspectives on antithrombotic agents: from unfractionated heparin to new antithrombotics

Giancarlo Agnelli, Francesco Sonaglia

Division of Internal and Cardiovascular Medicine, Department of Internal Medicine, University of Perugia, Italy

Background and Objectives. Unfractionated heparin (UFH) has been the antithrombotic agent of choice for the prevention and treatment of venous thromboembolism (VTE) for a long time. UFH is also widely used for the treatment of patients with acute coronary syndromes. However, UFH has some limitations such as the need for parenteral administration and close monitoring of its anticoagulant effect. UFH is also associated with bleeding, heparin-induced thrombocytopenia and osteoporosis.

Evidence and Information Sources. Low molecular weight heparins (LMWHs) are produced by the depolymerization of UFH. LMWHs have pharmacologic advantages over UFH: a better bioavailability after subcutaneous administration, a longer plasma half-life and a more predictable anticoagulant effect. These improved features allow once or twice daily subcutaneous injection of weight-adjusted doses of LMWHs without requiring laboratory monitoring in patients with VTE or unstable angina.

Perspectives. A number of new antithrombotic agents are currently under development. These include direct antithrombins and factor Xa inhibitors. The results of the main clinical trials with LMWHs as well as those of the studies with the new antithrombotic agents will be reviewed in this article.

Key words: unfractionated heparin, low molecular weight heparins, venous thromboembolism, acute coronary syndromes, direct antithrombins, pentasaccharide.

Correspondence: Giancarlo Agnelli M.D. Division of Internal and Cardiovascular Medicine, Department of Internal Medicine, University of Perugia, via Enrico dal Pozzo, 06126 Perugia, Italy. Phone: international +39.075.5783395. Fax: international +39.075.5722011 or 5733642. E-mail: agnellig@unipg.it

Unfractionated heparin (UFH) has, for a long time, been the anticoagulant agent of choice for the prevention and treatment of deep vein thrombosis (DVT) and pulmonary embolism (PE). UFH has also been extensively evaluated in the management of arterial thrombosis, in patients with acute myocardial infarction to speed-up thrombolysis or to prevent mural ventricular thrombosis, in patients with unstable angina and in patients undergoing vascular surgery and percutaneous transluminal coronary angioplasty (PTCA) to prevent acute arterial thrombosis. UFH has some pharmacological limitations mainly related to its non-specific binding to plasma proteins (fibrinogen, factor VIII, vitronectin and fibronectin), endothelial cells and macrophages. This binding complicates the clearance of UFH, reduces UFH bioavailability, and is responsible for the marked inter-individual variation in the dose-anticoagulant response. UFH also has biophysical limitations due to the inability of the heparin/antithrombin complex to inactivate factor Xa in the prothrombinase complex. Moreover, UFH is not able to inactivate thrombin when this is bound to fibrin or to exposed subendothelial matrix. Subcutaneous injection of UFH at low doses is associated with low bioavailability and a short half-life. Moreover, UFH may cause heparin-induced thrombocytopenia (HIT), a disease that is often complicated by arterial and/or venous thrombosis (HITT) and has a severe prognosis.

Low molecular weight heparins (LMWHs) are derivatives of UFH, produced by its depolymerization. LMWHs are composed of mixtures of saccharide chains with a mean molecular weight close to 5,000 Daltons. Approximately one third of the heparin chains contain the pentasaccharide sequence that constitutes the heparin-binding site to antithrombin. LMWHs bind less avidly to plasma proteins and endothelial cells than UFH. They have an almost complete bioavailability after subcutaneous administration of low doses, a more linear relationship between dose and anticoagulant effect and a longer plasma half-life than UFH. Finally, HIT is less commonly associated with the use of LMWHs.

LMWHs in the prevention of post-operative venous thromboembolism

General surgery

Without prophylaxis, patients undergoing general surgery have a 19% incidence of venographically-detected DVT. The incidence of proximal DVT (popliteal or more proximal veins) is 6-7%; the incidence of clinically overt PE and fatal PE is 1.6 and 0.9%, respectively.[1] The most extensively investigated anticoagulants are low doses of UFH (5,000 IU every 8 or 12 hours) (LDUFH) and LMWHs. A pooled analysis of 29 trials including more than 8,000 patients randomized to LDUFH (started 2 hours before surgery) or placebo showed that LDUFH reduces the incidence of DVT from 25 to 8%. Data from a meta-analysis[2] and large clinical trials indicate that LDUFH also reduces the occurrence of fatal PE.[3] LMWHs were compared with LDUFH in a series of studies and several meta-analyses. Enoxaparin was investigated in five trials on the prevention of VTE after general surgery. Samama *et al.* compared enoxaparin 20, 40 and 60 mg given once daily with LDUFH every 8 hours.[4] DVT was diagnosed by a fibrinogen uptake test (FUT) confirmed by venography. The incidence of DVT was 3.8, 2.8 and 2.9% in patients receiving enoxaparin at doses of 20, 40 and 60 mg and 7.8, 2.7 and 3.8% in LDUFH patients (p=ns). Dalteparin given at a dose of 2,500 to 5,000 anti-Xa IU once daily showed a similar efficacy and safety to LDUFH, while the 7,500 anti-Xa IU dose of dalteparin was associated with an increased incidence of bleeding. Three studies compared nadroparin (2,850 anti-Xa IU) with LDUFH in the prevention of VTE in general surgery.[5-7] Two studies showed the superiority of nadroparin over LDUFH.[5,6] DVT was assessed by FUT and confirmed by venography. In the third study the incidence of DVT, assessed by Doppler ultrasound and plethysmography, confirmed by venography, was low in both treatment groups.[7] Nadroparin was not associated with an increase in bleeding. Reviparin sodium was investigated in a large multicenter trial at a dose of 1,750 anti-Xa IU, and showed a similar efficacy to LDUFH every 12 hours but a lower incidence of bleeding.[8]

Therefore, LMWHs are at least as effective as LDUFH in the prevention of VTE in general surgery patients. The overall incidence of DVT with LMWHs is 6%. LMWHs have the advantage of their once daily administration. Bleeding complications are related to the dose of LMWHs. Indeed, doses of LMWHs higher than 3,400 anti-Xa IU/day are associated with a higher incidence of bleeding complications than LDUFH, whereas doses lower than 3,400 IU/day are associated with a lower incidence of bleeding than that associated with LDUFH.

Cancer surgery

The incidence of DVT diagnosed by bilateral venography in patients undergoing surgery for cancer without prophylaxis is 29%. Patients treated with LDUFH have a 13.7% incidence of DVT. Enoxaparin at a dose of 40 mg once daily was compared with LDUFH every 8 hours in patients undergoing elective abdominal or pelvic surgery for cancer in a large, randomized double-blind venography study.[9] The incidence of DVT was 14.7% in the enoxaparin group and 18.2% in the LDUFH group (p=ns). No difference was observed in terms of bleeding episodes. The results of the ENOXACAN II study have been recently published.[10] This was a randomized, double-blind, venography study evaluating the efficacy and safety of post-discharge prophylaxis with enoxaparin in cancer surgery patients. After an open phase with in-hospital prophylaxis of enoxaparin (40 mg once daily for a week), patients were randomized to 3 weeks of prophylaxis with enoxaparin (40 mg once daily) or to placebo. Patients underwent bilateral venography at the end of the double-blind period. Prolonged prophylaxis with enoxaparin was associated with a significant reduction in the incidence of VTE. The incidence of DVT was 4.8% in patients randomized to enoxaparin and 12% in placebo-treated patients. Moreover, the incidence of proximal DVT was 0.6 and 1.8%, respectively. No increase in major bleeding was associated with prolonged enoxaparin prophylaxis.

Major orthopedic surgery

Patients undergoing major orthopedic surgery (elective total hip replacement, elective total knee replacement or surgery for hip fracture) have a particularly high risk of post-operative VTE.

Patients undergoing total hip replacement (THR) have a high incidence of post-operative VTE despite prophylaxis. Mechanical methods have a moderate efficacy, making pharmacologic prophylaxis necessary. The use of aspirin is associated with a 56% incidence of venographic DVT and is not recommended for the prevention of VTE in these patients. LDUFH are not adequately effective in preventing VTE in THR patients. The most effective anticoagulant regimens in the prevention of VTE in these patients are adjusted dose UFH[11] or oral anticoagulants[12] and LMWHs. Adjusted dose UFH or oral anticoagulants are both effective but require strict laboratory monitoring. Two meta-analyses indicate that LMWHs are more effective

than LDUFH in patients undergoing elective THR.[13,14] Moreover, several randomized trials showed that LMWHs are at least as safe and effective as adjusted dose UFH or oral anticoagulants in the prevention of VTE in this setting.[15-17]

The incidence of venographically-detected DVT in patients undergoing elective total knee replacement (TKR) without prophylaxis is close to 60%; approximately 25% of these DVT are proximal. LDUFH and aspirin are barely effective in these patients. A meta-analysis of randomized controlled clinical trials showed that LMWHs are more effective than LDUFH and oral anticoagulants.[18] The efficacy and safety of LMWHs in the prevention of VTE after TKR were investigated in several randomized, controlled clinical trials evaluating enoxaparin, ardeparin, tinzaparin and nadroparin.[17,19-26] Prophylaxis started post-operatively except in two trials and was continued for 4-14 days. DVT was diagnosed by bilateral venography assessed by experts unaware of treatment allocation. LMWHs showed a higher efficacy than oral anticoagulants, however the main difference was confined to isolated distal DVT. The incidence of overall DVT in patients treated with LMWHs was 30%.

