Heparin and Low-Molecular-Weight Heparin

Mechanisms of Action, Pharmacokinetics, Dosing, Monitoring, Efficacy, and Safety

Jack Hirsh, MD, FCCP, Chair; Theodore E. Warkentin, MD; Stephen G. Shaughnessy, PhD; Sonia S. Anand, MD; Jonathan L. Halperin, MD; Robert Raschke, MD, MS; Christopher Granger, MD; E. Magnus Ohman, MBBCh, FCCP; and James E. Dalen, MD, MPH, Master FCCP

Abbreviations: ACT = activated clotting time; APTT = activated partial thromboplastin time; AT = antithrombin; AT-III = antithrombin III; CI = confidence interval; DVT = deep vein thrombosis; GP = glycoprotein; HIT = heparin-induced thrombocytopenia; INR = international normalized ratio; LMWH = low-molecular-weight heparin; MI = myocardial infarction; NQMI = non-Q-wave myocardial infarction; OR = odds ratio; PE = pulmonary embolism; PF4 = platelet factor 4; PTCA = percutaneous coronary angioplasty; RR = relative risk; sc = subcutaneous; tPA = tissue plasminogen activator; UA = unstable angina; UFH = unfractionated heparin

(CHEST 2001; 119:64S–94S)

Heparin and its derivative, low-molecular-weight heparin (LMWH), are the anticoagulants of choice when a rapid anticoagulant effect is required, because their onset of action is immediate when administered by IV injection. Both types of heparins are administered in lower doses for primary prophylaxis than for treatment of venous thrombosis or acute myocardial ischemia. Heparin has pharmacokinetic limitations[1] not shared by LMWHs. Based on these pharmacokinetic limitations, heparin therapy is usually restricted to the hospital setting, where its effect can be monitored and its dosage adjusted frequently. In contrast, LMWH preparations can be administered in either the in-hospital or out-of-hospital setting because they can be administered subcutaneously (sc) without the need for laboratory monitoring. When long-term anticoagulant therapy is indicated, heparin or LMWH administration is usually followed by treatment with oral anticoagulants. However, long-term out-of-hospital treatment with heparin or LMWH is used when anticoagulant therapy is indicated in pregnancy and in patients who develop recurrent venous thromboembolism while treated with appropriate doses of oral anticoagulants.

Since our report in 1998 (Supplement to *CHEST*, Vol. 114, iss 5), a number of LMWH preparations have been approved for use for the treatment of venous thrombosis and for the treatment of unstable angina (UA).

Clinical Indications

Heparin is effective and indicated for the prevention of venous thromboembolism; for the treatment of venous thrombosis and pulmonary embolism (PE); for the early treatment of patients with UA and acute myocardial infarction (MI); for patients who undergo cardiac surgery using cardiac bypass, vascular surgery, and coronary angioplasty; in patients with coronary stents; and in selected patients with disseminated intravascular coagulation.

LMWHs are effective and indicated for the prevention of venous thromboembolism, for the treatment of venous thrombosis, for the treatment of acute PE, and for the early treatment of patients with UA. The levels of evidence and grading of recommendations for the clinical use of heparin and LMWHs are discussed in the chapters that consider the evidence supporting antithrombotic therapy with these agents for the various clinical indications.

This chapter will review the mechanisms of action of heparin and LMWHs, their pharmacokinetics, anticoagulant effects, side effects, and laboratory monitoring. The clinical uses of heparin and LMWHs and the results of clinical trials will also be discussed, although more details appear in other chapters.

Historical Highlights

Heparin was discovered by McLean[2] in 1916, and Brinkhous and associates[3] demonstrated that its anticoagulant effect requires a plasma cofactor later named antithrombin III (AT-III),[4] but is now known simply as antithrombin (AT). Rosenberg and Lam,[1] Rosenberg and Bauer,[5] and Lindahl et al[6] elucidated the mechanisms responsible for the heparin/AT interaction. It is now known that the active center serine of thrombin and other coagulation enzymes are inhibited by an arginine-reactive site on the AT molecule and that heparin binds to lysine site on AT, producing a conformational change at the arginine-reactive site that converts AT from a slow, progressive thrombin inhibitor to a very rapid inhibitor of thrombin and factor Xa.[5] AT binds covalently to the active serine centers of coagulation enzymes; heparin then dissociates from the ternary complex and can be reutilized (Fig 1).[5] Subsequently, it was discovered[1,5,6] that heparin binds to and potentiates the activity of AT through a unique glucosamine unit[1,5–7] contained within a pentasaccharide sequence,[8] the structure of which has been confirmed. A synthetic pentasaccharide has been developed and is undergoing clinical evaluation for prevention and treatment of venous thrombosis.[9,10]

Mechanism of Action

Only about one third of an administered dose of heparin binds to AT, and this fraction is responsible for most of its anticoagulant effect.[11,12] The remaining two thirds has minimal anticoagulant activity at therapeutic concentrations, but at concentrations greater than usually obtained clinically, both high-affinity and low-affinity heparin catalyze the AT effect of a second plasma protein, heparin cofactor II (Table 1).[13]

The heparin-AT complex inactivates a number of coagulation enzymes, including thrombin factor (IIa), factors Xa, IXa, XIa, and XIIa.[5] Of these, thrombin and factor Xa are most responsive to inhibition, and human thrombin is about 10-fold more sensitive to inhibition by the heparin-AT complex than factor Xa (Fig 2). To inhibit throm-

Correspondence to: Jack Hirsh, MD, FCCP, Director, Hamilton Civic Hospitals Research Centre, 711 Concession St, Hamilton, ON L8V 1C3, Canada

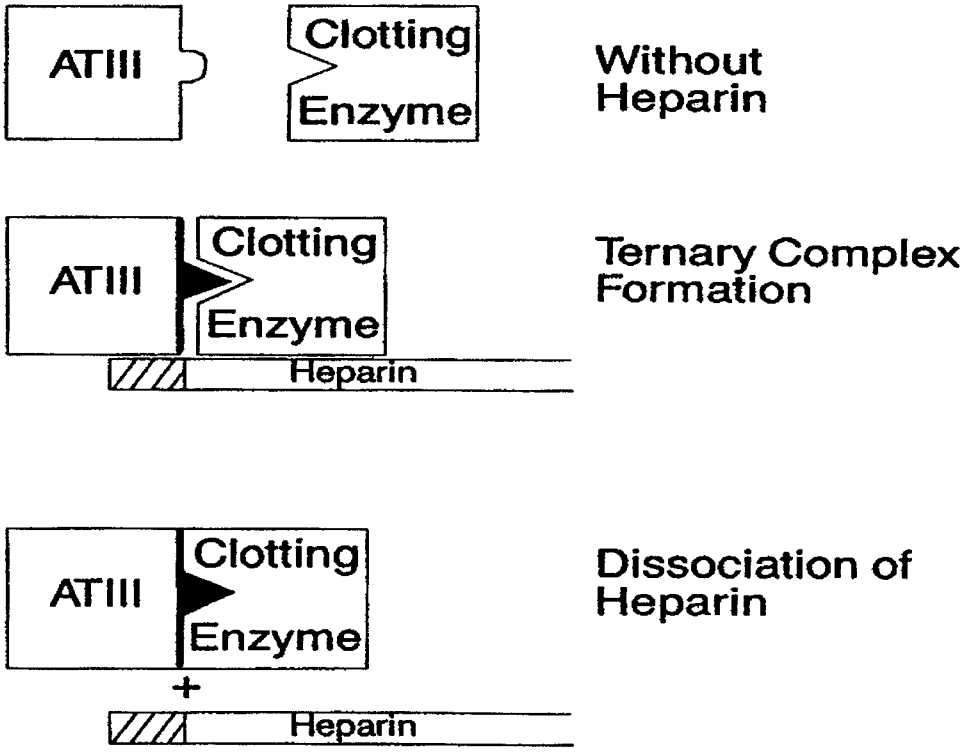

FIGURE 1. Inactivation of clotting enzymes by heparin. *Top panel:* AT-III is a slow inhibitor without heparin. *Middle panel:* Heparin binds to AT-III through high-affinity pentasaccharide and induces a conformational change in AT-III, thereby converting AT-III from a slow to a very rapid inhibitor. *Bottom panel:* AT-III binds covalently to the clotting enzyme, and the heparin dissociates from the complex and can be reutilized.

bin, heparin must bind to both the coagulation enzyme and AT, but binding to the enzyme is less important for the inhibition of activated factor X (factor Xa; Fig 3).[7] Molecules of heparin containing < 18 saccharides do not bind simultaneously to thrombin and AT and are therefore unable to catalyze thrombin inhibition. In contrast, very small heparin fragments containing the high-affinity pentasaccharide sequence catalyze inhibition of factor Xa by AT.[14–17] By inactivating thrombin, heparin not only prevents fibrin formation but also inhibits thrombin-induced activation of factor V and factor VIII.[18–20] Unfractionated heparin (UFH) and LMWH also induce secretion of tissue factor pathway inhibitor by vascular endothelial cells that reduce procoagulant activity of tissue factor VIIa complex, and this could contribute to the antithrombotic action of heparin and LMWH.[21–23]

Heparin is heterogeneous with respect to molecular size, anticoagulant activity, and pharmacokinetic properties (Table 2). Its molecular weight ranges from 3,000 to 30,000 d average, with a mean molecular weight of 15,000 d (approximately 45 monosaccharide chains; Fig 4).[24–26] Its anticoagulant activity varies because only one third of heparins have anticoagulant function and because its anticoagulant profile and clearance are influenced by the

Table 1—*Antihemostatic Effects of Heparin*

Effects	Comments
Binds to AT-III and catalyzes inactivation of factors IIa, Xa, IXa, and XIIa	Major mechanism for anticoagulant effect, produced by only one third of heparin molecules (those containing the unique AT-III-binding pentasaccharide)
Binds to heparin cofactor II and catalyzes inactivation of factor IIa	Anticoagulant effect requires high concentrations of heparin and occurs to the same degree whether or not the heparin has high or low affinity for AT-III
Binds to platelets	Inhibits platelet function and contributes to the hemorrhagic effects of heparin. High-molecular-weight fractions have greater effect than low-molecular-weight fractions

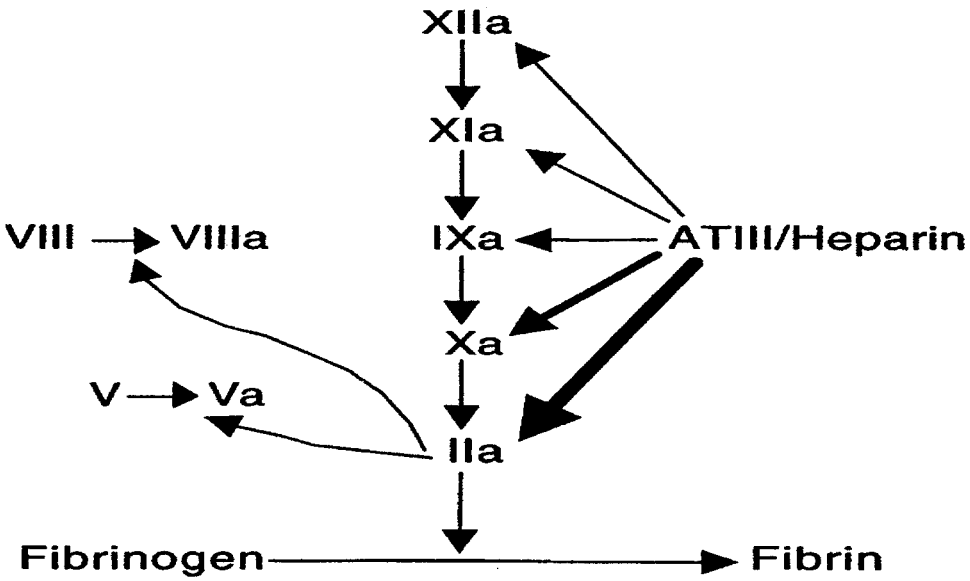

FIGURE 2. The heparin/AT-III complex inactivates the coagulation enzymes factor XIIa (XIIa), factor XIa (XIa), factor IXa (IXa), factor Xa (Xa), and thrombin (IIa). Thrombin and factor Xa are most sensitive to the effects of heparin/AT-III.

chain length of the molecules, with the higher-molecular-weight species cleared from the circulation more rapidly than the lower-molecular-weight species. This differential clearance results in accumulation *in vivo* of the lower-molecular-weight species, which have a lower ratio of antifactor IIa to antifactor Xa activity. The lower-molecular-weight species that are retained *in vivo* are measured by the antifactor Xa heparin assay, but these have little effect on the activated partial thromboplastin time (APTT). Binding of heparin to von Willebrand factor also inhibits von Willebrand factor-dependent platelet function.[27]

Heparin binds to platelets, and depending on the experimental conditions *in vitro*, can either induce or inhibit platelet aggregation.[28,29] Heparin prolongs the bleeding time in humans[30] and enhances blood loss from the microvasculature in rabbits.[31–33] The interaction of heparin with platelets[31] and endothelial cells[32] may contribute to heparin-induced bleeding by a mechanism independent of its anticoagulant effect.[33]

In addition to its anticoagulant effect, heparin increases vessel wall permeability,[32] inhibits the proliferation of vascular smooth muscle cells,[34] and suppresses osteoblast formation and activates osteoclasts that promote bone loss.[35,36] Each of these effects is independent of its anticoagulant activity, but only the osteopenic effect is likely to be relevant clinically.[37]

PHARMACOLOGY OF UFH

The preferred routes of UFH administration are continuous IV infusion and sc injection. When the sc route is selected, the initial dose must be sufficient to overcome the lower bioavailability associated with this route of administration.[38] An immediate anticoagulant effect requires an IV bolus.