The incidence of overall DVT without prophylaxis in patients undergoing surgery for hip fracture is close to 50%. About half of the DVT are proximal; moreover these patients have a high risk of PE. The incidence of DVT with LDUFH was 27%; few trials investigated LMWHs in this setting.[27-29] Dalteparin, 2,500 anti-Xa IU once daily, was compared with LDUFH every 8 hours in 90 patients undergoing surgery for hip fracture; this dose of dalteparin was less effective than LDUFH.[27] Dalteparin 5,000 anti-Xa IU was compared with placebo showing a moderate efficacy (DVT 30 and 58%, respectively).[28] A trial compared enoxaparin at 20 and 40 mg doses, showing that 40 mg once daily was more effective than the 20 mg dose with no increase in bleeding.[29] A meta-analysis of clinical trials on VTE prevention with LDUFH, LMWHs and mechanical methods, in patients undergoing surgery for hip fracture showed that LMWHs and LDUFH have a similar efficacy.[30] A recent trial compared enoxaparin, dalteparin and danaparoid in patients with hip fracture.[31] This randomized trial enrolled 197 patients. Prophylaxis was given for 9-11 days and then bilateral venography was performed. The results indicated no difference between enoxaparin, dalteparin and danaparoid.

The optimal duration of prophylaxis of VTE after major orthopedic surgery has been greatly debated. There are several data showing a clear reduction of venographic DVT associated with prolonged (3 weeks after discharge) out-of-hospital prophylaxis with enoxaparin and dalteparin after elective THR. However, the clinical relevance of an asymptomatic venographic DVT is not clear. Indeed, data from recently reported overviews indicate that prolonged prophylaxis with LMWHs or LDUFH is associated with a significant reduction of symptomatic VTE (1.3 vs 3.3%).[32] These results have been confirmed by a recent overview of randomized trials evaluating prolonged out-of-hospital prophylaxis with LMWHs after THR.[33]

Elective neurosurgery

Patients undergoing elective neurosurgery have a high incidence of VTE; on the other hand, clinicians are frightened of bleeding, especially intracranial. For this reason, the most investigated prophylactic methods are elastic stockings and intermittent pneumatic compression (IPC). IPC was associated with a reduction of the incidence of DVT from 23 to 6%. LMWHs were investigated in two large randomized clinical trials. In the first trial, post-operative nadroparin associated with elastic stockings was associated with a non-significant reduction of DVT (26.3, vs 18.7%, *p*=ns).[34] Nadroparin use caused an increase of bleeding (2.3 vs 0.8%). In the second trial, enoxaparin, 40 mg once daily, started after surgery, associated with elastic stockings was compared with elastic stockings alone. Enoxaparin reduced the incidence of DVT from 32.6 to 16.9% and the incidence of proximal DVT from 13.2 to 5.4% without an increase in bleeding.[35]

LMWHs in the initial treatment of venous thromboembolism

Until recently the standard initial treatment for VTE has been a course of 5 to 10 days of intravenous UFH followed by at least 3 months of oral anticoagulants. Following the promising results of the initial studies, LMWHs were investigated in two large randomized trials. These studies showed that subcutaneous LMWHs, at weight-adjusted doses, were at least as effective as intravenous UFH.[36,37] The results of the early meta-analyses assessing the efficacy of LMWHs in comparison with UFH in the treatment of VTE suggested that LMWHs are more effective and safer than UFH.[38,39] More recently, an updated meta-analysis comparing the efficacy and safety of UFH and LMWHs was published.[40] Thirteen studies were included in the analysis.[36,37,41-51] The results of the comparison between LMWHs and UFH did not show significant differences in the incidence of recurrent VTE, PE, and major bleeding.

However, the reduction in mortality in patients treated with LMWHs already observed in previous analyses, was confirmed. This meta-analysis also assessed the potential differences in efficacy and safety among various LMWHs. No difference in efficacy among dalteparin, enoxaparin, tinzaparin, reviparin and nadroparin was observed. A lower incidence of major bleeding was found in patients treated with nadroparin as compared with UFH.

LMWHs in the treatment of acute coronary syndromes

Aspirin and intravenous UFH have been the recommended treatment for patients with acute coronary syndromes for a long time.[52] However, the limitations of UFH led to the investigation of new agents such as LMWHs and direct thrombin inhibitors.[53] Five large clinical trials investigated three LMWHs (dalteparin, enoxaparin, and nadroparin) in patients with acute coronary syndromes without ST elevation.[54]

In the FRISC trial, 1,506 patients were randomized to dalteparin or placebo within 72 hours from the onset of symptoms.[55] Dalteparin-treated patients received a twice daily injection of 120 U/kg in the acute phase (6 days) and were then randomized to dalteparin 7,500 IU once daily or placebo in the chronic phase (until day 45). At day 6 the incidence of the composite end-point death, myocardial infarction and urgent revascularization was significantly lower in dalteparin treated patients 5.4% vs 10.3% (p=0.005). This difference remained significant at day 40 (p=0.005).

The FRIC study compared dalteparin (120 IU/kg twice daily) with UFH (5,000 IU intravenous bolus followed by 1,000 IU/hour for 48 hours and then by two daily injection of 12,500 IU) until day 6 in patients with unstable angina within 72 hours from symptom onset.[56] From day 7 to 45, all patients were randomized to dalteparin (once daily dose of 7,500 IU) or placebo. Dalteparin showed a similar efficacy to UFH in reducing the incidence of the combined end-point, death, myocardial infarction and recurrent angina.

The FRAXIS study investigated nadroparin in 3,468 patients with unstable angina within 48 hours from symptoms' onset. Patients were randomized to UFH for 6 days (intravenous bolus of 5,000 IU followed by continuous infusion at doses adjusted by the aPTT) or to 6 days of nadroparin (bolus of 86 IU anti-Xa/kg followed by twice daily subcutaneous injections of 86 IU anti-Xa/kg) or to 14 days of nadroparin at the same dose regimen.[57] At day 6 the incidence of the combined end-point of cardiac death, myocardial infarction, and refractory or recurrent angina was similar in the three groups. Patients treated with 14 days of nadroparin had a higher incidence of bleeding complications.

The ESSENCE study enrolled 3,171 patients with unstable angina within 24 hours from symptoms' onset.[58] Patients were randomized to 2-8 days' treatment with subcutaneous enoxaparin (1 mg/kg every 12 hours) or to intravenous UFH (5,000 IU bolus followed by an infusion at an aPTT target of 55 to 85 seconds). Follow-up was performed at 30 days and at one year. Enoxaparin was more effective than UFH; the combined end-point death, myocardial infarction and recurrent angina at day 14 occurred in 16.6% and in 19.8% of patients, respectively (p=0.02). At day 30, the incidence of the end-point was 19.8 and 23.3% (p=0.02). No increase in major bleeding was observed.

In the TIMI 11B trial,[59] patients treated with enoxaparin received an initial bolus of 30 mg followed by enoxaparin, 1 mg/kg twice daily for 3-8 days. After day 8, enoxaparin dose was reduced to 40 mg/twice daily for patients with a body weight less than 65 kg and to 60 mg/twice daily for patients over 65 kg. Subcutaneous enoxaparin was continued until day 43. At day 14, enoxaparin was associated with a 15% reduction of the combined end-point, death, myocardial infarction and urgent revascularization in comparison with the incidence of the same end-point in patients treated with UFH (14.2 vs 16.7%, p=0.03). At day 43, this difference was no longer statistically significant.

The meta-analysis of the ESSENCE and TIMI 11B trials showed the superiority of enoxaparin versus UFH. Enoxaparin was associated with a 20% reduction of the composite end-point at day 2, 8, 14 and 43 in comparison to UFH without an increase in major bleeding.[60] The results of the one-year follow-up of the ESSENCE study confirmed the superiority of enoxaparin over UFH concerning death, myocardial infarction or residual angina (p=0.022). In conclusion, the results of large randomized clinical trials in acute coronary syndromes indicate that enoxaparin is more effective than UFH, without increasing bleeding. Dalteparin and nadroparin showed an efficacy similar to UFH in this clinical setting. Enoxaparin was recently investigated as an adjunct to thrombolytic therapy in patients affected by acute Q-wave myocardial infarction.[61] The ASSENT III was a randomized, open trial conducted in 6,095 patients with acute myocardial infarction. Patients were randomized, to three regimens: full-dose tenecteplase and enoxaparin for a maximum of 7 days (enoxaparin group; n=2,040), full-dose

tenecteplase with weight-adjusted UFH for 48 hours (UFH group; n=2,038), or half-dose tenecteplase with weight-adjusted UFH and a 12-hour infusion of abciximab, a platelet glycoprotein IIb/IIIa inhibitor (abciximab group; n =2,017). The cumulative primary efficacy end-point was 30-day mortality, in-hospital reinfarction, and in-hospital refractory ischemia. Enoxaparin (11.4% vs 15.4%; p=0.0002) and abciximab plus UFH (11.1% vs 15.4%; p<0.0001) were more effective than UFH. Considering its ease of administration, tenecteplase plus enoxaparin seems an attractive alternative reperfusion regimen.