In the circulation, heparin binds to a number of plasma proteins (Fig 5), which reduces its anticoagulant activity at low concentrations, thereby contributing to the variability of the anticoagulant response to heparin among patients with thromboembolic disorders[39] and to the laboratory phenomenon of heparin resistance.[40] Heparin also binds

FIGURE 3. Inhibition of thrombin requires simultaneous binding of heparin to AT-III through the unique pentasaccharide sequence and binding to thrombin through a minimum of 13 additional saccharide units. Inhibition of factor Xa (Xa) requires binding heparin to AT-III through the unique pentasaccharide without the additional requirements for binding to Xa. 5 indicates unique high-affinity pentasaccharide.

Table 2—*Heterogenicity of UFH*

Attributes	Characteristics
Molecular size	Mean molecular weight, 15,000 d Range, 3,000 to 30,000 d
Anticoagulant activity	Only one third of heparin molecules contain the high-affinity pentasaccharide required for anticoagulant activity
Clearance	High-molecular-weight moieties are cleared more rapidly than lower-molecular-weight moieties

to endothelial cells[41] and macrophages, which further complicates its pharmacokinetics.

Heparin clearance involves a combination of a rapid saturable and a much slower first-order mechanisms (Fig 6).[42–44] The mechanism of the saturable phase of heparin clearance is through binding to receptors on endothelial cells[45,46] and macrophages[47] where it is depolymerized (Fig 5),[48,49] while the slower unsaturable mechanism is renal (Fig 6). At therapeutic doses, heparin is cleared predominantly through the rapid saturable, dose-dependent mechanism and its anticoagulant effects are nonlinear, with both the intensity and duration of effect rising disproportionately with increasing dose. As a result, the half-life of heparin increases from approximately 30 min following an IV bolus of 25 U/kg, to 60 min with a bolus of 100 U/kg, and to 150 min with a bolus of 400 U/kg.[42–44]

Plasma recovery of heparin is reduced[50] when administered by sc injection in low (5,000 U q12h) or moderate (12,500 to 15,000 U q12h) doses.[38,51] At high therapeutic doses (> 35,000 U/24 h), however, plasma recovery is almost complete.[52] The difference between the bioavailability of heparin administered by sc or IV injection was demonstrated in patients with venous thrombosis[38] randomized to receive either 15,000 q12h by sc injection or 30,000 U by continuous IV infusion; both regimens were preceded by a 5,000-U bolus. Therapeutic heparin levels and APTT ratios were achieved at 24 h in only 37% of patients given sc heparin, compared with 71% of those given the same total dose by continuous IV infusion.

Laboratory Monitoring

Randomized trials show a relationship among heparin dose, efficacy,[38,51,53] and safety.[54,55] Since the anticoagulant response to heparin varies among patients with thromboembolic disorders,[56–60] it is standard practice to adjust the dose of heparin and monitor its effect by measurement of the APTT that is sensitive to the inhibitory effects of heparin on thrombin, factor Xa, and factor IXa. Although a relationship exists between heparin dose and therapeutic efficacy for patients with venous thromboembolism, such a relationship has not been established for patients with acute coronary ischemia, although those receiving concomitant thrombolytic therapy or glycoprotein (GP) IIb/IIIa (GPIIb/IIIa) antagonists given heparin in a dose used to treat venous thrombosis have an unacceptably high rate of bleeding.

Although a close relationship between an effect of heparin *ex vivo* on the APTT and its clinical effect *in vivo* has been assumed, the data supporting this assumption are derived from retrospective subgroup analysis of cohort studies[38,51,57,58,60,61] (Table 3) and are inconsistent with the results of a randomized trial[62] and meta-analyses of contemporary cohort studies.[63,64] Furthermore, there was no

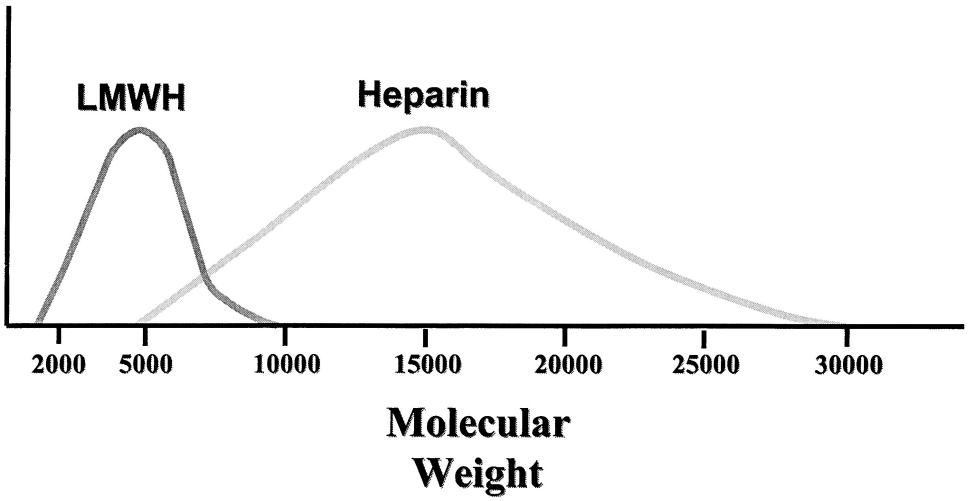

Figure 4. Molecular weight distributions of LMWHs and heparin.

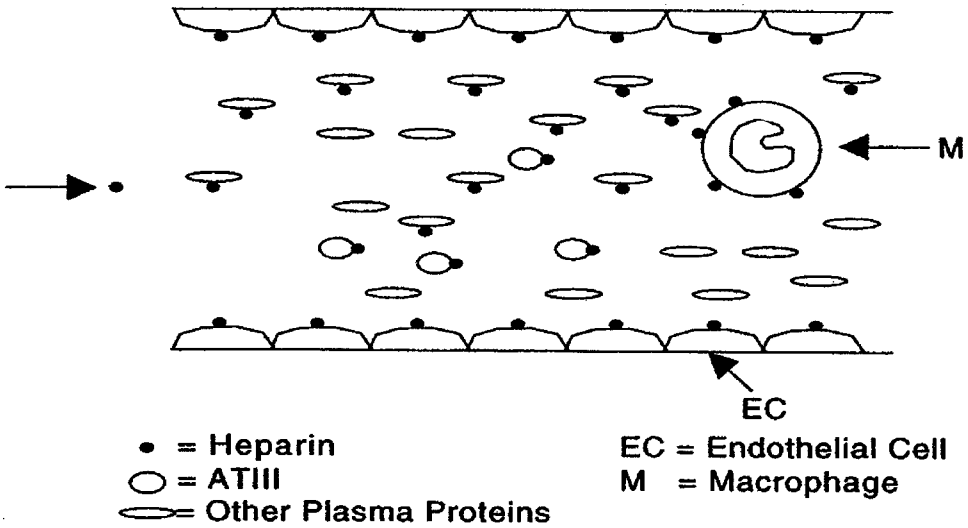

FIGURE 5. As heparin (small, filled circles) enters the circulation, it binds to heparin-binding proteins (elongated circles), endothelial cells (EC), macrophages (M), and AT-III (egg-shaped circles). Only heparin with the high-affinity pentasaccharide binds to AT-III, but binding to other proteins and to cells is nonspecific and occurs independently of the AT-III binding site.

direct relationship between APTT and efficacy observed in the subgroup analysis of the GUSTO I study[65] in patients with acute MI who were treated with thrombolytic therapy followed by heparin. And even if the APTT were predictive of clinical efficacy, its value would be limited by the variable responsiveness of commercial APTT reagents to heparin.[66]

The risk of heparin-associated bleeding increases with dose[67,68] and with concomitant thrombolytic therapy[69–72] or the GPIIb/IIIa antagonist abciximab.[54,55] The risk of bleeding is also increased by recent surgery, trauma, invasive procedures, or concomitant hemostatic defects.[73]

Despite its limitations, the APTT remains the most frequently used method for monitoring the anticoagulant response to heparin. The APTT should be measured approximately 6 h after the bolus dose of heparin, and the continuous IV dose should be adjusted based on the result.

When heparin is given by sc injection in a dose of

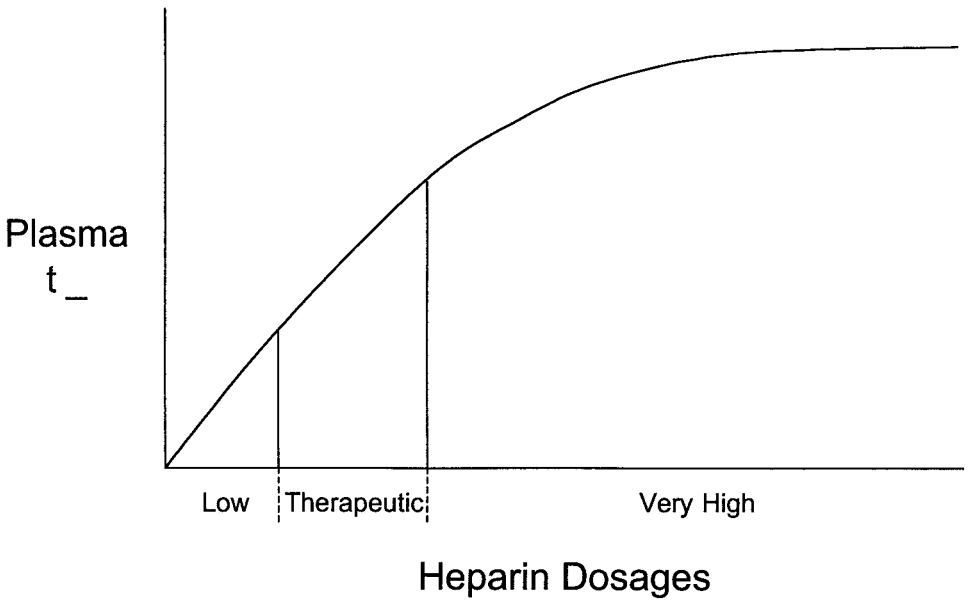

FIGURE 6. Low doses of heparin clear rapidly from plasma through saturable (cellular) mechanism of clearance. Therapeutic doses of heparin are cleared by a combination of the rapid, saturable mechanism and the slower, nonsaturable dose-independent mechanism of renal clearance. Very high doses of heparin are cleared predominantly through the slower nonsaturable mechanism of clearance.

Table 3—*Relation Between Failure to Reach Lower Limit of Therapeutic Range of APTT and Thromboembolic Events From Subgroup Analysis of Prospective Studies*

Source	Condition	Outcome	RR*
Hull et al[38]	DVT	Recurrent venous thromboembolism	15.0
Basu et al[61]	DVT	Recurrent venous thromboembolism	10.7
Turpie et al[51]	Acute MI	Left ventricular mural thrombosis	22.2
Kaplan et al[58]	Acute MI	Recurrent MI/angina pectoris	6.0
Camilleri et al[57]	Acute MI	Recurrent MI/angina pectoris	13.3

*RR refers to the relative increase in event rates when the rates in patients with subtherapeutic APTT times are compared with the rates in patients whose values are in the therapeutic range.

35,000 U/24 h in two divided doses,[52] the anticoagulant effect is delayed for approximately 1 h and peak plasma levels occur at approximately 3 h.

Dosing Nomograms

Audits of physician-directed heparin therapy have demonstrated a great deal of variability in dosing decisions.[74–77] A number of methods for standardizing the management of IV heparin therapy have been published, including heparin dose-adjustment nomograms[56,78–83] and computer algorithms.[84,85] Nomograms and computer-assisted dosage adjustment have also been used to manage heparin in conjunction with thrombolytic therapy for patients with MI.[65,81,85] The weight-adjusted nomogram has been incorporated into the Agency for Health Care Policy and Research guideline for treatment of UA.[86,87]

A weight-based nomogram using a starting dose of 18 U/kg/h heparin infusion (1,260 U/h for a 70-kg patient; Fig 7) reduced recurrent thromboembolism in a randomized controlled trial (relative risk [RR] = 0.2; 95% confidence interval [CI], 0.05 to 0.91)[78,88]; the control group, however, received an inadequate initial heparin infusion (1,000 U/h). Several other nomograms utilize initial heparin infusion doses as low as 12 U/kg/h,[89,90] but the APTT was determined unconventionally[91] and therefore might not be valid.

Two nomograms have been validated independently[92,93]; both significantly reduced latency to therapeutic APTT levels. Over a 5-year period, voluntary physician use of the nomogram approached 95% at one institution and this was associated with significantly higher initial heparin dosage, shorter time to therapeutic APTT, and no increase in bleeding.[94]

Weight-adjusted nomograms have also been evaluated in clinical trials in patients with UA. These have used a lower initial infusion rate of 15 U/kg/h.[95,96] In the OASIS-2 study,[95] a bolus dose of 5,000 U was followed by an infusion of 15 U/kg/h. More than 80% of patients reached the therapeutic APTT range (60 s and 100 s) within 24 h. In the TIMI-11B study,[96] a 70-U/kg bolus was followed by an infusion of 15 U/kg/h and the APTT reached 55 to 58 s in 42% of patients within 12 h. A weight-adjusted nomogram has been incorporated into the guidelines for treatment of UA promulgated by the Agency for Health Care Policy and Research.[86,87]

When a nomogram is used, it is important to determine the appropriate therapeutic range based on the local laboratory reagent and to adapt the recommended dosage adjustments accordingly. For patients with venous thrombosis or PE, the targeted APTT should be equivalent to a heparin level or 0.3 to 0.7 U/mL by antifactor Xa heparin levels.[97,98] A lower therapeutic range is recommended for patients with acute myocardial ischemia receiving thrombolytic or GPIIb/IIIa antagonist agents, since a lower dose of heparin proved safer and no less effective in these circumstances than the higher-dose regimen established for patients with venous thrombosis. Recognizing that the traditional heparin dosing regimens cause excessive bleeding in patients with acute MI who receive thrombolytic therapy, a therapeutic range corresponding to antifactor Xa levels of 0.14 to 0.34 seems reasonable.[89] Failure to adapt nomograms to the therapeutic range could result in dangerous errors in heparin therapy.

Heparin Resistance

Some patients require higher-than-average doses of heparin to prolong APTT to the therapeutic range. These patients are designated heparin resistant if their daily

Initial dose	80 U/kg bolus, then 18 U/kg/h
APTT, < 35 s (<1.2 x control)	80 U/kg bolus, then 4 U/kg/h
APTT, 35 to 45 s (1.2 to 1.5 x control)	40 U/kg bolus, then 2 U/kg/h
APTT, 46 to 70 s (1.5 to 2.3 x control)	No change
APTT, 71 to 90 s (2.3 to 3 x control)	Decrease infusion rate by 2 U/kg/h
APTT, > 90 s (>3 x control)	Hold infusion 1 h, then decrease infusion rate by 3 U/kg/h

Figure 7. Weight-based nomogram.

heparin requirement is > 35,000 U/24 h,[62,99,100] and approximately 25% of patients with venous thromboembolism fulfill this criterion.[38,52,101–103] Heparin resistance has been associated with AT deficiency,[5,91] increased heparin clearance,[104] elevations in heparin binding proteins,[40,105,106] and elevations of factor VIII,[62,107] fibrinogen,[107] and platelet factor 4 (PF4).[108] Aprotinin and nitroglycerin have been reported to cause drug-induced heparin resistance,[109,110] though the association with nitroglycerin is controversial.[111] Factor VIII or fibrinogen levels are elevated in response to acute illness or pregnancy.[91,112,113] Elevation of the levels of factor VIII alters the response of the APTT to heparin without diminishing the antithrombotic effect,[62] as the anticoagulant effect of heparin (measured by the APTT) and the antithrombotic effect measured by anti-Xa activity become dissociated.[91,100] Studies in experimental animals demonstrated that the infusion of factor VIII significantly lowers APTT values without interfering with the antithrombotic effect of heparin. Under these experimental circumstances, heparin concentration was unperturbed and was a more accurate measure of thrombus inhibition than the APTT.[114] A randomized, controlled trial has shown that adjusting dosage by anti-Xa heparin concentrations results in favorable clinical outcomes in heparin-resistant patients despite lower doses of heparin and subtherapeutic APTT levels.[62]

For patients who require > 35,000 U of UFH per 24 h, the dose should be adjusted to maintain anti-Xa heparin levels of 0.35 to 0.70 IU/mL.[91,106,112] In a randomized, controlled trial in 131 patients with venous thromboembolism requiring > 35,000 U of heparin per day, monitoring the APTT was compared to anti-Xa heparin activity with no significant differences in clinical outcomes, but the group monitored using anti-Xa heparin levels required significantly less heparin with no difference in bleeding.[62] This approach is especially useful for patients at high risk of bleeding when continued heparin therapy is necessary. Substitution of LMWH may be inadvisable in such patients due to its long half-life and the lack of an effective neutralizing agent. Although measurement of AT levels has also been recommended in the management of heparin resistance,[91] low values are usually secondary to heparin therapy,[115,116] rather than the cause of heparin resistance.

Limitations

The limitations of heparin are based on its pharmacokinetic, biophysical, and its nonanticoagulant biological properties.[117] All of these limitations are caused by the AT-independent, charge-dependent binding properties of heparin to proteins and surfaces. Pharmacokinetic limitations are caused by the following: (1) AT-independent binding of heparin to plasma proteins,[118] to proteins released from platelets,[119] and possibly to endothelial cells, which result in the variable anticoagulant response to heparin and to the phenomenon of heparin resistance[62]; and (2) AT-independent binding to macrophages and endothelial cells, which result in its dose-dependent mechanism of clearance.

The biophysical limitations occur because the heparin/AT complex is unable to inactivate factor Xa in the prothrombinase complex and thrombin bound to fibrin or to subendothelial surfaces. The biological limitations of heparin include osteopenia and heparin-induced thrombocytopenia (HIT). Osteopenia is caused as a result of the binding of heparin to osteoblasts,[35] which then release factors that activate osteoclasts, whereas HIT results from heparin binding to PF4 to form an epitope to which the HIT antibody binds.[120,121] The pharmacokinetic and nonanticoagulant biological limitations of heparin are less evident with LMWH,[122] while the limited ability of the heparin-AT complex to fibrin- bound thrombin and factor Xa is overcome by several new classes of AT-independent thrombin and factor Xa inhibitors.[123]

The anticoagulant effect of heparin is modified by platelets, fibrin, vascular surfaces, and plasma proteins. Platelets limit the anticoagulant effect of heparin by protecting surface factor Xa from inhibition by heparin/AT[124,125] and by secreting PF4, a heparin-neutralizing protein.[126] Fibrin limits the anticoagulant effect of heparin by protecting fibrin-bound thrombin from inhibition by heparin/AT.[127] Heparin binds to fibrin and bridges between fibrin and the heparin binding site on thrombin. As a result, heparin increases the affinity of thrombin for fibrin, and by occupying the heparin binding site on thrombin, protects fibrin-bound thrombin from inactivation by heparin/AT.[128,129] Thrombin also binds to subendothelial matrix proteins, where it is protected from inhibition by heparin.[130] These observations explain why in experimental animals[131,132] heparin is less effective than the AT-independent thrombin and factor Xa inhibitors[123] at preventing thrombosis at sites of deep arterial injury and may explain why hirudin is more effective than heparin in UA and non-Q-wave myocardial infarction (NQMI).[95]

Therapeutic Use

Heparin is indicated for prevention of venous thromboembolism; for treatment of venous thrombosis and PE for the early treatment of patients with UA and acute MI; for patients who undergo cardiac surgery using cardiopulmonary bypass, vascular surgery, coronary angioplasty, and stents; and in selected patients with disseminated intravascular coagulation. LMWHs are indicated for prevention of venous thromboembolism, for treatment of venous thrombosis, for treatment of acute PE, and for the early treatment of patients with UA. Levels of evidence and grading of recommendations for the clinical use of heparin and LMWHs are discussed in the chapters discussing antithrombotic therapy for the various clinical indications.

In patients with venous thromboembolism or UA, the dose of heparin is usually adjusted to maintain the APTT at an intensity equivalent to an antifactor Xa level of 0.35 to 0.7 U/mL. For many APTT reagents, this is equivalent to a ratio (patient/control APTT) of 1.5 to 2.5. This therapeutic range[38,61] is recommended based on animal studies[114] and subgroup analysis of prospective studies of patients with established deep vein thrombosis (DVT),[38] and studies of prevention of mural thrombosis after MI[51] and prevention of recurrent ischemia following coronary

thrombolysis.[57,58] Recommended heparin regimens for venous and arterial thrombosis are summarized in Table 4.

Treatment of Venous Thromboembolism

The efficacy and safety of continuous IV infusion of heparin has been compared with intermittent IV injection in seven studies[135–141] and with high-dose sc injection in five studies.[52,101,142–144] From these studies, it is difficult to determine the optimal route of heparin administration because most were underpowered, total doses varied, and disparate criteria were used to assess outcome. A pooled analysis of 11 clinical trials involving 15,000 patients treated with either IV UFH (administered as an initial bolus of 5,000 U followed by 30,000 to 35,000 U/24 h with APTT monitoring) or sc LMWH[145] found the mean incidence of recurrent venous thromboembolism 5.4% (fatal in 0.7%) and major bleeding 1.9% (fatal in 0.2%).

A 5-day course of heparin therapy appears as effective as a 10-day course for the treatment of DVT (Table 5),[103,146] and brevity has obvious appeal, reducing both hospital stay and the risk of HIT. While adequate for most patients with venous thromboembolism, a 5-day course of heparin therapy may not be sufficient for those with extensive iliofemoral venous thrombosis or PE who were underrepresented in these studies.[103,146]

Prophylaxis Against Venous Thromboembolism

Heparin in a fixed low dose of 5,000 U sc every 8 to 12 h results in 60 to 70% risk reduction for venous thrombosis and fatal PE.[147,148] The incidence of fatal PE in general surgical patients was reduced from 0.7% in control subjects to 0.2% in one analysis ($p < 0.001$),[147] and from 0.8 to 0.3% ($p < 0.001$) in a larger analysis that included orthopedic surgical patients.[148] There was also a statistically significant decrease in mortality, from 3.3 to 2.4% ($p < 0.02$).[148] The use of low-dose heparin was associated with a small excess of wound hematoma,[147–149] but there was no statistically significant increase in major bleeding. Low-dose heparin therapy is also effective for prevention of venous thromboembolism in patients with MI and other serious medical disorders,[150] reducing in-hospital mortality by 31% ($p < 0.05$) in a study of 1,358 general medical patients > 40 years old.[151] The incidence of DVT remains substantial (20 to 30%) after hip surgery,[148] despite low-fixed-dose heparin prophylaxis, and risk can be reduced further by administering either adjusted-dose heparin[152] or fixed-dose LMWH.[122] Moderate-dose warfarin therapy is also effective in patients undergoing major orthopedic surgical procedures,[153,154] but direct comparisons of low-dose heparin and warfarin therapy have not been performed in patients undergoing major orthopedic surgery in sufficiently powered trials.

Coronary Artery Disease

Coronary thrombosis is important in the pathogenesis of UA, acute MI, and sudden cardiac death, and affects the outcomes of patients with acute MI treated with thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA). Heparin is no longer used as the sole antithrombotic drug in patients with acute coronary syndromes, but it is combined with aspirin in eligible patients with acute MI,[155] with thrombolytic therapy in patients with evolving MI, and with GPIIb/IIIa antagonists in high-risk patients with UA[156,157] or in those undergoing high-risk PTCA.[54,55,157] Heparin increases the risk of bleeding when given in full doses combined with aspirin,[155,158] thrombolytic therapy, and GPIIb/IIIa antagonists, so the dose is usually reduced in these settings.[55]

Unstable Angina and NQMI

Heparin has been evaluated in a number of randomized, double-blind, placebo-controlled clinical trials for the short-term treatment of patients with UA or NQMI.[159–162] When used alone in patients with UA, heparin reduces the risk of developing recurrent angina or acute MI.[160–162] Meta-analysis of short-term results suggests that the combination of heparin and aspirin reduces cardiovascular death and MI by about 30% over that achieved by aspirin alone.[159]

Theroux et al[160] compared the relative efficacy and safety of heparin, aspirin, or the combination in 479 patients with UA. The incidence of MI during the acute period was 11.9% in the placebo group and was reduced to 3.3% in the aspirin group ($p = 0.012$), 0.8% in the heparin group ($p < 0.0001$), and 1.6% with the combination ($p = 0.001$; all comparisons to placebo). The incidence of refractory angina, 22.9% in the placebo group, was re-

Table 4—*Clinical Use of Heparin*

Conditions	Recommended Heparin Regimen*
Venous thromboembolism	
Prophylaxis of DVT and PE	5,000 U sc q8h or q12h or adjusted low-dose heparin
Treatment of DVT	5,000 U IV bolus followed by 32,000 U q24h by IV infusion or 35,000 to 40,000 U q24h sc, adjusted to maintain APTT in the therapeutic range
Coronary heart disease	
UA or acute MI without thrombolytic therapy	5,000 U IV bolus followed by 32,000 U q24h IV infusion adjusted to maintain APTT in the therapeutic range
Acute MI after thrombolytic therapy†	5,000 U IV bolus followed by 24,000 U q24h adjusted to maintain APTT in the therapeutic range

*APTT varies in responsiveness to heparin.
†Role of heparin unproven.

Table 5—*Comparison of Long and Short Courses of Heparin Therapy in the Treatment of Proximal Vein Thrombosis

Variables	Gallus et al[146] (n = 266)		Hull et al[103] (n = 199)	
	Short Course, 4 d	Long Course, 9.5 d	Short Course, 5 d	Long Course, 10 d
Recurrent VTE, %				
During heparin treatment	3.6	4.7	7.7	7.7
During warfarin treatment	3.3	1.6		
Total during treatment	6.9	6.3		

*VTE = venous thromboembolism.

duced to 8.5% with heparin ($p = 0.002$), 10.7% with heparin plus aspirin ($p = 0.11$), but it was 16.5% in the aspirin group. In a second study,[163] these investigators compared heparin with aspirin, eliminating the placebo and combination therapy groups. Fatal or nonfatal MI occurred in 4 of 362 heparin-treated patients, compared with 23 of 362 patients treated with aspirin (odds ratio = 0.16; $p < 0.005$).

In contrast, the Research Group in Instability in Coronary Artery Disease Investigators[159] found heparin (10,000 U q6h for 24 h and 7,500 U q6h for 5 days thereafter) no more effective than aspirin (75 mg/d) in 796 men with UA or NQMI. The incidence of MI or death 5 days after enrollment was significantly reduced only in the group given the combination of aspirin plus heparin (1.4%, $p = 0.027$), and not in the groups receiving either heparin or aspirin alone. After 30 days and 90 days, both the aspirin and aspirin-plus-heparin groups fared significantly better than those given placebo, but the outcome in the group receiving heparin alone was no different than placebo.

A meta-analysis of published data from six randomized trials, each relatively small (composite n = 1,353) including the foregoing, found a risk reduction of 33% in cardiovascular death and MI (95% CI, 2 to 56%) with the combination of IV UFH and aspirin compared to placebo, but this was of borderline statistical significance (Fig 8).[155] When data from the FRISC trial,[228] which compared LMWH to placebo in patients treated with aspirin, are also considered, the combination of heparin and aspirin appears more effective than aspirin alone in patients with UA.