New antithrombotic agents

The limitations of UFH and LMWHs promoted the development of new antithrombotic agents that could potentially overcome the shortcomings. New antithrombotic agents have specific targets at different levels of the coagulation cascade. In this article, the agents in more advanced phase of clinical investigation will be briefly reviewed.

New antithrombotic agents can be classified in three main categories: direct thrombin (factor IIa) inhibitors, inhibitors of factor Xa, inhibitors of the factor VIIa/tissue factor (TF) complex.

Direct thrombin inhibitors

Human thrombin is a serine protease composed of an A *light* chain (39 amino acids) and a B *heavy* chain of 259 amino acids linked by a disulphide bridge. Thrombin converts fibrinogen to fibrin. Thrombin also activates factor V, factor VIII, thrombomodulin and factor XIII. Moreover, thrombin is a potent platelet agonist. Based on these considerations, thrombin is a crucial target for the development of new antithrombotic agents. Direct antithrombins inhibit thrombin without a plasma cofactor and inhibit both free and fibrin-bound thrombin.[62] Moreover, since direct antithrombins do not bind to plasma proteins, they have a more linear and predictable anticoagulant effect than UFH. Another advantage of direct antithrombins is that their use is not associated to HIT. The most investigated direct antithrombins are hirudin, its synthetic fragment hirulog and the low molecular weight inhibitors of the thrombin active site such as melagatran and its oral prodrug ximelagatran.

Hirudins. Hirudin, a 65-amino acid polypeptide produced by the salivary glands of a medicinal leech (*Hirudo medicinalis*) is the most potent known natural anticoagulant and is now produced through DNA recombinant technology.[63] Hirudin is a specific thrombin inhibitor that acts through the generation of an almost irreversible stoichiometric complex (1:1) with thrombin. It blocks both the active site and the fibrinogen recognition site of thrombin. Hirudin is cleared by the kidneys, and has a plasma half-life of 40 min after intravenous administration and of 120 min after subcutaneous injection.

Desirudin (CGP39393) was investigated in large randomized trials on VTE prevention after THR.[64] In the first trial, 1,119 patients undergoing elective THR were randomized to twice daily subcutaneous injection of desirudin at 10, 15 or 20 mg, or to UFH 5,000 IU three times daily. The incidence of DVT was 23.9, 18.9 and 18.2% in patients treated with desirudin and 34.2% in patients treated with UFH. The rate of proximal DVT in patients treated with the 3 regimens of desirudin was 8.5, 3.1 and 2.4%, respectively. The incidence of proximal DVT in patients treated with UFH was 19.6%. A subsequent trial compared the twice daily injections of 15 mg desirudin with UFH (5,000 IU every 8 hours) in 445 patients undergoing THR. The incidence of DVT was 7.5% in the desirudin group and 23.2% in the UFH group (p <0.0001). The incidence of proximal DVT was 3.4% in the desirudin group and 16.4% in the UFH group (p<0.0001). Desirudin was more effective than enoxaparin in the prevention of VTE after THR in a large, randomized, double blind, clinical trial. Patients (n = 2,079) were assigned to twice daily injection of 15 mg of desirudin, starting 30 minutes before surgery, or to enoxaparin 40 once daily, started the evening before surgery. Prophylaxis was continued for 8-12 days. Patients treated with desirudin had an 18.4% incidence of DVT, while enoxaparin-treated patients had an incidence of DVT of 25.5% (p=0.001). The rate of proximal DVT was 4.5% in the desirudin group and 7.5% in the enoxaparin group (p=0.01). The safety profiles of desirudin and enoxaparin were similar. Therefore desirudin is highly effective in the prophylaxis of DVT in patients undergoing THR and has an acceptable safety profile.

The other form of recombinant hirudin, lepirudin (HBW023) was evaluated in the treatment of VTE in a multicenter, randomized, dose-finding trial. This trial compared 3 doses of lepirudin (0.75, 1.25 and 2 mg/kg twice daily) given subcutaneously with the standard UFH regimen.[65] The 0.75 and 1.25 mg/kg doses of lepirudin were at least as effective as intravenous UFH and were not associated with increased bleeding; by contrast, the 2 mg/kg lepirudin dose was associated with a 3% rate of major bleeding.

The GUSTO IIb study was a prospective, double-blind, randomized comparison of 72 hours' therapy with UFH or desirudin in 12,142 patients with

acute coronary syndromes.[66] Patients without ST elevation were randomized to UFH (5,000 IU bolus followed by 1,000 IU/hour infusion, target aPTT 60 to 90 seconds) or to desirudin (0.1 mg/kg bolus followed by a 0.1 mg/kg/hour infusion). The primary composite end-point was death or non-fatal myocardial infarction at 30 days. The incidence of this end-point was 8.3% in patients treated with desirudin and 9.1% in UFH treated patients (*p*=ns). Desirudin was associated with a higher incidence of intracranial bleeds (0.2 and 0.02%; *p*= ns) and with a slight increase in severe or moderate bleeding. However, desirudin was associated with a reduction in mortality and in the incidence of myocardial infarction during the first 24 hours over UFH (1.3 vs 2.1%; *p*=0.001).

Lepirudin was investigated in OASIS 2, an international, large-scale, randomized, double-blind trial conducted in patients with acute coronary syndromes without ST elevation.[67] This trial compared a 72-hour infusion of lepirudin (n=5,083), bolus of 0.4 mg/kg followed by an infusion of 0.15 mg/kg/hour, with UFH (n=5,058). The incidence of the primary end-point of cardiovascular death or new myocardial infarction at 7 days was lower in the lepirudin group than in UFH group, but this difference was not statistically significant. The incidence of the secondary end-point cardiovascular death, myocardial infarction or refractory angina, was significantly lower in lepirudin-treated patients (5.6 vs 6.7%; p=0.012). Patients treated with lepirudin had a higher incidence of major bleeding (1.2 vs 0.7%; p=0.01). However, the rate of potentially fatal bleeding and strokes was similar.

In the GUSTO IIa study, patients with myocardial infarction were randomized to UFH, 5,000 IU bolus followed by infusion at a target aPTT from 60 to 90 seconds, or to desirudin: 0.6 mg/kg bolus followed by 0.2 mg/kg/hour infusion.[68] Patients with ST elevation were randomized to streptokinase or rt-PA. The trial was prematurely interrupted because of the high incidence of intracranial bleeding in both groups. Patients receiving thrombolytic therapy had a 1.8% incidence of hemorrhagic strokes, while this event occurred in only 0.3% of the patients not exposed to thrombolytics (*p*<0.001). The TIMI 9A trial was a randomized comparison of UFH and desirudin in patients with myocardial infarction treated with streptokinase or rt-PA.[69] The doses of UFH and desirudin were the same as those in the GUSTO IIa study. The TIMI 9A trial was also prematurely discontinued because of the high incidence of major bleeding.

In the HIT III trial, patients with myocardial infarction receiving rt-PA were randomized to UFH (70 IU/kg bolus followed by 15 IU/kg/hour infusion) or to lepirudin (0.4 mg/kg bolus followed by 0.15 mg/kg/hour infusion).[70] Given the high rate of intracranial bleeding in the lepirudin group, this study was prematurely terminated. The incidence of major bleeding was 6.8% in the lepirudin-treated patients and 1.9% in UFH-treated patients.

Given the high rate of intracranial bleeding observed in the GUSTO IIa and TIMI 9A, lower doses of hirudin were tested in subsequent studies.

In the TIMI 9B study, 3,002 patients with acute coronary syndromes were randomized to UFH at the doses of the GUSTO I trial or to a lower dose of desirudin (0.1 mg/kg bolus followed by 0.1 mg/kg/hour infusion).[71] No difference in death or myocardial infarction was observed between the two treatment groups. Intracranial bleeding occurred in 0.7% of UFH-treated patients and in 0.4% of patients assigned to desirudin. The incidence of other bleeding episodes was about 5% in both groups.

The GUSTO IIb trial investigated reduced doses of desirudin and UFH as an adjunct to thrombolytics (70% of patients received rt-PA and 30% streptokinase) in patients with myocardial infarction. Desirudin was given as intravenous bolus of 0.1 mg/kg followed by a 0.1 mg/kg/hour infusion for 3-5 days. UFH was given as 5,000 IU bolus followed by 1,000 IU/hour infusion (aPTT target 60-85 seconds). The primary outcome was death or non-fatal myocardial infarction or early reinfarction. The incidence of the primary end-point in patients receiving hirudin was 9.9% whereas in patients treated with UFH it was 11.3% (*p* = ns). The incidence of death or myocardial infarction at 24 and 48 hours was reduced in patients with ST elevation treated with desirudin. There was no difference in the incidence of major bleeding in patients with ST segment elevation, but minor bleeding was more frequent in patients receiving desirudin. The benefit of desirudin over UFH observed in the first 48 hours was partially lost at 30 days. The results of the meta-analysis of the GUSTO IIb and TIMI 9B trials indicate that desirudin is associated with a significant (*p*=0.024) reduction in reinfarction rate at 30 days.[72] The relative risk reduction (RRR) is 14% (95% confidence interval 0.75-0.98).