Acute MI

The benefit of heparin in patients with acute MI not given thrombolytic therapy may not be applicable to the current clinical practice of treating these patients with aspirin. In randomized trials before the thrombolytic era, heparin reduced reinfarction by an average of 22% and mortality by 17%.[164]

Heparin also reduces the incidence of mural thrombosis.[56] A fixed dose of 12,500 U sc q12h reduced the incidence of mural thrombosis detected by two-dimensional echocardiography by 72% compared with no treatment,[165] and by 58% compared with low-dose heparin (5,000 U sc q12h; $p < 0.06$ for each study).[166]

Coronary Thrombolysis

Although in the past it was generally accepted that heparin is effective after coronary thrombolysis, the results of recent studies have cast doubt on this view. In both the International Study Group[167] and the ISIS-3[168] studies, adjunctive heparin, 12,500 U sc q12h, in patients receiving thrombolytic therapy plus aspirin produced only marginal benefit at the price of increased bleeding. The delayed sc heparin regimen used in

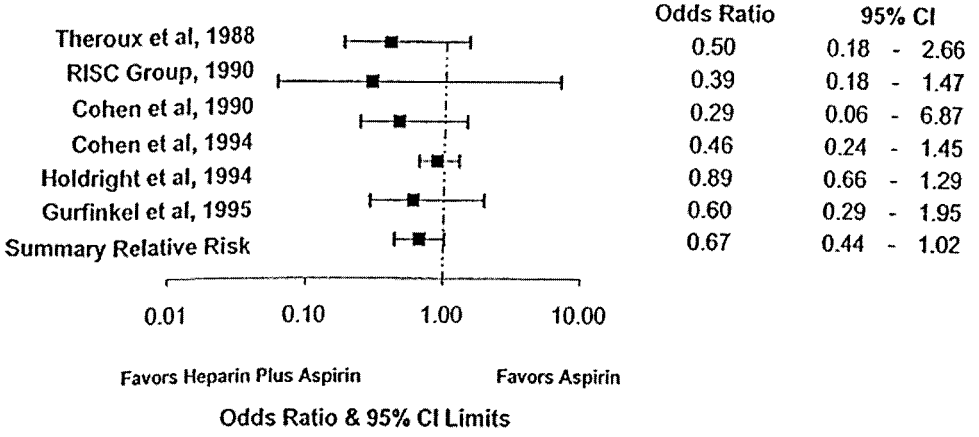

Figure 8. RR of MI or death during hospitalization.

the ISIS-3 trial proved no better or worse than high-dose IV heparin (5,000 U initially followed by infusion of 1,000 to 1,200 U/h to maintain APTT 60 to 85 s) among patients with acute MI receiving streptokinase in the GUSTO trial in terms of patency of the infarct-related artery, reinfarction hemorrhage, or mortality.[65]

In a much smaller study of 250 patients randomized to treatment with either aspirin alone or aspirin plus weight-adjusted IV heparin infusion beginning 4 h after anisoylated plasminogen streptokinase activator complex infusion, there were no differences in ischemic outcomes, but overall bleeding was significantly greater with heparin (32% vs 17.2%; $p = 0.006$).[169]

A meta-analysis of trials of thrombolytic therapy in patients with acute MI, largely driven by the International Study Group and the ISIS-3 studies, found that when aspirin is also used, heparin reduced mortality by only 6% (95% CI, 0 to 10%; $p = 0.03$), translating to five fewer deaths per 1,000 treated patients; three fewer reinfarctions ($p = 0.04$), and one fewer PE ($p = 0.01$; Table 6). This relatively small benefit was counterbalanced by three major bleeds per 1,000 patients treated with heparin ($p < 0.0001$) and an insignificant trend toward excess stroke.[170]

Data on the role of adjunctive heparin in patients treated with tissue plasminogen activator (tPA) are limited. From contemporary studies, Kruse and associates[171] concluded that the role of heparin as adjunctive treatment to accelerated tPA is still an open issue. Among six randomized trials involving tPA therapy, there was a trend toward reduction of in-house mortality (odds ratio [OR] = 0.91; 95% CI, 0.59 to 1.39), but a significantly higher rate of hemorrhagic complications with heparin.[172] Accordingly, the American College of Cardiology/American Heart Association guidelines for management of patients with acute MI suggest adjusting the intensity of heparin based on the type of thrombolytic agent used and the presence or absence of risk factors for systemic embolism.[173]

Coronary Angioplasty

PTCA can be complicated by early thrombotic occlusion. It is standard practice to administer either as an IV bolus of 10,000 U with repeated smaller injections as required, or as a weight-adjusted dose of 100 to 175 U/kg followed by infusion of 10 to 15 U/kg/h adjusted to maintain the activated clotting time (ACT) at > 300 to 350 s. There is evidence that the complication rate is higher with lower ACT values.[174] When these high-dose regimens are used in combination with abciximab and aspirin, however, heparin increases the risk of major bleeding.[54,55] The risk can be reduced without compromising efficacy by lowering the initial bolus dose of heparin to 70 U/kg, adjusting subsequent doses to raise ACT of > 200 s, and removing arterial sheaths when ACT falls to < 150 to 180 s.[55] Postprocedural heparin infusions are not needed for most patients after coronary angioplasty, who are treated with a combination of aspirin and ticlopidine or aspirin and clopidogrel.

LMWHs

Historical Perspective

The development of LMWHs for clinical use was stimulated by three main observations. Compared to UFH, LMWH displayed the following: (1) reduced antifactor IIa activity relative to antifactor Xa activity[12,175]; (2) a more favorable benefit-risk ratio[176–181] in experimental animals; and (3) superior pharmacokinetic properties.[182–187] Of these potential advantages, only the pharmacokinetics are of clear clinical importance.[122,188]

In 1976, Johnson et al[175] and Andersson et al[12] reported that LMWH fractions prepared from standard commercial-grade heparin had progressively less effect on the APTT, as they were reduced in molecular size, while still inhibiting activated factor X (factor Xa). The APTT activity of heparin reflects mainly its antifactor IIa activity. The disassociation of antifactor Xa activity from its effect on antithrombin (IIa) activity (expressed as an APTT measurement) challenged the prevailing biophysical model for the anticoagulant effect of heparin, which predicted that any heparin molecule, irrespective of chain length, would catalyze the inactivation of serine protease coagulation enzymes equally, provided it contained the high-affinity binding site for AT. The difference in anticoagulant profile between LMWH and UFH was elucidated in the United States and in Sweden (Table 7).[15,16,189–193]

Evidence that LMWH produces less microvascular bleeding than UFH in experimental models[176–181] has not been borne out in human studies involving prevention and treatment of venous thrombosis, PE, or UA. In these studies, LMWH and UFH were associated with similar rates of bleeding.

Table 6—*Effect of Heparin With or Without Aspirin in Coronary Thrombolysis: Overview of 26 Randomized Trials

Variables	No Aspirin (n = 5,459)	p Value	Aspirin (n = 68,090)	p Value
Death	35	0.002	5	0.03
Reinfarction	15	0.08	3	0.04
Stroke	10	0.01	1	0.01
Major bleeding	10 more	0.01	3 more	< 0.001

*Data are presented as reduction per 1,000 patients assigned heparin. Adapted from Collins et al.[158]

Table 7—*Relationship Between Molecular Weight and Anticoagulant Activity of Heparin Fractions

Heparin Oligosaccharides	Molecular Weight	Anticoagulant Activity	
		Anti-Xa	Anti-IIa
8	2,400	1.3	Nil
12	3,600	2.58	Nil
16	4,800	1.60	Nil
18	5,400	0.95	0.51
24	7,200	1.30	1.21

*Data from Lane et al.[15]

Structure and Pharmacology

LMWHs are derived from heparin by chemical or enzymatic depolymerization, yielding fragments approximately one third the size of heparin. The various LMWHs approved for use in Europe, Canada, and the United States are shown in Table 8. Since they are prepared by different methods of depolymerization, they differ to some extent in pharmacokinetic properties and anticoagulant profile, and may not be clinically interchangeable. LMWHs have a mean molecular weight of 4,500 to 5,000 d, with a distribution of 1,000 to 10,000 d.

The anticoagulant, pharmacokinetic, and other biological differences between UFH and LMWH can be explained by the relatively lower binding properties of LMWH to circulating and cellular proteins (Table 9). Compared to UFH, LMWHs have less ability to inactivate thrombin because the smaller fragments cannot bind simultaneously to both AT and thrombin. However, since bridging between AT and factor Xa is less critical for antifactor Xa activity, the smaller fragments inactivate factor Xa almost as well as do larger molecules.[24,194–196] LMWHs are cleared principally by the renal route, and their biological half-life is prolonged in patients with renal failure.[197–199] LMWH preparations have a longer plasma half-life and better bioavailability at low doses than UFH, and a more predictable dose response.[182–185,200] These findings provided the rationale for comparing unmonitored weight-adjusted LMWH with APTT-guided UFH in patients with DVT or UA.

Anticoagulant Effects

Like UFH, LMWHs produce their major anticoagulant effect by activating AT. The interaction with AT is mediated by a unique pentasaccharide sequence[7,201] found on fewer than one third of LMWH molecules. Since a minimum chain length of 18 saccharides (including the pentasaccharide sequence) is required to form ternary complexes among heparin, AT, and thrombin, only the minority of LMWH species that are above this critical chain length are available to inactivate thrombin. In contrast, all LMWH chains containing the high-affinity pentasaccharide catalyze the inactivation of factor Xa (Fig 3). Since virtually all heparin molecules contain at least 18 saccharide units,[190,191] UFH has an antifactor Xa to antifactor IIa ratio near unity. In contrast, the ratios of commercial LMWHs range between 2 and 4 depending on molecular weight distribution.

Monitoring LMWH in Patients With Obesity or Renal Failure

Laboratory monitoring of LMWH therapy is usually not necessary. In certain clinical situations, such as morbid obesity and renal failure, however, the dose can be difficult to determine. Although dosing studies have not been carried out in these special populations, monitoring has been suggested.[202–206] Several laboratory assays have been proposed, including the Heptest (Sigma-Aldrich Canada; Oakville, Ont) and the chromogenic anti-Xa assay[204,205,207]; the latter is more widely available and currently recommended by the College of American Pathologists.[208] The minimal therapeutic levels have not been established definitively, but anti-Xa levels were found to be inversely related to thrombus propagation in some studies.[209,210] High anti-Xa levels ($>$ 0.8 U/mL at steady state) in patients receiving therapeutic doses of LMWH have also been associated with bleeding in some experimental animal and clinical studies.[211–215] The best time to perform an anti-Xa assay (if indicated in renal failure or morbid obesity) is 4 h after sc injection of a weight-adjusted dose of LMWH.[50,205,208,216] For twice-daily administration, a conservative therapeutic range is 0.6 to 1.0 IU/mL.[204,208,216] The target range at 4 h is less clear in patients treated with LMWH once daily, but 1.0 to 2.0 IU/mL is reasonable.[208] Although weight-adjusted dosing has not been evaluated in patients with severe obesity,[217–228] it would be prudent to check an anti-Xa level after the first few doses in these patients, but once the correct dose is established, repeated testing is unnecessary.

Randomized trials of LMWH either excluded patients with renal failure[217,219,220,223,224,227,228] or failed to specify

Table 8—*Commercial LMWHs and a Heparinoid: Methods of Preparation*

Agents	Manufacturer/Location	Method of Preparation
Nadroparin calcium (Fraxiparin)	Sanofi/Gentilly, France	Nitrous acid depolymerization
Enoxaparin sodium (Lovenox/Clexane)	Aventis/Collegeville, PA	Benzylation followed by alkaline depolymerization
Dalteparin (Fragmin)	Pharmacia/Peakack, NJ	Nitrous acid depolymerization
Ardeparin (Normiflo)	Wyeth-Ayerst/Philadelphia, PA	Peroxidative depolymerization
Tinzaparin (Innohep)	Leo Laboratories/Dublin, Ireland	Enzymatic depolymerization with heparinaze
Reviparin (Clivarine)	Knoll/Markham, Ont	Nitrous acid depolymerization
Danaparoid sodium (Orgaran)	NV Organon/Oss, Netherlands	Prepared from animal gut mucosa; contains heparin sulfate (84%), dermatan sulfate (12%), and chondroitin sulfate (4%)

Table 9—*Biological Consequences of Reduced Binding of LMWH to Proteins and Cells*

Binding Targets	Biological Effects	Clinical Consequences
Thrombin	Reduced anti-IIa to anti-Xa ratio	Unknown
Proteins	More predictable anticoagulant response	Monitoring of anticoagulant effect unnecessary
Macrophages	Cleared through renal mechanism	Longer plasma half-life; once-daily sc treatment effective
Platelets	Reduced incidence of heparin-dependent antibody	Reduced incidence of HIT
Osteoblasts	Reduced activation of osteoclasts	Lower incidence of osteopenia

whether such patients were recruited.[218,222,225,226] When LMWH is administered to patients with renal failure, it is prudent to check anti-Xa levels periodically to avoid accumulation to toxic levels.

Anti-Xa assays in use throughout North America are highly variable: one specimen tested in 46 laboratories yielded mean LMWH anti-Xa activity of 0.7 IU/mL with a SD of 0.1, and a range of 0.8 to 1.0 IU/mL.[206]

Oral Heparin

Heparin is not normally absorbed from the GI tract.[229] Chemical delivery agents (N-acylated amino acids) have been synthesized that can form noncovalent bonds with heparin and facilitate absorption across intestinal epithelium.[230,231] Animal studies have demonstrated antithrombotic effects,[232] and 30,000 U of an oral preparation increased APTT values in healthy volunteers from 28 to 42 s ($p < 0.01$) and anti-Xa levels to 0.2 ± 0.05 IU/mL.[233] Several clinical trials are now underway to investigate clinical applications of oral heparin preparations.