Desirudin was compared with UFH after elective PTCA for unstable angina in the HELVETICA study, a multicenter, randomized, double-blind trial.[73] Patients (n = 1,141) were assigned to UFH (bolus of 10,000 IU followed by a 24-hour infusion and subcutaneous placebo twice daily for 3 days; n=382), or to one of two different desirudin regimens: a

bolus of 40 mg followed by a 24 hour-infusion followed by subcutaneous placebo twice daily for 3 days (n=381), or to the same initial regimen of desirudin followed by subcutaneous twice daily administration of 40 mg of desirudin for 3 days (n= 378). The primary end-point was event-free survival after 7 months. Secondary end-points were cardiac events during the first 96 hours, bleeding episodes and other drug-related side-effects and the angiographic assessment of coronary luminal diameter at the 6-month follow-up. The event-free survival rate was similar among the three groups: 67.3% in the UFH group, 63.5% in the group receiving intravenous desirudin alone, and 68.0% in the group randomized to intravenous plus subcutaneous desirudin. Desirudin markedly reduced early cardiac events (p= 0.023). By contrast, the mean minimal luminal diameters at the 6-month angiographic follow-up were similar among the three groups.

Desirudin was investigated in HIT, an immune condition that is often associated with thromboembolic complications.[74] Direct antithrombins have no structural analogy with UFH and do not cross-react with HIT antibody, therefore are an ideal agent for patients for HIT. The results of two prospective trials investigating lepirudin in patients with documented HIT type II have been reported.[75,76] In the first trial, patients were treated with one of 4 doses of lepirudin.[75] Patients with HIT and thrombosis (HITT) received a 0.4 mg/kg bolus followed by an infusion of 0.15 mg/kg/hour; patients with HITT treated with thrombolytics received a bolus of 0.2 mg/kg followed by 0.1 mg/kg/hour. Patients without thrombosis were treated with lepirudin at 0.1 mg/kg/hour, whereas patients undergoing cardiopulmonary bypass[4] received a bolus of lepirudin of 0.25 mg/kg and then boluses of 5 mg as needed. Laboratory evaluation criteria were an increase of the platelet count of more than 30% until 100,000 mm^3 and an aPTT ratio between 1.5 to 3 with no more than 2 dose adjustments. Platelet count rose rapidly in the 88.7% of the patients affected by HIT treated with lepirudin. The combined clinical end-point was death, amputations, and new thromboembolic complications. Results were compared with those from a historical cohort of 120 patients affected by documented HIT. The primary combined end-point incidence was lower in the lepirudin-treated patients at days 7 and 35 (9.9% vs 23% and 25.4% vs 52%; p = 0.014). The incidence of bleeding was similar.

In the second study, 112 patients with confirmed HIT were treated with lepirudin at 3 different doses (treatment of HITT 0.4 mg/kg bolus followed by 0.15 mg/kg/hour infusion, treatment of HITT in conjunction with thrombolytics 0.2 mg/kg and 0.1 mg/kg/hour infusion, patients without thrombosis 0.1 mg/kg/hour infusion).[76] The incidence of the primary combined end-point of death, limb amputation and new thromboembolic event at day 35 was 30.4% while it was 52.0% in the historical control group. In this study, the difference was not statistically significant. There was an increase in the incidence of bleeding with respect to that in the historical control group (44.6 vs 27.2%; p=0.0001) however; there was not an increase in transfusions. Lepirudin was also investigated in 57 patients with HIT undergoing cardiopulmonary bypass;[77] 95% of patients had a favorable clinical outcome. Based on these results, the clinical use of hirudin in patients affected by HIT has been approved in the United States and in Europe.

Hirulog. Hirulog is a synthetic, bivalent antithrombin agent. Hirulog was developed by connecting hirugen, through a tetraglycine linker, to a peptide specific for the inhibition of the catalytic site of thrombin. Hirugen is a synthetic dodecapeptide composed of the carboxy-terminal region of hirudin, which blocks the interaction of thrombin with fibrinogen. Hirulog was investigated in the prevention of VTE after major orthopedic surgery in a dose-finding study in 222 patients.[78] Prophylaxis with subcutaneous hirulog started post-operatively and was continued until day 11. The dose varied from 0.3 mg/kg/12 hours to 1.0 mg/kg/8 hours. The lowest incidence of DVT (17%) and of proximal DVT (2%) was observed in patients treated with the highest dose of hirulog (1.0 mg/kg every 8 hours). Bleeding was observed in less than 5% of the patients. These results suggest that 1.0 mg/kg of hirulog given subcutaneously three-times daily is effective and safe for the prophylaxis of VTE in major orthopedic surgery patients when given post-operatively.

A randomized, double-blind, dose-finding trial investigated hirulog in 410 patients with unstable angina.[79] Patients received 3 days of continuous hirulog infusion at four different doses: 0.02 (n = 160), 0.25 (n = 81), 0.5 (n = 88), and 1.0 (n = 81) mg/kg/hour. The primary combined end-point was death, non-fatal myocardial infarction, rapid clinical deterioration, or recurrent ischemic pain at rest associated with ECG changes during 72 hours. The rate of the primary end-point was similar among the four groups. There was a dose-related reduction in the incidence of the secondary end-point (death or non-fatal myocardial infarction through hospi-

tal discharge): 10% in patients receiving the lowest dose of hirulog and 3.2% in patients treated with the 3 highest regimens of hirulog (p=0.008). Only 2 patients had major bleeding. These results prompted further hirulog evaluation.

The results of the HERO 2 trial have been recently reported.[80] The HERO 2 was a randomized, open-label, assessor-blind trial comparing hirulog with UFH in patients undergoing fibrinolysis with streptokinase for acute myocardial infarction with ST elevation. Patients (n=17,073) were randomized to hirulog (intravenous bolus followed by 48 hour infusion) or to UFH, together with a 1.5 million unit dose of streptokinase. The primary end-point was 30-day mortality. Secondary end-points included reinfarction within 96 hours and bleeding. Analysis was by intention-to-treat. Mortality at day 30 was 10.8% the hirulog group and 10.9% in the UFH group (p=ns). There were significantly fewer reinfarctions within 96 hours in the hirulog group (p=0.001). Severe bleeding and intracerebral bleeding were similar in both groups. The incidence of moderate and mild bleeding was higher in the hirulog group. Hirulog did not reduce mortality compared to UFH, but did reduce the rate of adjudicated reinfarction within 96 hours by 30%. Bivalirudin seems to have a potential role as a new anticoagulant option in patients with acute myocardial infarction treated with streptokinase. Hirulog was investigated in a large, double-blind, randomized clinical trial in patients undergoing PTCA for unstable or post-infarction angina.[81] Patients (n = 4,098) were treated with UFH or hirulog immediately before PTCA. Hirulog infusion was continued for 24 hours. The primary composite end-point was in-hospital death, myocardial infarction, abrupt coronary occlusion, or rapid clinical deterioration from cardiac origin. The incidence of the primary end-point was similar in the two groups (11.4 and 12.2%, respectively). However, hirulog was associated with a lower incidence of bleeding (3.8 vs 9.8%; p<0.001). The cumulative rate of death, myocardial infarction, and repeated revascularization during the 6 months following angioplasty was similar (20.5 versus 25.1%). Hirulog appears to be at least as effective as UFH in patients undergoing PTCA for unstable angina, showing a better safety profile.

Low molecular weight active site inhibitors. Several low molecular weight active site thrombin inhibitors have been recently synthesized. These agents are competitive inhibitors that bind thrombin in a non-covalent manner. Argatroban, a synthetic derivative of arginine is the precursor of these agents. Argatroban has a short half-life and requires continuous intravenous administration. Argatroban has been successfully investigated in a wide cohort of patients affected by HIT and has been recently approved for this indication.

Melagatran, together with its oral prodrug (ximelagatran) is the most extensively investigated of these agents. Melagatran is a synthetic antithrombin that acts as a competitive and reversible inhibitor of the thrombin active site. Melagatran is administered by parenteral route, has a predictable anticoagulant activity and does not require laboratory monitoring. Ximelagatran has a reasonable gastrointestinal absorption and must be converted to melagatran to exert its antithrombotic activity.[82] Ximelagatran has a half-life of 3 hours and is administered every 12 hours. Melagatran and ximelagatran were initially investigated in the prevention of VTE in major orthopedic surgery in the METHRO I trial.[83] After the promising results of this trial a large phase IIb dose-finding study (METHRO II) compared melagatran (started immediately before surgery) and ximelagatran, with dalteparin (5,000 IU anti Xa) in about 1,900 patients undergoing major orthopedic surgery.[84] Patients were subdivided into 5 treatment groups. Doses ranged from 1 mg of melagatran every 12 hours followed by 8 mg of ximelagatran every 12 hours to 3 mg every 12 hours of melagatran followed by 24 mg of ximelagatran every 12 hours. The incidence of DVT in the group treated with the highest doses of melagatran and ximelagatran was 15.1%. The incidence of DVT in the dalteparin group was 28.2%. Thus, the dose of 3 mg melagatran followed by 24 mg of ximelagatran every 12 hours reduced the incidence of DVT by 47% in comparison to dalteparin. This dose also reduced the incidence of proximal DVT by half (3% vs 7%). The results obtained with the highest regimen of melagatran and ximelagatran were more evident in patients undergoing THR (incidence of DVT 12% and 25%, respectively) than in patients undergoing TKR (DVT incidence of 21% and 32%, respectively). The highest doses of melagatran and ximelagatran were associated with a moderate increase in major bleeding with respect to dalteparin.