Possible Antineoplastic Effects of LMWH

In 1992, two clinical trials comparing LMWH to UFH in the treatment of venous thromboembolism demonstrated unexpected reductions in death from cancer.[102,220] A meta-analysis[145] of 629 cancer patients from nine clinical trials demonstrated that the odds ratio for 3-month cancer mortality was 0.65 (95% CI, 0.40 to 0.93) for LMWH compared to UFH. The hypothesis that LMWH might have anticancer effects was not considered *a priori* in the design of these trials, so the results observed in the meta-analysis must be considered exploratory. However, the observed results are consistent with animal studies, which have shown that LMWH inhibits metastasis[212,213] and angiogenesis necessary for tumor growth[234,235] Currently, several trials are underway to evaluate the effects of LMWH preparations on mortality in patients with cancer and thrombosis.

Clinical Applications

Prevention of Venous Thrombosis

LMWHs were first evaluated for the prevention of venous thrombosis in high-risk surgical patients in the mid-1980s, and considerable experience has been gained for this indication. In general surgical patients and in high-risk medical patients, low doses of LMWHs administered sc once-daily are at least as effective and safe as low-dose UFH administered sc two or three times daily. LMWH has become the anticoagulant of choice for the prevention of venous thrombosis following major orthopedic surgery and in anticoagulant-eligible victims of major trauma. The risk of bleeding with LMWH is small and comparable to that with low-dose UFH.

General Surgery

LMWHs proved safe and effective for prevention of thromboembolism in two well-designed randomized trials of patients undergoing noncardiovascular surgery. One trial[236] in 4,498 patients found a statistically significant reduction in thromboembolic mortality with LMWH (0.07%) compared with UFH (0.36%). In contrast, a meta-analysis[237] of randomized trials comparing low-dose UFH with LMWH reported minimal differences between the two forms of prophylaxis.

Orthopedic Surgery

Compared to placebo, LMWHs produced a 70 to 79% risk reduction for venous thrombosis in two studies[238,239] without an increase in major bleeding. There was a small increase in minor bleeding with LMWH in a third study,[240] but none was sufficiently powered to exclude a modest increase in major bleeding.

LMWHs have been compared with a variety of other methods of prophylaxis, including low-dose UFH,[241–243] adjusted-dose heparin,[244,245] dextran,[246,247] and warfarin.[248] In most studies performed in North America, LMWH therapy was started postoperatively, the first dose administered 12 to 24 h after surgery, which increased the acceptability of prophylaxis among surgeons. This approach is particularly appealing in view of concerns about the risk of spinal cord hematoma when anticoagulant prophylaxis is used in conjunction with spinal anesthesia. In such cases, the first dose of LMWH should be delayed until after the epidural catheter has been removed; when this is not possible, the catheter should be removed at least 8 h after the last dose of LMWH. Under these circumstances, other drugs that impair hemostasis (such as nonsteroidal anti-inflammatory agents) should be avoided.

A meta-analysis[249] of randomized studies comparing prophylactic LMWH with fixed low-dose or adjusted-dose UFH reported an incidence of venous thrombosis of 15.9% in the group given LMWH and 21.7% in the heparin groups ($p = 0.01$), with a lower incidence of

proximal venous thrombosis in the LMWH group (5.4% vs 12.5%; $p < 0.0001$). There was no difference in the rates of bleeding between the two groups (Table 10). These results are comparable to those of an earlier meta-analysis.[237]

In two studies, LMWH was compared to low-dose UFH for prevention of venous thrombosis after elective knee arthroplasty. The incidences of venous thrombosis were 23% and 24.6% in the LMWH groups compared with 27% and 34.2% in the heparin groups, respectively.[250,251] The difference was statistically significant in one study[250] but not the other study,[251] and there was no difference in the incidence of bleeding between the two types of heparin in either study.

Comparison of LMWH With Oral Anticoagulants

LMWH preparations have been compared with warfarin and other oral anticoagulants in six studies involving high-risk orthopedic surgical patients.[252–259] The efficacy of the LMWH preparations was equal to warfarin in patients undergoing elective hip replacement, but superior in patients undergoing major knee surgery (Table 11). In several of these studies, LMWH was associated with a slightly greater incidence of major bleeding.

Hip Fracture

There have been two randomized trials with the LMWH danaparoid sodium in patients with fractured hips. In one, the incidence of thrombosis was 13% in patients given danaparoid sodium compared with 35% in patients given dextran ($p < 0.001$).[246] Transfusion requirements were significantly higher in the dextran group. In the other trial,[260] venous thrombosis occurred in 44.8% of patients treated with aspirin vs 27.8% treated with danaparoid sodium ($p < 0.05$); there was no difference in bleeding rates.

Multiple Trauma

LMWH (enoxaparin sodium; 30 mg sc q12h) was compared with UFH (5,000 U q12h); treatment with each was started within 36 h of multiple trauma. The incidence of venous thrombosis was 31% among 129 patients in the LMWH group vs 44% among 136 patients allocated to UFH ($p = 0.014$). The incidence of proximal venous thrombosis was 6% in the LMWH group vs 15% in the low-dose UFH group ($p = 0.09$).[261] Major bleeding occurred in 0.6% with UFH and 2.9% with LMWH.

Summary of LMWH in Orthopedic Surgery and Trauma

Overall, LMWHs appear effective for prevention of venous thomboembolism and safe in high-risk patients undergoing major orthopedic surgical procedures. Compared to placebo, the RR reduction for all thrombi and for proximal vein thrombi is approximately 70%. LMWHs are more effective than low-dose UFH, at least as effective as warfarin, and more effective than dextran or aspirin in patients undergoing total hip arthroplasty, and more effective than warfarin, aspirin, or dextran in patients undergoing major knee surgery. Similarly, LMWHs are more effective than aspirin in patients with hip fracture. The risk of bleeding with LMWH is comparable to low-dose UFH or warfarin.

Neurosurgery

In a study[262] comparing LMWH plus compression stockings with compression stockings alone, those assigned to LMWH had a risk reduction of 48%. There was no difference in major bleeding.

Medical Patients

LMWH has been compared to placebo in two studies of patients with ischemic stroke[263,264] and has been compared with low-dose heparin in two studies.[265,266] Compared with placebo, LMWHs reduce the risk of venous thrombosis between 40% and 86% without an increase in clinically important bleeding, and in studies comparing LMWH with UFH, the reduction in risk of thrombosis was > 70% with LMWH.[144,266]

Treatment of Venous Thromboembolism

A number of well-designed studies have compared LMWH preparations with UFH in patients with venous thrombosis or PE. A meta-analysis[267] of randomized studies published up to 1996, in which four different LMWH preparations administered by sc injection in unmonitored weight-adjusted doses were compared with IV UFH, found similar rates of recurrent thromboembolism and major bleeding with each type LMWH and with APTT-guided IV UFH (Tables 12, 13). Following publication of this analysis, five additional trials[155,217,218,225,226] have been completed, two involving patients with venous thrombosis,[218,225] one in patients with venous thrombosis or PE,[226] one in patients with PE,[217] and one in patients with proximal vein thrombosis were also randomized to inferior

Table 10—*Meta-analysis of Randomized Studies Comparing LMWH and Heparin in Elective Hip Surgery*

Factors	Proximal Venous Thrombosis	Total Venous Thrombosis	Major Bleeding	Minor Bleeding	PE
Common OR*	0.40	0.70	0.64	0.90	0.30
95% CI	0.28–0.59	0.53–0.92	0.34–1.23	0.61–1.33	0.09–1.02

*OR < 1 favors LMWH over heparin. Data from Anderson et al.[249]

Table 11—*Controlled Trials With LMWH in Elective Total Knee Surgery

Source	LMWH	DVT	Venous Thrombosis Proximal	Major Hemorrhage	Control	DVT	Venous Thrombosis Proximal	Major Hemorrhage
Hull et al[255]	Tinzaparin	116/258 (45)	20/258 (18)	9/317 (3)	Warfarin	152/277 (55)	34/227 (12)	3/324 (1)
RD Heparin Group[252]	Ardeparin	41/149 (28)	7/149 (5)	NA	Warfarin	60/147 (41)	15/147 (10)	NA
Leclerc et al[256]	Enoxaparin sodium	8/41 (19)	0/41 (0)	0/66 (0)	Placebo	35/54 (65)	11/54 (20)	1/65 (2)
Leclerc et al[257]	Enoxaparin sodium	79/206 (37)	24/206 (12)	7/336 (2)	Warfarin	109/211 (52)	22/211 (10)	6/334 (2)
Spiro et al[253]	Enoxaparin sodium	41/108 (38)	3/108 (3)	9/173 (5)	Warfarin	72/122 (59)	16/122 (13)	4/176 (2)
Fauno et al[251]	Enoxaparin sodium	29/92 (23)	3/92 (3)	NA	Heparin	25/93 (27)	5/93 (5)	NA
Colwell et al[250]	Enoxaparin sodium	54/145 (37)	4/145 (2)	3/28 (1)	Heparin	74/143 (52)	22/143 (15)	3/225 (1)

*Data are presented as No./total patients (%). NA = not available. Data from Colwell et al.[250]

vena cava filter insertion.[219] In two of the studies,[218,225] patients assigned to LMWH were encouraged to administer treatment at home, while those assigned to UFH were treated conventionally with a continuous IV infusion in hospital. Patients with symptomatic PE or previous venous thrombosis were excluded. Out-of-hospital administration of LMWH to eligible patients with DVT was as effective and safe as IV heparin administered in hospital (Table 14).[218,225] In the COLUMBUS study,[226] 1,021 patients with venous thrombosis or PE were randomized to treatment with either sc LMWH (reviparin sodium) or IV adjusted-dose UFH for 8 days. Warfarin therapy was started concomitantly and continued for 3 months. The mean hospital stay was 3 days shorter in patients assigned to LMWH, while rates of recurrent thromboembolism, bleeding, and mortality were similar in both groups.

The relative efficacy and safety of LMWH and UFH have also been investigated in a larger population: 612 patients with acute PE who did not require thrombolytic therapy or pulmonary embolectomy were randomized to regimens of LMWH (tinzaparin, 175 anti-Xa U/kg sc qd) or heparin (50 U/kg IV bolus followed by an infusion of 500 U/kg/d) adjusted to an APTT ratio of 2.0 to 3.0, for prevention of recurrent thromboembolism, major bleeding, and death. By day eight, 9 of 308 patients (2.9%) assigned to UFH and 9 of 304 patients (3.0%) assigned to LMWH developed primary events; by day 90, 22 patients (7.1%) assigned to UFH and 18 patients (5.9%) assigned to LMWH developed events ($p = 0.54$; Table 15). The rate of major bleeding was similar in both groups (2.6% and 2.0%, respectively; not significant). There were 3 deaths at 8 days and 14 deaths (4.5%) at 90 days in those assigned to UFH, and 4 deaths at 8 days and 12 deaths (3.9%) at 90 days in patients assigned to LMWH. Five deaths in the heparin group were treatment related (three from PE and two from major bleeding), compared to four deaths in the LMWH group (three from PE and one from bleeding). Taken together with the results of the COLUMBUS study, the data indicate that sc LMWH is as effective and safe as IV UFH.

A meta-analysis[145] of 11 randomized studies comparing IV UFH and sc LMWH in about 3,500 patients with acute DVT (Table 16) found less major bleeding with LMWH (OR = 0.57; $p = 0.05$). The frequency of recurrent thromboembolic events did not differ significantly between treatment groups (OR = 0.85; $p = 0.28$), but mortality was lower in those assigned LMWH (OR = 0.71; $p = 0.02$). Most deaths were not ascribed to PE, so the mechanism for this reduction is uncertain.

Most studies evaluating LMWH preparations for the treatment of venous thromboembolism evaluated a twice-daily, weight-adjusted regimen. However, two studies using tinzaparin, one in patients with acute venous thrombosis[220] and the other in patients with acute PE,[217] found

Table 12—*LMWH vs Heparin in the Treatment of DVT: Symptomatic Recurrent Venous Thromboembolic Complications During Initial Treatment and During 3-mo to 6-mo Follow-up

Agents	LMWH	Heparin	RR Reduction (95% CI)	p Value
Nadroparin	20/361 (5.5)	32/355 (9.0)	40 (−5–66)	0.07
Tinzaparin	6/213 (2.8)	15/219 (6.9)	59 (−1–83)	0.07
Enoxaparin	13/314 (4.1)	20/320 (6.3)	35 (−32–68)	0.23
Dalteparin	16/322 (5.0)	8/339 (2.4)	−110 (−374–7)	0.07

*Data are presented as No./total patients (%) unless otherwise indicated. Data from Kuijer et al.[267]

Table 13—*LMWH vs Heparin: Incidence of Major Bleeding Complications During Initial Heparin Treatment, Including 48 h After Heparin Treatment Cessation

Agent	LMWH	Heparin	RR Reduction (95% CI)	p Value
Nadroparin	4/446 (0.9)	10/436 (2.3)	59 (−16–86)	0.09
Tinzaparin	1/213 (0.5)	11/219 (5.0)	91	< 0.01
Enoxaparin	5/314 (1.6)	3/320 (0.9)	−70 (−580–58)	> 0.2
Dalteparin	2/433 (0.5)	5/464 (1.0)	55 (−99–90)	> 0.2

*Data are presented as No./total patients (%) unless otherwise indicated. Data from Kuijer et al.[267]

Table 14—*Efficacy and Safety of Two Trials Using Outpatient LMWH for the Treatment of Venous Thromboembolism*

Source	Treatment	Patients, No.	Recurrent Thrombosis, No.	Major Bleeding, No.	Mean Length of Hospital Stay, d
Levine et al[218]	UFH	253	17	3	6.5
	LMWH	247	13	5	1.1
Koopman et al[225]	UFH	198	17	4	1.1
	LMWH	202	14	1	

Table 15—*Relative Efficacy and Safety of LMWH and Heparin in Two Trials That Included Patients With PE

Source	Treatment	Patients, No.	Recurrent Thrombosis	Major Bleeding	Death
Columbus[226]	UFH	511	25 (4.9)	8 (1.6)	39 (7.6)
	LMWH	510	27 (5.3)	10 (2.0)	36 (7.1)
Simonneau et al[217]	UFH	308	6 (1.9)	5 (1.6)	14 (4.5)
	LMWH	304	5 (1.6)	3 (1.0)	12 (3.9)

*Data are presented as No. (%) unless otherwise indicated.

once-daily administration of 175 anti-Xa U/kg as effective and safe as twice-daily dosing.[221,227]

Unstable Angina and NQMI

Although the combination of heparin and aspirin is effective for short-term treatment of patients with UA, within 1 month between 6% and 15% progress to MI or death, despite continuing aspirin therapy.[69,268] LMWHs have been evaluated in seven randomized trials of patients with UA or NQMI (Table 17). The combined rate of MI, recurrent angina, and urgent coronary revascularization was significantly lower in a small (n = 219) open trial with the LMWH nadroparin plus aspirin than with UFH plus aspirin alone.[222] A larger, double-blind, placebo-controlled trial in 1,506 aspirin-treated patients with UA or NQMI[228] compared dalteparin 120 anti-Xa u/kg bid for 6 days followed by 7,500 U qd, with placebo treatment. LMWH reduced the risk of death or MI by approximately 80% at 6 days compared to placebo treatment. The composite rate of death, MI, and revascularization was significantly lower in patients treated with LMWH (10.3% vs 5.4%) over 35 to 45 days, but after 4 to 5 months, the difference between these event rates in the placebo and dalteparin groups was

Table 16—*LMWH vs Heparin for Treatment of DVT: Summary of Meta-analysis Results

Variables	Total Subjects, No.	Summary OR†	NNT, No.	Frequency in UFH Group, %
Major bleeding	3,674	0.57‡	164	1.9
RTE	3,566	0.85	114	5.4
Mortality (overall)	3,566	0.71‡	61	6.8

*RTE = recurrent thromboembolism; NNT = number needed to treat. Adapted from Gould et al.[145]
†A summary OR < 1.0 favors LMWH; a summary OR > 1.0 favors UFH.
‡$p < 0.05$.

no longer statistically significant. This study established the short-term effectiveness of LMWH (dalteparin) in combination with aspirin for treatment of UA and NQMI, and supported a beneficial effect of heparin plus aspirin over aspirin alone in this population.

In a third study,[223] which used an open randomized design in 1,492 patients with UA or NQMI, dalteparin (120 anti-Xa U/kg bid) was compared with UFH (5,000 U IV bolus followed by 1,000 U/h infusion for 6 days), followed by a second, double-blind phase comparing LMWH at a dose of 7,500 U qd with placebo treatment. All patients received aspirin. Both regimens were equivalent in terms of efficacy and safety. The ESSENCE trial,[224] one of two that compared enoxaparin with UFH, randomized 3,171 patients with UA or NQMI to enoxaparin, 1 mg/kg sc (100 anti-Xa U) q12h, or UFH as an IV bolus followed by a continuous infusion, for 2 to 8 days (median, 2.6 days; Table 17). There was a 17% reduction in death, MI, or recurrent angina at 14 days with LMWH ($p = 0.019$), and a 15% reduction at 30 days ($p = 0.016$). This difference was due mainly to a lower incidence of recurrent angina in patients assigned to LMWH. There was no difference in major bleeding (6.5% with LMWH vs 7.0% with UFH at 30 days), but minor bleeding was more frequent with LMWH (18.4% vs 14.2%), mainly bruising at injection sites. The difference in primary event rates remained significant after a year ($p = 0.022$).[269]

The TIMI-11B study was the other double-blind study comparing enoxaparin with IV UFH in 3,910 patients with UA or NQMI. The primary outcome was death, MI, or urgent revascularization at 43 days.[96] Patients randomized to LMWH therapy who did not experience bleeding or surgery continued treatment at a lower dose, and those randomized to UFH therapy received placebo until day 43, when a 12% risk reduction was observed ($p = 0.048$). An absolute risk reduction of 2.3% was apparent at 14 days, and no additional benefit of LMWH developed with longer treatment.

In the FRAXIS trial,[270] patients with UA or NQMI were randomly assigned to treatment with abbreviated sc nadroparin (q12h for 6 days, then placebo from day 7 to day 14), sustained sc nadroparin (q12h for 14 days), or initial IV UFH for 6 days followed by placebo until day 14. The rate of vascular death, MI, or recurrent angina was no different among the three groups at 6 days, or between short-term and long-term treatment with LMWH by 14 days. In the FRISC-II trial,[271] 2,267 patients with UA or NQMI received dalteparin, 120 U/kg q12 h for 5 to 7 days, and then either dalteparin, 5,000 to 7500 U q12h, or placebo for 90 days. The primary outcome, death or MI at 3 months, did not differ between groups, but bleeding was more frequent in patients who received dalteparin. Compared with UFH, an overview of trials reveals a 15 to 20% relative reduction in risk of MI or death during 7 to 14 days of treatment with LMWH.[91,224] Based on available data from approximately 10,000 patients with UA, however, treatment with LMWH does not offer long-term benefit over placebo in reducing MI or death (OR = 1.04; 95% CI, 0.79 to 1.37; Fig 9).

Q-Wave MI

In patients with Q-wave acute MI, experience with LMWH is limited to two small studies, in which most patients received thrombolytic therapy. The Fragmin in Acute Myocardial Infarction study[272] enrolled 776 patients with acute anterior MI in a randomized, double-blind comparison of LMWH (dalteparin, 150 U/kg sc bid) with placebo. Thrombolytic therapy (streptokinase) and aspirin were administered to > 90% of patients. The mean time to the start of treatment was approximately 12 h. After an average of 9 days, left ventricular mural thrombus or system embolism developed in 35 of 247 patients (14.2%) receiving dalteparin vs 59 of 270 patients (21.9%) in the placebo group ($p = 0.03$). Benefit was limited to inhibition of left ventricular thrombus formation (RR = 0.63; 95% CI, 0.43 to 0.92; $p = 0.02$) with LMWH, as there were too few events to detect differences between treatments with respect to arterial embolism, reinfarction, or death. LMWH therapy was associated with increases in major (2.9% vs 0.3%; $p = 0.006$) and minor hemorrhage (14.8% vs 1.8%; $p < 0.001$). Thus, LMWH reduced mural thrombus formation in patients with acute anterior MI at the price of increased bleeding that may have been related to

Table 17—*Trials of LMWH in Acute Coronary Syndrome Without ST-Segment Elevation

Trials	Patients, No.	Short-term Comparison	Long-term Comparison
Gurfinkel et al[222]	219	Nadroparin 214 IU sc bid vs placebo	N/A
FRISC[228]	1,506	Dalteparin 120 IU/kg sc bid vs placebo	Dalteparin 7,500 IU sc qd vs placebo
Klein et al[223]	1,482	Dalteparin 120 IU/kg sc bid vs UFH 5,000 IU bolus, 1,000 IU/h IV then 12,500 IU sc bid	Dalteparin 7,500 IU sc qd vs placebo
Cohen et al[224]	3,171	Enoxaparin 1 mg/kg sc bid 2 to 8 d vs UFH 5,000 IU APTT 55 to 85 s	N/A
Antman et al[96]	3,912	Enoxaparin 3,000 IU bolus, 100 IU/kg bid vs UFH 70 IU bolus, 15 U/kg APTT 1.5 to 2.0	Enoxaparin 4,500 to 6,000 IU sc bid vs placebo
Leizorivicz[270]	3,468	Nadroparin 87 IU sc bid vs UFH 5,000 IU bolus 1,250/h target local APTT	Nadroparin 87 IU sc vs placebo
FRISC II[271]	2,457	Dalteparin 120 IU/kg bid vs placebo	Dalteparin 5,000 IU or 7,500 IU sc bid vs placebo

*N/A = not applicable.

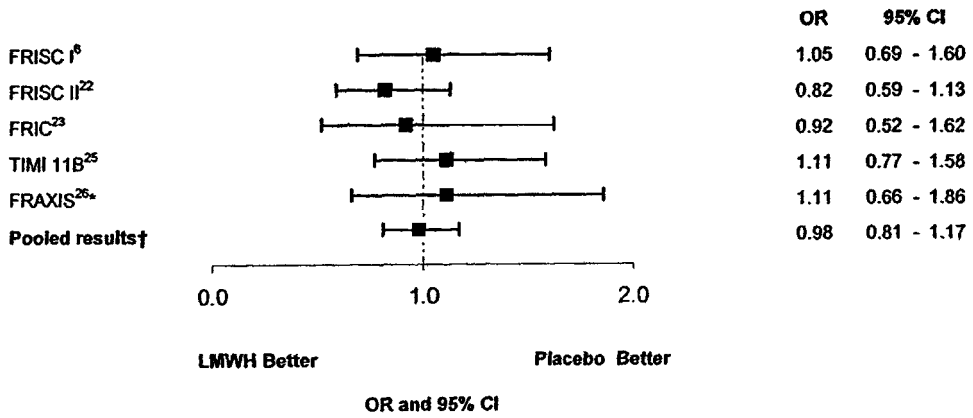

FIGURE 9. Comparison of LMWH with no treatment (control) on long-term outcome in patients with unstable angina or non-Q-Wave MI.

concomitant thrombolytic therapy and use of a higher dose of dalteparin than given in either the Fragmin in Acute Myocardial Infarction Study[272] or the Fragmin in Unstable Coronary Artery Disease Trial.[223] In a study[273] of 103 streptokinase-treated patients randomly assigned to enoxaparin or placebo therapy within 5 days of acute MI, 2 of 43 patients in the enoxaparin group (4.3%) developed recurrent MI within 30 days, compared to 12 of 60 patients receiving placebo treatment (20%; $p = 0.02$).

Coronary Angioplasty

Animal studies indicating that LMWH suppresses neointimal proliferation following arterial balloon injury[274,275] prompted clinical trials to evaluate the effect of LMWH on restenosis after angioplasty. In the Enoxaparin Restenosis after Angioplasty trial,[276] there was no difference in rates of restenosis among patients randomized to treatment with enoxaparin, 40 mg sc qd, or placebo for 1 month following successful coronary angioplasty. Although minor bleeding was more frequent in the enoxaparin group, rates of major bleeding did not differ significantly. The Enoxaparin and MaxEPA for the Prevention of Angioplasty Restenosis study[277] randomized 653 patients to treatment with either enoxaparin (30 mg sc bid) or placebo for 6 weeks following successful angioplasty after factorial randomization to either fish oil or control a median of 6 days earlier. Quantitative coronary angiography revealed no significant difference in rates of restenosis either per patient or per lesion. These two studies leave little doubt about the lack of efficacy of enoxaparin for prevention of restenosis when applied in doses up to 60 mg/d (6,000 anti-Xa U/d) for 6 weeks.

Overcoming the Anticoagulant Effect of Heparin

The anticoagulant effect of UFH can be neutralized rapidly by IV protamine, a cationic protein derived from fish sperm that binds strongly to (anionic) heparin in a ratio of approximately 100-U UFH per milligram of protamine; 50 mg of protamine would therefore be required immediately following a 5,000-U IV heparin bolus to counteract the anticoagulant effect. When infused, only the heparin given during the preceding several hours should be included in the dose calculation, since the half-life of IV UFH is approximately 60 min. A patient receiving an infusion of 1,250 U/h needs approximately 30 mg of protamine. Neutralization of heparin after a sc dose may require a prolonged infusion or a repeated injection of protamine. A fall in APTT can be used to confirm heparin neutralization.[278]

The risks of severe adverse reactions to protamine, such as hypotension and bradycardia, are reduced by slow administration over 1 to 3 min.[279] Allergic reactions, including anaphylaxis, are associated with previous exposure to protamine-containing insulin, *eg*, neutral protamine Hagedorn insulin, vasectomy, and hypersensitivity to fish.[278,280,281] Patients at risk of developing antiprotamine antibodies can be pretreated with corticosteroid and antihistamine medications.[278]

Other methods to overcome the effects of heparin include hexadimethrine,[282,283] heparinase (neutralase),[284] PF4,[285,286] extracorporeal heparin removal devices,[287,288] and synthetic protamine variants,[289] but these are not widely available.

Protamine neutralizes the antithrombin activity of LMWH, normalizing the APTT and thrombin time, but the cationic protein neutralizes the antifactor Xa activity incompletely,[290–297] because protamine exhibits reduced binding to low-molecular-weight components.[122,296,298] The clinical significance of incomplete anti-Xa neutralization by protamine is unclear. In animals, protamine overcomes microvascular bleeding in animal models despite incomplete restoration of factor Xa-activity,[298–300] but to our knowledge, no published clinical studies demonstrate a beneficial effect of protamine on bleeding complications of LMWH. In animal studies,[301–304] synthetic protamine variants have been highly effective in neutralizing LMWH (including anti-Xa activity) and are less toxic than protamine. Of the other reversing agents under investigation,

including synthetic heparin-binding peptide, heparinase-I, and lactoferrin,[305,306] none are yet available for clinical use.

Recommendations for treatment of LMWH overdose listed below are consistent with package labeling but are clinically untested. Within 8 h of administering LMWH, the dose of protamine is 1 mg/100 anti-Xa u for enoxaparin (1 mg = approximately 100 anti-Xa u). If bleeding continues, a second dose of 0.5 mg protamine/100 anti-Xa U LMWH may be administered. Smaller doses are needed beyond 8 h after LMWH administration.