METHRO III was a randomized, double-blind, double-dummy, parallel-group study in patients undergoing THR or TKR comparing the efficacy and safety of subcutaneous melagatran 3 mg started 4-12 h after surgery, followed by oral ximelagatran 24 mg twice daily, with subcutaneous enoxaparin 40 mg once daily started the evening before surgery.[85] Both treatments were continued for 8-11 days. Efficacy was evaluated by DVT assessed by bilateral

venography on the final day of treatment, and clinically suspected and verified DVT and PE during treatment. Of 2,788 patients, 2,268 (81.3%) had an evaluable venogram. The VTE rate was 31% and 27% in the melagatran plus ximelagatran and in the enoxaparin groups, respectively. The rate of proximal DVT or PE was 5.7% in the melagatran plus ximelagatran group and 6.2% in the enoxaparin group. Total bleeding was similar in the two groups. In a *post-hoc* analysis, the possible effect of timing of the first post-operative dose was evaluated; the population was divided by the median time of the first post-operative dose. The time interval between surgery and the first dose of anticoagulant was found to be crucial to ensure optimal efficacy.

The pharmacokinetic features of ximelagatran are rapid absorption and conversion to melagatran, reasonable bioavailability, low dose-time variability, bioavailability independent of food intake, predictable anticoagulant activity and good tolerability. These features make ximelagatran an attractive antithrombotic agent for the long-term treatment of both venous and arterial thromboembolism. On these bases, ximelagatran is currently under investigation in large, multicenter, international, randomized trials in the treatment of VTE and in the prevention of thromboembolic complications in patients with chronic atrial fibrillation.

Factor Xa inhibitors

Direct inhibitors of factor Xa. Direct factor Xa inhibitors bind to factor Xa and block all its activities. These agents include natural inhibitors of factor Xa such as the tick anticoagulant peptide (TAP) and antistasin. Based on the promising results of TAP in experimental animal models, a series of low molecular weight inhibitors that act against the active site of factor Xa have been recently synthesized. Some of these inhibitors of factor Xa, such as DX 9095a have been recently investigated in phase II trials in patients affected by unstable angina.

Indirect inhibitors of factor Xa. Pentasaccharide is a selective factor-Xa inhibitor composed of five saccharide units and is the smallest heparin-based molecule (molecular weight of 1,728 Daltons) that retains antithrombotic activity. Pentasaccharide has a plasma half-life of about 14 hours after intravenous or subcutaneous administration and has a complete bioavailability after subcutaneous injection. The peak concentration is reached in 1-3 hours. Pentasaccharide is excreted mainly through the urine.

The most clinically relevant data have been obtained in the prevention of VTE after major orthopedic surgery. The PENTATHLON study was a large (933 patients) phase IIB dose-finding trial in the prophylaxis of VTE after elective THR.[86] The dose ascending regimens of pentasaccharide were 0.75 mg, 1.5, 3, 6 and 8 mg, given once daily; the comparator was enoxaparin 30 mg started 12 hours after surgery and then given twice daily. The diagnosis of DVT was made with bilateral venography performed at day 5-10. A clear dose-response effect was observed for the first 3 groups of patients. The incidence of DVT was 11.8% in the 0.75 mg group, 6.7% in the 1.5 mg group and 1.7% in the group treated with 3 mg. The 6 and 8 mg groups were discontinued because of the high incidence of bleeding. The incidence of DVT in the enoxaparin group was 9.4%. Therefore, the 3 mg dose of pentasaccharide led to a highly significant reduction in the incidence of DVT with respect to enoxaparin (p=0.009, RRR of 81%) without a significant increase in major bleeding (4.5% vs 3.5%).

A dose-finding trial was also conducted in patients undergoing elective TKR: the PENTATAK study. The doses of pentasaccharide were the same as those adopted in the PENTATHLON study. The incidence of DVT was 40% in the 0.75 mg group, 30% in the 1.5 mg group and 15% in the 3 mg group. There was a clear dose-effect from the 0.75 to the 3 mg dose. Again, the 6 and 8 mg doses were associated with excessive bleeding. On the bases of the results of these trials, the dose chosen for phase III trials was 2.5 mg.

This dose was investigated in a very large phase III trial program in major orthopedic surgery enrolling 7,344 patients in four trials in patients undergoing elective THR (EPHESUS in Europe and PENTATHLON 2000 in North America), elective TKR (PENTAMAKS in North America) and surgery for hip fracture (PENTHIFRA in Europe). In the EPHESUS trial 2,200 patients undergoing elective THR were randomized to pentasaccharide 2.5 mg once daily starting at least 6 hours after surgery or to enoxaparin 40 mg once daily starting the evening before surgery.[87] The incidence of DVT was 4.1% in the pentasaccharide group and 9.2% in the enoxaparin group with a RRR of 56%; the incidence of proximal DVT was 0.7 and 2.5%, respectively. The incidence of major bleeding was 8.0 and 6.2%.

The efficacy of pentasaccharide in patients undergoing THR was confirmed by the results of the PENTATHLON 2000 study.[88] In this trial, 2,200 patients were randomized to pentasaccharide 2.5 mg at least 6 hours after surgery or to enoxaparin 30 mg twice daily starting at least 12 hours after surgery. The incidence of DVT was 6.2% in the pen-

tasaccharide group and 8.3% in the enoxaparin group, RRR=25%; there was no difference in proximal DVT. The incidence of major bleeding was also similar, being 3% and 3.2%, respectively.

The results of the PENTAMAKS study showed the superiority of pentasaccharide (2.5 mg/die once daily starting at least 6 hours after surgery) over enoxaparin (30 mg twice daily starting at least 12 hours after surgery) in 1,000 patients undergoing elective TKR.[89] The incidence of DVT was 12.5% and 27.8%, respectively with a RRR of 55%. Proximal DVT occurred in 2.4% of patients treated with pentasaccharide and in 5.4% of enoxaparin-treated patients. The incidence of major bleeding was 4.5% and 3.9%, respectively.

In the PENTHIFRA trial, 1,700 patients undergoing surgery for hip fracture were randomized to pentasaccharide 2.5 mg once daily starting at least 6 hours after surgery or to enoxaparin 40 mg once daily starting the evening before surgery.[90] The incidence of VTE was 8.3% in the pentasaccharide group and 19.1% in the enoxaparin group, RRR 56%; the incidence of proximal DVT was 0.9 and 4.1%. There was an increase in major bleeding events in pentasaccharide-treated patients (6.3 vs. 4.4%).

The results of these four large trials have been summarized in an overview analysis. The overall analysis showed a 50% RRR for VTE over enoxaparin. The incidence of PE and death until day 11 was similar in the two groups. The overall safety analysis indicated a higher incidence of major bleeding in the pentasaccharide group (2.3 vs. 1.4%) that was not statistically significant. The results of these trials represent a great step forward for the prophylaxis of VTE after major orthopedic surgery.

Pentasaccharide was investigated in a randomized, parallel group, phase IIb comparison trial with dalteparin in the treatment of patients affected by proximal DVT. The primary outcome measure was a change in the thrombus mass and improvement of the basal lung scan repeated at day 7. The results were similar across treatment groups (pentasaccharide once daily 5 mg, 7.5 mg, 10 mg or dalteparin 100 IU/kg twice daily).[91] Moreover the incidence of symptomatic VTE recurrence was 2.4% in the 334 patients treated with pentasaccharide and 5% in the 119 dalteparin-treated patients: pentasaccharide was not associated with an increase of major bleeding at any dose. On the basis of these results pentasaccharide is being tested in large trials in patients affected by VTE. Pentasaccharide has recently been investigated as an adjuvant to thrombolytic therapy in patients affected by acute myocardial infarction.[92] Patients (n=333) with ST elevation were treated with aspirin and alteplase and randomized to UFH, given intravenously during 48 to 72 h, or to a low, medium or high dose of pentasaccharide, administered once daily for 5 to 7 days, intravenously on the first day, then subcutaneously. Coronary angiography was performed at 90 min and on days 5 to 7. TIMI flow grade 3 rates at 90 min were similar in the four treatment groups. Among patients with TIMI 3 flow at 90 min who did not undergo a coronary intervention (n=155), a trend towards less reocclusion of the infarct-related vessel on days 5 to 7 was observed with pentasaccharide: 0.9% vs 7.0% with UFH (p=0.065). The incidence of the primary safety end-point of intracranial bleeding and transfusion requirement was identical for the pentasaccharide and UFH groups (7.1%). In this study, pentasaccharide together with alteplase was as safe and as effective as UFH in restoring coronary artery patency.

Inhibitors of the factor VIIa/tissue factor pathway

The coagulation cascade starts at the level of the factor VIIa/tissue factor (TF) complex. Renewed consideration of this element has prompted the evaluation of the strategy that blocks coagulation at this level. Two inhibitors of the factor VIIa/TF complex are included in this class of compounds: tissue factor pathway inhibitor (TFPI) and the nematode anticoagulant protein c2 (NAPc2).

TFPI is a naturally occurring coagulation inhibitor that blocks thrombin generation through two steps. TFPI initially binds to factor Xa and inactivates factor Xa, and then the TFPI bound to factor Xa forms a complex with factor VIIa and inactivates it. This process of inactivation of factor VIIa intervenes within the factor VIIa/TF complex. TFPI has a short plasma half-life and is usually administered by continuous intravenous infusion. TFPI is undergoing clinical investigation in patients affected by severe sepsis in order to treat disseminated intravascular coagulation, which is common in such patients.

NAPc2 is derived from an intestinal nematode (*Ankylostoma caninum*), NAPc2 acts by binding to a non-catalytic site located on factor X or on factor Xa and by inhibition of factor VIIa within the factor VIIa/TF complex. NAPc2 is administered subcutaneously, and has an antithrombotic activity that lasts for about 50 hours. NAPc2 has been recently evaluated in a phase IIa study in patients undergoing elective TKR. NAPc2 was given intravenously at dose-ascending regimens from 1.5

µg/kg to 5 µg/kg, prophylaxis was started postoperatively and then the injections were given on alternative days (days 1, 3, 5 and 7). At the end of treatment, patients underwent unilateral venography. The primary safety end-point was the incidence of major bleeding. Of the 293 patients studied, 251 were included in the efficacy analysis. The results showed that the 3 µg/kg dose administered within 1 hour after surgery was associated with the lowest incidence of DVT (12.2%) and with an acceptable rate of major bleeding. These results are comparable with those of the currently adopted prophylactic methods in patients undergoing TKR.[93]

References

1. Geerts WH, Heit JA, Clagett GP, Pineo GF, Colwell CW, Anderson FA Jr, et al. Prevention of venous thromboembolism. Chest 2001; 119:132S-75S.
2. Collins R, Scrimgeour A, Yusuf S, Peto R. Reduction in fatal pulmonary embolism and venous thrombosis by perioperative administration of subcutaneous heparin. Overview of results of randomized trials in general, orthopedic, and urologic surgery. N Engl J Med 1988; 318:1162-73.
3. Anonymous. Prevention of fatal postoperative pulmonary embolism by low doses of heparin. An international multicentre trial. Lancet 1975; 2:45-51.
4. Samama M, Bernard P, Bonnardot JP, Combe-Tamzali S, Lanson Y, Tissot E. Low molecular weight heparin compared with unfractionated heparin in prevention of postoperative thrombosis. Br J Surg 1988; 75:128-31.
5. Kakkar V, Murray WJ. Efficacy and safety of low-molecular-weight heparin (CY216) in preventing postoperative venous thrombo-embolism: a co-operative study. Br J Surg 1985; 72:786-91.
6. Anonymous. Comparison of a low molecular weight heparin and unfractionated heparin for the prevention of deep vein thrombosis in patients undergoing abdominal surgery. The European Fraxiparin Study (EFS) Group. Br J Surg 1988; 75:1058-8.
7. Eurin B. Efficacy and tolerance of fraxiparin in the prevention of deep vein thrombosis in general surgery performed with medullar conduction anesthesia. Ann Fr Anesth Reanim 1994; 13:311-7.
8. Kakkar VV, Boeckl O, Boneu B, Bordenave L, Brehm OA, Brucke P, et al. Efficacy and safety of a low-molecular-weight heparin and standard unfractionated heparin for prophylaxis of postoperative venous thromboembolism: European multicenter trial. World J Surg 1997; 21:2-9.
9. Anonymous. Efficacy and safety of enoxaparin versus unfractionated heparin for prevention of deep vein thrombosis in elective cancer surgery: a double-blind randomized multicentre trial with venographic assessment. ENOXACAN Study Group. Br J Surg 1997; 84:1099-103.
10. Bergqvist D, Agnelli G, Cohen AT, Eldor A, Nilsson PE, Le Moigne-Amrani A, et al. Duration of prophylaxis against venous thromboembolism with enoxaparin after surgery for cancer. The ENOXACAN II Investigators. N Engl J Med 2002; 346:975-80.
11. Leyvraz PF, Richard J, Bachmann F, Van Melle G, Treyvaud JM, Livio JJ, et al. Adjusted versus fixed-dose subcutaneous heparin in the prevention of deep-vein thrombosis after total hip replacement. N Engl J Med 1983; 309:954-8.
12. Francis CW, Marder VJ, Evarts CM, Yaukoolbodi S. Two-step warfarin therapy. Prevention of postoperative venous thrombosis without excessive bleeding. JAMA 1983; 249:374-8.
13. Nurmohamed MT, Rosendaal FR, Buller HR, Dekker E, Hommes DW, Vandenbroucke JP, et al. Low-molecular-weight heparin versus standard heparin in general and orthopaedic surgery: a meta-analysis. Lancet 1992; 340:152-6.
14. Leizorovicz A, Haugh MC, Chapuis FR, Samama MM, Boissel JP. Low molecular weight heparin in the prevention of perioperative thrombus. BMJ 1992; 305:913-20.
15. Anonymous. Prevention of deep vein thrombosis with low molecular-weight heparin in patients undergoing total hip replacement. A randomized trial. The German Hip Arthroplasty Trial (GHAT) Group. Arch Orthop Trauma Surg 1992; 111:110-20.
16. Dechavanne M, Ville D, Berruyer M, Trepo F, Dalery F, Clermont N, et al. Randomized trial of a low molecular weight heparin (Kabi 2165) versus adjusted-dose subcutaneous standard heparin in the prophylaxis of deep vein thrombosis after elective hip surgery. Haemostasis 1989; 1:5-12.
17. Anonymous. RD heparin compared with warfarin for prevention of venous thromboembolic disease following total hip or knee arthroplasty. RD Heparin Arthroplasty Group. J Bone Joint Surg Am 1994; 76:1174-85.
18. Howard AW, Aaron SD. Low molecular weight heparin decreases proximal and distal deep venous thrombosis following total knee arthroplasty. A meta-analysis of randomized trials. Thromb Haemost 1998; 79:902-6.
19. Leclerc JR, Geerts WH, Desjardins L, Jobin F, Laroche F, Delorme F, et al. Prevention of deep vein thrombosis after major knee surgery: a randomized, double-blind trial comparing a low molecular weight heparin fragment (enoxaparin) to placebo. Thromb Haemost 1992; 67:417-23.
20. Fauno P, Suomalainen O, Rehnberg V, Hansen TB, Kroner K, Soimakallio S, et al. Prophylaxis for the prevention of venous thromboembolism after total knee arthroplasty. A comparison between unfractionated and low-molecular-weight heparin. J Bone Joint Surg Am 1994; 76: 1814-8.
21. Colwell CW Jr, Spiro TE, Trowbridge AA, Stephens JW, Gardiner GA Jr, Ritter MA. Efficacy and safety of enoxaparin versus unfractionated heparin for the prevention of deep venous thrombosis after elective knee arthroplasty. Enoxaparin Clinical Trial Group. Clin Orthop 1995; 12:19-27.
22. Leclerc JR, Geerts WH, Desjardins L, Laflamme GH, L'Esperance B, Demers C, et al. Prevention of venous thromboembolism after knee arthroplasty. A randomized, double-blind trial comparing enoxaparin with warfarin. Ann Intern Med 1996; 124:619-26.
23. Levine MN, Gent M, Hirsh J, Weitz J, Turpie AG, Powers P, et al. Ardeparin (low-molecular-weight heparin) vs graduated compression stockings for the prevention of venous thromboembolism. A randomized trial in patients undergoing knee surgery. Arch Intern Med 1996; 156: 851-6.