Nonhemorrhagic Side Effects

HIT

HIT is an antibody-mediated, adverse reaction to heparin, which can cause venous and arterial thrombosis. HIT should be considered a clinicopathologic syndrome, the diagnosis of which is based on both clinical and serologic features.[307,308] HIT antibody formation accompanied by otherwise unexplained fall in platelet count $> 50\%$ (even if the nadir remains $> 150 \times 10^9$/L)[309] or skin lesions at injection sites[310] are manifestations of the HIT syndrome.

The antigen is a multimolecular complex of heparin and PF4 (a platelet granule protein of the CXC family of chemokines).[120,121,311–313] HIT antibodies bind to one or more PF4 regions conformationally modified by interaction with heparin or other anions, *eg*, hypersulfated chondroitin sulfate,[314] pentosan polysulfate,[315] or polyvinylsulfonate.[316] Although at least 18 saccharides units are necessary to bind heparin simultaneously to thrombin and AT, a smaller number (12 to 14 saccharides) is the minimum needed to form the antigenic complex with PF4[312,313]; thus, LMWH molecules $>$ 4,000 d can cause HIT, but the risk of antibody formation and clinical HIT is lower during treatment with LMWH than with UFH.[317,318] Theoretically, very small LMWH preparations (*eg*, pentasaccharide[319]) or specially engineered heparin preparations (*eg*, highly sulfated heparin moieties bridged by nonsulfated spacer regions[320]) will not cause HIT.

Figure 9 illustrates the central role of thrombin in the pathogenesis of HIT. *In vivo* platelet activation results from binding of heparin-PF4-IgG immune complexes to platelet FcIIa receptors.[321–324] Evidence for thrombin generation *in vivo* includes markedly elevated levels of thrombin-AT complexes (median, 43 to 44 μg/L),[325,326] much higher than occur in control patients with postoperative DVT (median, 7.6 μg/L).[325,326] Factors contributing to thrombin generation could include formation of procoagulant, platelet-derived microparticles[323,327]; tissue factor expression by endothelium injured by HIT antibodies cross-reacting with PF4 bound to endothelial glycosaminoglycans[328]; and neutralization of heparin by PF4 released from activated platelets. Marked thrombin generation explains several clinical features of HIT, including the hypercoagulable state associated with venous and arterial thrombosis,[318] decompensated (hypofibrinogenemic) disseminated intravascular coagulation in 5 to 10% of HIT patients,[328] and progression of DVT to gangrene in some patients with HIT who are treated with warfarin.[325,329] This iatrogenic syndrome results from disturbing the balance between procoagulant and anticoagulant states during coumarin therapy: warfarin causes severe acquired reduction of protein C, while it fails to control thrombin generation.[325,330] Typically, patients with warfarin-induced venous limb gangrene have international normalized ratio (INR) values > 3.5, due to severely reduced factor VII levels that parallel the fall in protein C.[325,331] The role for *in vivo* thrombin generation in HIT provides a rationale for current therapies that emphasize reduction of thrombin generation,[307] either via inhibition of factor Xa (*eg*, administration of danaparoid) or through direct inhibition of thrombin (*eg*, lepirudin, argatroban).

The frequencies of HIT antibody formation and clinical HIT vary in different clinical settings.[332,333] For example, patients undergoing cardiac surgery are more likely than orthopedic surgical patients to form HIT antibodies during treatment with UFH, but orthopedic patients who form HIT antibodies are more likely to develop clinical HIT.[317] Furthermore, HIT occurs less often in medical than surgical patients treated with UFH.[333]

There are two main laboratory assays to detect HIT antibodies.[334,335] *Activation assays* exploit the potent platelet-activating properties of HIT serum or plasma in the presence of heparin. Although assays are widely performed with platelet-rich plasma from normal donors, those utilizing washed platelets (*eg*, platelet serotonin release assay,[336] or the heparin-induced platelet activation assay)[337,338] are more sensitive and specific, and have been validated in blinded assessment.[318] *Antigen assays* based on detecting antibodies that recognize PF4 bound to heparin[76,337] or polyvinylsulfonate[316] are now commercially available. Antigen assays detect clinically insignificant antibodies more often than activation assays do, however, so physicians must interpret antigen assays results cautiously in clinical context.[332,334]

Treatment of HIT-Associated Thrombosis

Both prospective and retrospective studies,[333,339] indicate that 50 to 75% of patients with HIT develop thrombosis requiring effective anticoagulant therapy, but to our knowledge, only one randomized trial[340] of HIT treatment has been performed to date. Danaparoid sodium is a mixture of anticoagulant glycosaminoglycans with predominant antifactor Xa activity.[341] The rate of thrombus resolution was higher with danaparoid and warfarin than with dextran and warfarin for patients with either mild thrombosis (92% vs 72%; $p < 0.04$) or severe thrombosis (92% vs 33%; $p < 0.001$).[340] This small study was unblinded, and only preliminary (1996) data are available.[340]

Although generally weak, cross-reactivity can be detected *in vitro* in a minority of HIT sera, $< 5\%$ of patients treated with danaparoid develop evidence suggesting *in vivo* cross-reactivity[342,343]; moreover, *in vivo* cross-reactivity cannot be predicted from *in vitro* observations.[342,343] This, plus the generally favorable clinical experience with danaparoid for treatment of HIT, means treatment should not be delayed for cross-reactivity testing.[307,341] The anticoagulant effect of danaparoid is monitored by chromogenic antifactor Xa assay. Most patients achieve therapeutic levels with standard dosing (Table 18), yet monitoring

is recommended for very small or large patients, those with renal failure, and those with HIT threatening life or limb. Danaparoid does not interfere with INR measurements, which facilitates monitoring of overlapping coumarin anticoagulation. Danaparoid has been approved for DVT prophylaxis in the United States, Canada, and most of Europe, although its use for treatment of HIT is predominantly off-label.[341]

Lepirudin is a hirudin derivative manufactured using recombinant technology.[344] Two prospective cohort studies[345,346] of lepirudin for the treatment of HIT-associated thrombosis using a prespecified dosing schedule and historical control subjects (Table 18) led to its approval for this indication in both the European Union (March 1997) and the United States (March 1998). In one study,[345] the composite end point of mortality, amputation, and thromboembolism was significantly reduced compared with historical control subjects (10% vs 23% at day 7, and 25% vs 52% at day 35, respectively; $p = 0.014$). In the other study,[346] there was a trend favoring lepirudin. A subgroup analysis[326] of the two studies (pooled) found that a subtherapeutic APTT ratio (< 1.5) was associated with an increased risk for thrombosis, whereas an APTT ratio above the therapeutic range (> 2.5) was associated with bleeding. Even within the therapeutic range, however, bleeding was significantly greater in treated patients than control subjects (RR = 3.21; 95% CI, 1.7 to 6.0; $p < 0.001$).

The anticoagulant effect of lepirudin is monitored by the APTT. Lepirudin is renally excreted, and the risks of accumulation and bleeding are high in patients with renal failure.[344] The half-life of lepirudin is about 1.3 h. A high proportion of patients develop antihirudin antibodies,[344,347] which occasionally result in an increase in anticoagulant effects.[344] Thus, ongoing monitoring is recommended throughout the course of lepirudin treatment, even when the initial anticoagulant effect appears stable.

Argatroban is a direct thrombin inhibitor that has been reported to be associated with a lower thrombotic event rate in a prospective cohort study[348] than in historical control subjects. Like lepirudin, the half-life of argatroban is short (< 1 h). Argatroban is excreted normally in patients with moderate renal failure,[349] but the dose must be reduced in patients with hepatic failure. Argatroban recently was approved by the US Food and Drug Administration for the treatment and prevention of thrombosis in patients with HIT. A dosing schedule is shown in Table 18.

Warfarin: It has been suggested that warfarin therapy can lead to venous limb gangrene in patients with HIT.[325,329,330] However, the frequency of venous limb gangrene among HIT patients treated with coumarin anticoagulants is uncertain. Venous limb gangrene was found in 8 of 66 patients (12%) with HIT- associated DVT who were treated with warfarin in a retrospective study[325] in Hamilton. In some patients, high initial doses of warfarin or concomitant use of ancrod (a defibrinogenating snake venom that is associated with increased thrombin generation)[349] may have contributed to this complication; however, even "usual" doses of warfarin that result in a rise in the INR to supratherapeutic levels can be associated with limb necrosis.[325] Since none of the patients appeared to have an underlying congenital mutation involving the protein C anticoagulant system,[325] it is possible

Table 18—*Treatment Protocols for Danaparoid, Lepirudin, and Argatroban*

Drugs	Recommendations	Indications	Dose	Maintenance
Danaparoid	Grade 1B	Rapid therapeutic anticoagulation (IV infusion)	Loading:* 2,250-U bolus (1,500 U if < 50 kg; 3,000 U if 75 to 90 kg; 3,750 U if > 90 kg), followed by 400 U/h for 4 h, then 300 U/h for 4 h	†150 to 200 U/h to maintain anti-Xa levels between 0.5 U/mL and 0.8 U/mL‡
		Prophylactic anticoagulation (sc injection)	750 U q12h§ (750 U q8h if 75 to 90 kg; 1,500 U q12h if > 90 kg)	
Lepirudin (recombinant hiruden)	Grade 1C	Rapid therapeutic anticoagulation (IV infusion)	Loading: 0.4 mg/kg bolus IV	0.15 mg/kg/h IV, with adjustments to maintain APTT 1.5 to 2.5 times the median of the normal laboratory range.
Argatroban	Grade 2C	Rapid therapeutic anticoagulation (IV infusion)	Initial: 2 μg/kg/min	Above initial dose adjusted to maintain APTT 1.5 to 3.0 times the initial baseline value (not to exceed 100 s)

*All bolus doses are based on 750-U ampule availability.

†If preferred, following initial bolus, danaparoid can still be administered sc (generally, 1,500 U q8h to q12h).

‡If antifactor Xa levels are not available, danaparoid can still be administered safely for most patients, as there is a high probability of achieving the target anticoagulant range with this regimen, and bleeding complications are uncommon. However, monitoring is recommended for very small and large patients, patients with renal failure, and patients with life- or limb-threatening thrombosis.

§Generally, the anti-Xa level will be 0.1 U/mL on day 1, and rise to 0.15 to 0.35 U/mL by the fifth day (measured midinterval, 6 h after the morning dose).

that increased thrombin generation from HIT contributed to the development of warfarin-induced venous gangrene in these patients. Supporting this supposition were higher ratios of thrombin-AT complexes to protein C activity among patients developing venous gangrene.[325]

Although there have been other case reports[350–352] of warfarin-induced venous limb gangrene, a recent retrospective cohort study[353] did not identify any among 51 HIT patients treated with warfarin. Only 16 patients had active DVT when warfarin therapy was commenced, however, and the initial doses of warfarin were lower than in the Hamilton study. Given the risk of thrombosis over the first few days after stopping heparin therapy for HIT, the delayed onset of anticoagulation with coumarin, and the potential for gangrene, we recommend that warfarin not be given as monotherapy for acute HIT or in combination with ancrod. However, warfarin has not been reported to cause gangrene when combined with an anticoagulant that reduces thrombin generation in HIT (*eg*, danaparoid or lepirudin).

LMWH: Although LMWH is less likely to cause HIT antibody formation,[317,318] it is as reactive as UFH in activation assays using washed platelets and HIT sera.[318,354] Furthermore, a risk of thrombosis during treatment of HIT with LMWH has been observed.[355]

Platelet transfusions should be considered as relatively contraindicated in prophylaxis of bleeding in patients with acute HIT[356] because petechiae and other signs of bleeding are not clinical features of HIT, despite thrombocytopenia,[328] and in anecdotal reports, platelet transfusions have been associated with thrombotic events.[357,358] However, if bleeding caused by local lesions or other factors complicates HIT, therapeutic platelet transfusions may be appropriate.

Treatment of Isolated HIT

Discontinuation of heparin therapy has long been the cornerstone of management of HIT, but this step alone is not enough even for patients with isolated thrombocytopenia.[326,339,345,346,359,360] The risk of thrombosis is still 10% at 2 days, 40% at 7 days, and 50% at 30 days despite stopping heparin therapy in one study[339] and 38% in another.[353] The incidence of thrombosis was no lower when heparin therapy was stopped < 48 h after onset of HIT than when the diagnosis was made later (45% vs 34%; $p = 0.26$). A high initial rate of thrombosis (10.4% over a mean of 1.7 days before starting lepirudin therapy) was also observed in prospective cohort studies.[326]

In 16 patients with isolated HIT examined by compression ultrasonography or contrast venography, DVT was identified in 8 patients (all but 1 proximal). These patients were treated with danaparoid in therapeutic dosage, while those without thrombosis received danaparoid in lower prophylactic dosage. One death occurred due to thrombosis.[361] The high frequency of DVT when the diagnosis of isolated HIT was established suggests that subclinical thrombosis early during the course of HIT may account for the prevalence of subsequent clinical thromboembolism among patients managed solely by withdrawal of heparin therapy.

Given the unfavorable natural history of isolated HIT and the efficacy and safety of alternative anticoagulation in patients with HIT-associated thrombosis, we recommend administration of another rapidly acting anticoagulant in patients with isolated HIT until the platelet count is restored. Prophylactic doses may be appropriate when subclinical DVT has been excluded by objective studies, but this approach has not been prospectively evaluated.