24. Heit JA, Berkowitz SD, Bona R, Cabanas V, Corson JD, Elliott CG, et al. Efficacy and safety of low molecular weight heparin (ardeparin sodium) compared to warfarin for prevention of venous thromboembolism after total knee replacement surgery: a double-blind, dose-ranging study. Ardeparin Arthroplasty Study Group. Thromb Haemost 1997; 77:32-8.
25. Hull R, Raskob G, Pineo G, Rosenbloom D, Evans W, Mallory T, et al. A comparison of subcutaneous low-molecular-weight heparin with warfarin sodium for prophylaxis against deep-vein thrombosis after hip or knee implantation. N Engl J Med 1993; 329:1370-6.
26. Hamulyak K, Lensing AW, van der Meer J, Smid WM, van Ooy A, Hoek JA. Subcutaneous low-molecular weight heparin or oral anticoagulants for the prevention of deep-vein thrombosis in elective hip and knee replacement? Fraxiparine Oral Anticoagulant Study Group. Thromb Haemost 1995; 74:1428-31.
27. Monreal M, Lafoz E, Navarro A, Granero X, Caja V, Caceres E, et al. A prospective double-blind trial of a low molecular weight heparin once daily compared with conventional low-dose heparin three times daily to prevent pulmonary embolism and venous thrombosis in patients with hip fracture. J Trauma 1989; 29:873-5.
28. Jorgensen PS, Knudsen JB, Broeng L, Josephsen L, Bjerregaard P, Hagen K, et al. The thromboprophylactic effect of a low-molecular-weight heparin (Fragmin) in hip fracture surgery. A placebo-controlled study. Clin Orthop 1992; 278:95-100.
29. Barsotti J, Gruel Y, Rosset P, Favard L, Dabo B, Andreu J, et al. Comparative double-blind study of two dosage regimens of low-molecular weight heparin in elderly patients with a fracture of the neck of the femur. J Orthop Trauma 1990; 4:371-5.
30. Handoll HH, Farrar MJ, McBirnie J, Tytherleigh-Strong G, Awal KA, Milne AA, et al. Heparin, low molecular weight heparin and physical methods for preventing deep vein thrombosis and pulmonary embolism following surgery for hip fractures. Cochrane Database Syst Rev 2000; CD000305.
31. Anonymous. Thromboprophylaxis in hip fracture surgery: a pilot study comparing danaparoid, enoxaparin and dalteparin. The TIFDED Study Group. Haemostasis 1999; 29:310-7.
32. Eikelboom JW, Quinlan DJ, Douketis JD. Extended-duration prophylaxis against venous thromboembolism after total hip or knee replacement: a meta-analysis of the randomised trials. Lancet 2001; 358:9-15.
33. Hull RD, Pineo GF, Stein PD, Mah AF, MacIsaac SM, Dahl OE, et al. Extended out-of-hospital low-molecular-weight heparin prophylaxis against deep venous thrombosis in patients after elective hip arthroplasty: a systematic review. Ann Intern Med 2001; 135:858-69.
34. Nurmohamed MT, van Riel AM, Henkens CM, Koopman MM, Que GT, d'Azemar P, et al. Low molecular weight heparin and compression stockings in the prevention of venous thromboembolism in neurosurgery. Thromb Haemost 1996; 75:233-8.
35. Agnelli G, Piovella F, Buoncristiani P, Severi P, Pini M, D'Angelo A, et al. Enoxaparin plus compression stockings compared with compression stockings alone in the prevention of venous thromboembolism after elective neurosurgery. N Engl J Med 1998; 339:80-5.
36. Koopman MM, Prandoni P, Piovella F, Ockelford PA, Brandjes DP, van der Meer J, et al. Treatment of venous thrombosis with intravenous unfractionated heparin administered in the hospital as compared with subcutaneous low-molecular-weight heparin administered at home. The Tasman Study Group. N Engl J Med 1996; 334: 682-7.
37. Levine MN, Gent M, Hirsh J, Leclerc J, Anderson D, Weitz J, et al. A comparison of low-molecular-weight heparin administered primarily at home with unfractionated heparin administered in the hospital for proximal deep-vein thrombosis. N Engl J Med 1996; 334:677-81.
38. Siragusa S, Cosmi B, Piovella F, Hirsh J, Ginsberg JS. Low-molecular-weight heparins and unfractionated heparin in the treatment of patients with acute venous thromboembolism: results of a meta-analysis. Am J Med 1996; 100:269-77.
39. Lensing AW, Prins MH, Davidson BL, Hirsh J. Treatment of deep venous thrombosis with low-molecular-weight heparins. A meta-analysis. Arch Intern Med 1995; 155: 601-7.
40. Dolovich LR, Ginsberg JS, Douketis JD, Holbrook AM, Cheah G. A meta-analysis comparing low-molecular-weight heparins with unfractionated heparin in the treatment of venous thromboembolism: examining some unanswered questions regarding location of treatment, product type, and dosing frequency. Arch Intern Med 2000; 160:181-8.
41. Bratt G, Alberg W, Johansson M, Tornebohm E, Granqvist S, Locner D. Two daily subcutaneous injections of fragmin as compared with intravenous standard heparin in the treatment of deep venous thrombosis (DVT). Thromb Haemost 1990; 64:506-10.
42. Prandoni P, Vigo M, Cattelan AM, Ruol A. Treatment of deep venous thrombosis by fixed doses of a low-molecular-weight heparin (CY216). Haemostasis 1990; 20 Suppl 1:220-3.
43. Anonymous. A randomised trial of low molecular weight heparin (CY 216) compared with intravenous unfractionated heparin in the treatment of deep vein thrombosis. A collaborative European multicentre study. Thromb Haemost 1991; 65:251-6.
44. Hull RD, Raskob GE, Pineo GF, Green D, Trowbridge AA, Elliott CG, et al. Subcutaneous low-molecular-weight heparin compared with continuous intravenous heparin in the treatment of proximal-vein thrombosis. N Engl J Med 1992; 326:975-82.
45. Prandoni P, Lensing AW, Buller HR, Carta M, Cogo A, Vigo M, et al. Comparison of subcutaneous low-molecular-weight heparin with intravenous standard heparin in proximal deep-vein thrombosis. Lancet 1992; 339:441-5.
46. Simonneau G, Charbonnier B, Decousus H, Planchon B, Ninet J, Sie P, et al. Subcutaneous low-molecular-weight heparin compared with continuous intravenous unfractionated heparin in the treatment of proximal deep vein thrombosis. Arch Intern Med 1993; 153:1541-6.
47. Lindmarker P, Holmstrom M, Granqvist S, Johnsson H, Lockner D. Comparison of once-daily subcutaneous Fragmin with continuous intravenous unfractionated heparin in the treatment of deep vein thrombosis. Thromb Haemost 1994; 72:186-90.
48. Fiessinger JN, Lopez-Fernandez M, Gatterer E, Granqvist S, Kher A, Olsson CG, et al. Once-daily subcutaneous dalteparin, a low molecular weight heparin, for the initial treatment of acute deep vein thrombosis. Thromb Haemost 1996; 76:195-9.

49. Luomanmaki K, Grankvist S, Hallert C, Jauro I, Ketola K, Kim HC, et al. A multicentre comparison of once-daily subcutaneous dalteparin (low molecular weight heparin) and continuous intravenous heparin in the treatment of deep vein thrombosis. J Intern Med 1996; 240:85-92.
50. Anonymous. Low-molecular-weight heparin in the treatment of patients with venous thromboembolism. The Columbus Investigators. N Engl J Med 1997; 337:657-62.
51. Simonneau G, Sors H, Charbonnier B, Page Y, Laaban JP, Azarian R, et al. A comparison of low-molecular-weight heparin with unfractionated heparin for acute pulmonary embolism. The THESEE Study Group. Tinzaparine ou Heparine Standard: Evaluations dans l'Embolie Pulmonaire. N Engl J Med 1997; 337:663-9.
52. Cairns JA, Lewis HD, Meade TW, Sutton GC, Theroux P. Antithrombotic agents in coronary artery disease. Fourth ACCP Consensus Conference on Antithrombotic Therapy Chest 1995; 108 Suppl 4:380-400.
53. Agnelli G, Sonaglia F. Clinical status of direct thrombin inhibitors. Crit Rev Onc Haematol 1999: 31:97-116.
54. Agnelli G, Sonaglia F. Low-molecular-weight heparins in the management of unstable angina. Haemostasis 2000; Suppl 2:158-67.
55. Anonymous. Low-molecular-weight heparin during instability in coronary artery disease, Fragmin during instability in coronary artery disease (FRISC) study group. Lancet 1996; 347:561-8.
56. Klein W, Buchwald A, Hillis SE, Monrad S, Sanz G, Turpie AG, et al. Comparison of low-molecular-weight heparin with unfractionated heparin acutely and with placebo for 6 weeks in the management of unstable coronary artery disease. Fragmin in unstable coronary artery disease study (FRIC). Circulation 1997; 96:61-8.
57. Anonymous. Comparison of two treatment durations (6 days and 14 days) of a low molecular weight heparin with a 6-day treatment of unfractionated heparin in the initial management of unstable angina or non-Q wave myocardial infarction: FRAX.I.S. (FRAxiparine in Ischaemic Syndrome). Eur Heart J 1999; 20:1553-62.
58. Cohen M, Demers C, Gurfinkel EP, Turpie AG, Fromell GJ, Goodman S. A comparison of low molecular weight heparin with unfractionated heparin for unstable coronary artery disease. N Engl J Med 1997; 337:447-52.
59. Antman EM, Mc Cabe CH, Premmereur J, McCabe C, Rush J, Radley D, et al. Enoxaparin for the acute and chronic management of unstable angina/non Q wave myocardial infarction. Results of the TIMI 11B. Circulation 1998; 98: 504.
60. Antman EM, Cohen M, Radley D, McCabe C, Rush J, Premmereur J, et al. Assessment of the treatment effect of enoxaparin for unstable angina/non-Q-wave myocardial infarction. TIMI 11B-ESSENCE meta-analysis.Circulation 1999; 100:1602-8.
61. Anonymous. Efficacy and safety of tenecteplase in combination with enoxaparin, abciximab, or unfractionated heparin: the ASSENT-3 randomised trial in acute myocardial infarction. The Assessment of the Safety and Efficacy of a New Thrombolytic Regimen (ASSENT)-3 Investigators. Lancet 2001; 358:605-13.
62. Weitz JI, Leslie B, Hudoba M. Thrombin binds to soluble fibrin degradation products where it is protected from inhibition by heparin-antithrombin III, but is susceptible to inactivation by antithrombin-independent inhibitors. Circulation 1998; 97:544-52.
63. Harvey RP, Degryse E, Stefani L, Schamber F, Cazenave JP, Courtney M, et al. Cloning and expression of a cDNA coding for the anticoagulant hirudin from bloodsucking leech, Hirudo medicinalis. Proc Natl Acad Sci USA 1986; 83:1084-8.
64. Agnelli G, Sonaglia F. Recombinant hirudin in the prevention of venous thromboembolism in patients undergoing elective hip surgery. Semin Thromb Hemost 1997; 23:143-8.
65. Schiele F, Lindgaerde F, Eriksson H, Bassand JP, Wallmark A, Hansson PO, et al. Subcutaneous recombinant hirudin (HBW 023) versus intravenous sodium heparin in treatment of established acute deep vein thrombosis of the legs: a multicentre prospective dose-ranging randomized trial. International Multicentre Hirudin Study Group. Thromb Haemost 1997; 77:834-8.
66. Anonymous. A comparison of recombinant hirudin with heparin for the treatment of acute coronary syndromes. The Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) IIb investigators. N Engl J Med 1996; 335:775-82.
67. Anonymous. Effects of recombinant hirudin (lepirudin) compared with heparin on death, myocardial infarction, refractory angina, and revascularisation procedures in patients with acute myocardial ischaemia without ST elevation: a randomised trial. Organisation to Assess Strategies for Ischemic Syndromes (OASIS-2) Investigators . Lancet 1999; 353:429-38.
68. Anonymous. Randomized trial of intravenous heparin versus recombinant hirudin for acute coronary syndromes. The Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) IIa Investigators. Circulation 1994; 90:1631-7.
69. Antmann EM. Hirudin in acute myocardial infarction. Safety report from the Thrombolysis and Thrombin Inhibition in Myocardial Infarction (TIMI) 9A Trial. Circulation 1994; 90:1624-30.
70. Neuhaus KL, von Essen R, Tebbe U, Jessel A, Heinrichs H, Maurer W, et al. Safety observations from the pilot phase of the randomized r-hirudin for improvement of thrombolysis (HIT-III) study. A study of the Arbeitsgemeinschaft Leitender Kardiologischer Krankenhausarzte (ALKK). Circulation 1994; 90:1638-42.
71. Antmann EM. Hirudin in acute myocardial infarction. Thrombolysis and Thrombin Inhibition in Myocardial Infarction (TIMI) 9B trial. Circulation 1996; 94:911-21.
72. Simes RJ, Granger CB, Antmann EM, Califf RM, Braunwald E, Topol EJ. Impact of hirudin on mortality and reinfarction in patients with acute coronary syndromes: A prospective meta-analysis of the GUSTO IIb and TIMI 9B trials. Circulation 1996; 94: Suppl 1:a430[abstract].
73. Serruys PW, Herrman JP, Simon R, Rutsch W, Bode C, Laarman GJ, et al. A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. Helvetica Investigators. N Engl J Med 1995; 333:757-63.
74. Greinacher A. Heparin-induced thrombocytopenia: pathogenesis and treatment. Thromb Haemost 1999; 82 Suppl 1:148-56.
75. Greinacher A, Volpel H, Janssens U, Hach-Wunderle V, Kemkes-Matthes B, Eichler P, et al. Recombinant hirudin (lepirudin) provides safe and effective anticoagulation in patients with heparin-induced thrombocytopenia: a prospective study. Circulation 1999; 99:73-80.
76. Greinacher A, Janssens U, Berg G, Bock M, Kwasny H, Kemkes-Matthes B, et al. Lepirudin (recombinant hirudin)

for parenteral anticoagulation in patients with heparin-induced thrombocytopenia. Heparin-Associated Thrombocytopenia Study (HAT) investigators. Circulation 1999; 100:587-93.
77. Koster A, Hansen R, Kuppe H, Hetzer R, Crystal GJ, Mertzlufft F. Recombinant hirudin as an alternative for anticoagulation during cardiopulmonary bypass in patients with heparin-induced thrombocytopenia type II: a 1-year experience in 57 patients. J Cardiothorac Vasc Anesth 2000; 14:243-8.
78. Ginsberg JS, Nurmohamed MT, Gent M, MacKinnon B, Sicurella J, Brill-Edwards P, et al. Use of hirulog in the prevention of venous thrombosis after major hip or knee surgery. Circulation 1994; 90:2385-9.
79. Fuchs J, Cannon CP. Hirulog in the treatment of unstable angina. Results of the Thrombin Inhibition in Myocardial Ischemia (TIMI) 7 trial. Circulation 1995; 92:727-33.
80. White H. Thrombin-specific anticoagulation with bivalirudin versus heparin in patients receiving fibrinolytic therapy for acute myocardial infarction: the HERO-2 randomised trial. The Hirulog and Early Reperfusion or Occlusion (HERO)-2 Trial Investigators. Lancet 2001; 358: 1855-63.
81. Bittl JA, Strony J, Brinker JA, Ahmed WH, Meckel CR, Chaitman BR, et al. Treatment with bivalirudin (Hirulog) as compared with heparin during coronary angioplasty for unstable or postinfarction angina. Hirulog Angioplasty Study Investigators. N Engl J Med 1995; 333:764-9.
82. Gustaffson D, Nystrom JE, Carlsson S. Pharmacodynamic properties of ximelagatran, a prodrug of the direct thrombin inhibitor melagatran intended for oral use. Blood 1999; 94:26a[abstract].
83. Eriksson BI, Arfwidsson AC, Frison L, Eriksson UG, Bylock A, Kalebo P, et al. A dose-ranging study of the oral direct thrombin inhibitor, ximelagatran, and its subcutaneous form, melagatran, compared with dalteparin in the prophylaxis of thromboembolism after hip or knee replacement: METHRO I. MElagatran for THRombin inhibition in Orthopaedic surgery. Thromb Haemost 2002; 87:231-7.
84. Eriksson BI, Lindbratt S, Kalebo P. METHRO II: dose-response study of the novel oral, direct thrombin inhibitor, H/376/95, and its subcutaneous formulation melagatran, compared with dalteparin as thromboembolic prophylaxis after total hip or knee replacement. Haemostasis 2000; 30 Suppl 1:20-1.
85. Eriksson BI, Ögren M, Agnelli G. The oral direct thrombin inhibitor ximelagatran (formerly H 376/95) and its subcutaneous form melagatran compared with enoxaparin as thromboprophylaxis after total hip or total knee replacement. Thromb Haemost 2001 Suppl 1638a [abstract].
86. Turpie AG, Gallus AS, Hoek JA. A synthetic pentasaccharide for the prevention of deep vein thrombosis after total hip replacement. N Engl J Med 2001; 344:619-25.
87. Lassen MR. Efficacy of the first synthetic factor Xa inhibitor, pentasaccharide ORG31540/SR90107A, versus low-molecular-weight heparin (LMWH) in the prevention of venous thromboembolism (VTE) following elective hip replacement surgery: the EPHESUS study. On behalf of the Pentasaccharide Orthopedic Prophylaxis Studies Investigators [abstract]. Thromb Haemost 2001; Suppl: 45a.
88. Turpie AG. Efficacy of the first synthetic factor Xa inhibitor, Pentasaccharide ORG31540/SR90107A, versus low molecular weight heparin (LMWH) in the prevention of venous thromboembolism (VTE) following elective hip replacement surgery: The Pentathlon 2000 Study. On Behalf of the Pentasaccharide Orthopedic Prophylaxis Studies Investigators. Thromb Haemost 2001; Suppl 48a [abstract].
89. Bauer KA, Eriksson BI, Lassen MR, Turpie AG. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after elective major knee surgery. Steering Committee of the Pentasaccharide in Major Knee Surgery Study. N Engl J Med 2001; 345:1305-10.
90. Eriksson BI, Bauer KA, Lassen MR, Turpie AG. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after hip-fracture surgery. Steering Committee of the Pentasaccharide in Hip-Fracture Surgery Study. N Engl J Med 2001; 345:1298-304.
91. Anonymous. Treatment of proximal deep vein thrombosis with a novel synthetic compound (SR90107A/ORG31540) with pure anti-factor Xa activity: a phase II evaluation. The Rembrandt Investigators. Circulation 2000; 22:2726-31.
92. Coussement PK, Bassand JP, Convens C, Vrolix M, Boland J, Grollier G, et al. A synthetic factor-Xa inhibitor (ORG31540/SR9017A) as an adjunct to fibrinolysis in acute myocardial infarction. The PENTALYSE study. Eur Heart J 2001; 22:1716-24.
93. Lee A, Agnelli G, Buller H, Ginsberg J, Heit J, Rote W, et al. Dose-response study of recombinant factor VIIa/tissue factor inhibitor recombinant nematode anticoagulant protein c2 in prevention of postoperative venous thromboembolism in patients undergoing total knee replacement. Circulation 2001; 104:74-8.