Heparin-Induced Osteoporosis

Heparin or LMWH is usually administered for short periods when an immediate anticoagulant effect is required and coumarins are used for long-term treatment. In pregnancy, however, long-term administration of heparin or LMWH is preferred to prevent and treat venous thromboembolism, to prevent systemic embolism in women with mechanical heart valves, and to prevent fetal loss in women with antiphospholipid antibodies. In patients who are not pregnant, long-term heparin or LMWH therapy is also indicated for recurrent venous thromboembolism after "adequate" oral anticoagulant therapy, and in some immobilized patients requiring prophylaxis for prolonged periods. Clinical trials are ongoing to determine whether long-term therapy with LMWH is useful in outpatients with symptomatic coronary artery disease. In all these instances, the long-term use of heparin or LMWH carries a risk of osteoporosis.[362–373]

Significant reductions in bone density have been reported in about 30% of patients, and symptomatic vertebral fractures occur in 2 to 3% of patients receiving heparin for 1 month or more. There was a 2.2% incidence of vertebral fracture among 184 women receiving long-term heparin therapy during pregnancy,[370] and spinal fractures occurred in 6 of 40 patients receiving 10,000-U UFH sc bid for 3 to 6 months. In the first study,[370] only women with severe back pain were tested for fracture; patients in the second study[373] were significantly older and were routinely screened for spinal fracture.

Progress in delineating the mechanisms of the effect of heparin on bone has been slowed by the lack of suitable animal models. Studies in Sprague Dawley rats have provided new information on the mechanism of heparin-induced osteoporosis. Animals treated with UFH at doses of 0.25 to 1.0 anti-Xa U/g for 28 days demonstrated a dose-dependent decrease in cancellous bone volume in the distal third of the femur.[374] Treatment was associated with a 45% decrease in the number of osteoblasts and a 81% decrease in the amount of unmineralized collagen (osteoid) lining the cancellous bone surface. Furthermore, heparin increased osteoclast surface by 58%, indicating that heparin causes bone loss both by decreasing the rate of bone formation and by increasing bone resorption.

The same rat model of heparin-induced osteoporosis was used to explore reversibility when heparin treatment was stopped.[375] Rats were randomized to once-daily sc UFH or saline solution for 28 days and followed an additional 28 days off treatment. Based on histomorphometric analysis of the distal third of the right femur proximal to the epiphyseal growth plate, heparin caused a 30% loss in cancellous bone volume over the first 28 days

accompanied by an increase in osteoclast surface and a decrease in both osteoblast and osteoid surface. Twenty-eight days after stopping heparin therapy, however, there was no improvement in any of these parameters, and serum alkaline phosphatase, a biochemical marker of bone formation, continued to decline. ^{125}I-labeled heparin accumulated in bone and was retained at least 28 days after treatment, which suggests that, following discontinuation of therapy, heparin-induced osteoporosis is not rapidly reversible because heparin is sequestered in bone for extended periods,[375] possibly bound to bone matrix proteins.

LMWHs may carry a lower risk of osteoporosis than UFH. Dalteparin, 5,000 anti-Xa U sc, was compared with UFH, 10,000 U sc bid, for 3 to 6 months in 80 patients with DVT.[373] Six of the 40 patients who received UFH developed spinal fractures, compared to 1 patient receiving dalteparin. There was a dose-dependent decrease in cancellous bone volume in rats treated with UFH or the LMWH tinzaparin (0.5 to 1.0 μg) for 32 days, but UFH caused significantly greater cancellous bone loss than LMWH.[376] Furthermore, although UFH and LMWH decreased osteoblast and osteoid surface similarly, only UFH increased osteoclast surface. Both UFH and LMWH reduced serum alkaline phosphatase, consistent with reduced bone formation, while there is a transient increase in urinary type 1 collagen cross-linked pyridinoline, consistent with an increase in bone resorption. Whereas UFH decreases cancellous bone volume both by decreasing the rate of bone formation and increasing the rate of bone resorption, LMWH causes less osteopenia, decreasing only the rate of bone formation.[376]

Using an *in vitro* assay of ^{45}Ca release in fetal rat calvaria, UFH was found to stimulate bone resorption at concentrations commonly used for prophylaxis and treatment of the thromboembolism.[35] In contrast, $>$ 50-fold higher concentrations of the LMWH preparations enoxaparin, dalteparin, tinzaparin, and ardeparin than used clinically were required for an equivalent effect. Both molecular weight and sulfation are major determinants of ^{45}Ca release by heparin, but affinity for AT-III is not an important factor.[35]

Based on studies in animal models, we hypothesize that heparin binds to bone matrix and interacts with a variety of cell types found within the bone microenvironment, including cells of the osteoblast lineage. Such interactions may alter mesenchymal stem cell differentiation reducing the number of mature osteoblasts,[377] reduce collagen synthesis by osteoblasts,[36] and release specific growth factors and/or cytokines capable of inducing the formation of osteoclasts from pluripotent mononuclear precursors in the bone marrow.

Conclusion

LMWH preparations are at least as effective and safe as UFH and are more convenient. Although more expensive than UFH, the expense is likely to be offset by savings from reduced hospital stay. The major appealing feature of LMWH is the more predictable relationship between dose and response with LMWH than UFH, which translates to weight-adjusted dosing without laboratory monitoring. The only study[200] that compared the predictability of the dose-response of LMWH to UFH demonstrated less variability with LMWH, but this was not abolished entirely. The efficacy and safety of LMWH might still be improved by monitoring antifactor Xa levels, but the anticipated improvement in clinical outcome is likely to be marginal and offset by inconvenience and expense. Weight-adjusted dosing could be misleading in patients with renal insufficiency or obesity, and further studies are required to determine whether monitoring is necessary in such cases. Based on current information, LMWH preparations should be administered in the majority of patients based on weight-adjusted dosing.

Recommendations

Treatment of HIT (see Nonhemorrhagic Side Effects)

1. We recommend the use of one of the following anticoagulant drugs to treat acute HIT complicated by thrombosis: danaparoid sodium (grade 1B), lepirudin (grade 1C), or argatroban (grade 1C).

2. We recommend that anticoagulation with one of these agents until the platelet count has recovered should also be considered for patients with acute HIT without thrombosis (isolated HIT), as there is a high risk for subsequent clinically evident thrombosis in these patients (all grade 2C in comparison to no treatment).

3. We recommend that clinicians do not use warfarin alone to treat acute HIT complicated by DVT because of the risk of causing venous limb gangrene (grade 1C).

4. Warfarin appears to be safe in acute HIT when it is given to a patient who is adequately anticoagulated with a drug that reduces thrombin generation in HIT, such as danaparoid, lepirudin, or argatroban, although it may be prudent to delay starting warfarin therapy until the platelet count has risen to $> 100 \times 10^9$/L. We recommend that if warfarin is given to patients with acute HIT, it should be administered together with a drug that reduces thrombin generation in HIT, until the platelet count has recovered. Then, warfarin can be continued alone (grade 1C).

5. LMWH is contraindicated in HIT. We recommend that clinicians do not administer LMWH for the treatment of acute HIT (grade 1C+).

6. We recommend that clinicians do not administer prophylactic platelet transfusions for the treatment of acute HIT (grade 2C).

References

1 Rosenberg RD, Lam L. Correlation between structure and function of heparin. Proc Natl Acad Sci USA 1979; 76:1218–1222

2 McLean J. The thromboplastic action of cephalin. Am J Physiol 1916; 41:250–257
3 Brinkhous KM, Smith HP, Warner ED, et al. The inhibition of blood clotting: an unidentified substance which acts in conjunction with heparin to prevent the conversion of prothrombin into thrombin. Am J Physiol 1939; 125:683–687
4 Abildgaard U. Highly purified antithrombin III with heparin cofactor activity prepared by disc electrophoresis. Scand J Clin Lab Invest 1968; 21:89–91
5 Rosenberg RD, Bauer KA. The heparin-antithrombin system: a natural anticoagulant mechanism. In: Colman RW, Hirsh J, Marder VJ, et al, eds. Hemostasis and thrombosis: basic principles and clinical practice. 3rd ed. Philadelphia, PA: JB Lippincott, 1994; 837–860
6 Lindahl U, Backstrom G, Hook M, et al. Structure of the antithrombin-binding site of heparin. Proc Natl Acad Sci U S A 1979; 76:3198–3202
7 Casu B, Oreste P, Torri G, et al. The structure of heparin oligosaccharide fragments with high anti-(factor Xa) activity containing the minimal antithrombin III-binding sequence. Biochem J 1981; 97:599–609
8 Choay J, Lormeau JC, Petitou M, et al. Structural studies on a biologically active hexasaccharide obtained from heparin. Ann NY Acad Sci 1981; 370:644–649
9 Vuillemenot A, Schiele F, Meneveau N, et al. Efficacy of a synthetic pentasaccharide, a pure factor Xa inhibitor, as an antithrombotic agent: a pilot study in the setting of coronary angioplasty. Thromb Haemost 1999; 81:214–220
10 Herbert JM, Herault JP, Bernat A, et al. Biochemical and pharmacological properties of SANORG 34006, a potent and long-acting synthetic pentasaccharide. Blood 1998; 91: 4197–4205
11 Lam LH, Silbert JE, Rosenberg RD. The separation of active and inactive forms of heparin. Biochem Biophys Res Commun 1976; 69:570–577
12 Andersson LO, Barrowcliffe TW, Holmer E, et al. Anticoagulant properties of heparin fractionated by affinity chromatography on matrix-bound antithrombin III and by gel filtration. Thromb Res 1976; 9:575–583
13 Tollefsen DM, Majerus DW, Blank MK. Heparin cofactor II: purification and properties of a heparin-dependent inhibitor of thrombin in human plasma. J Biol Chem 1982; 257:2162–2169
14 Lindahl U, Thunberg L, Backstrom G, et al. Extension and structural variability of the antithrombin-binding sequence in heparin. J Biol Chem 1984; 259:12368–12376
15 Lane DA, Denton J, Flynn AM, et al. Anticoagulant activities of heparin oligosaccharides and their neutralization by platelet factor 4. Biochem J 1984; 218:725–732
16 Oosta GM, Gardner WT, Beeler DL, et al. Multiple functional domains of the heparin molecule. Proc Natl Acad Sci U S A 1981; 78:829–833
17 Nesheim ME. A simple rate law that describes the kinetics of the heparin-catalyzed reaction between antithrombin III and thrombin. J Biol Chem 1983; 258:14708–14717
18 Ofosu FA, Sie P, Modi GJ, et al. The inhibition of thrombin-dependent feedback reactions is critical to the expression of anticoagulant effects of heparin. Biochem J 1987; 243:579–588
19 Ofosu FA, Hirsh J, Esmon CT, et al. Unfractionated heparin inhibits thrombin-catalyzed amplification reactions of coagulation more efficiently than those catalyzed by factor Xa. Biochem J 1989; 257:143–150
20 Beguin S, Lindhout T, Hemker HC. The mode of action of heparin in plasma. Thromb Haemost 1988; 60:457–462
21 Lupu C, Poulsen E, Roquefeuil S, et al. Cellular effects of heparin on the production and release of tissue factor pathway inhibitor in human endothelial cells. Arterioscler Thromb Vasc Biol 1999; 19:2251–2262
22 Altman R, Scazziota A, Rouvier J. Efficacy of unfractionated heparin, low molecular weight heparin, and both combined for releasing total and free tissue factor pathway inhibitor. Haemostasis 1998; 28:229–235
23 Gori AM, Pepe G, Attanasio M, et al. Tissue factor reduction and tissue factor pathway inhibitor release after heparin administration. Thromb Haemost 1999; 81:589–593
24 Andersson L-O, Barrowcliffe TW, Holmer E, et al. Molecular weight dependency of the heparin potentiated inhibition of thrombin and activated factor X: effect of heparin neutralization in plasma. Thromb Res 1979; 5:531–541
25 Harenberg J. Pharmacology of low molecular weight heparins. Semin Thromb Hemost 1990; 16:12–18
26 Johnson EA, Mulloy B. The molecular weight range of commercial heparin preparations. Carbohydr Res 1976; 51:119–127
27 Sobel M, McNeill PM, Carlson PL, et al. Heparin inhibition of von Willebrand factor-dependent platelet function *in vitro* and *in vivo*. J Clin Invest 1991; 87:1787–1793
28 Eika C. Inhibition of thrombin-induced aggregation of human platelets in heparin. Scand J Hematol 1971; 8:216–222
29 Kelton JG, Hirsh J. Bleeding associated with antithrombotic therapy. Semin Hematol 1980; 17:259–291
30 Heiden D, Mielke CH, Rodvien R. Impairment by heparin of primary hemostasis and platelet (14C)5-hydroxytryptamine release. Br J Hematol 1977; 36:427–436
31 Fernandez F, Nguyen P, Van Ryn J, et al. Hemorrhagic doses of heparin and other glycosaminoglycans induce a platelet defect. Thromb Res 1986; 43:491–495
32 Blajchman MA, Young E, Ofosu FA. Effects of unfractionated heparin, dermatan sulfate and low molecular weight heparin on vessel wall permeability in rabbits. Ann NY Acad Sci 1989; 556:245–254
33 Ockelford PA, Carter CJ, Cerskus A, et al. Comparison of the *in vivo* hemorrhagic and antithrombotic effects of a low antithrombin III affinity heparin fraction. Thromb Res 1982; 27:679–690
34 Clowes AW, Karnovsky MJ. Suppression by heparin of smooth muscle cell proliferation in injured arteries. Nature 1977; 265:625–626
35 Shaughnessy SG, Young E, Deschamps P, et al. The effects of low molecular weight and standard heparin on calcium loss from the fetal rat calvaria. Blood 1995; 86:1368–1373
36 Bhandari M, Hirsh J, Weitz J, et al. The effects of standard and low molecular weight heparin on bone nodule formation *in vitro*. Thromb Haemost 1998; 80:413–417
37 Castellot JJ, Favreau LV, Karnovsky MJ, et al. Inhibition of vascular smooth muscle cell growth by endothelial cell-derived heparin: possible role of a platelet endoglycosidase. J Biol Chem 1982; 257:11256–11260
38 Hull RD, Raskob GE, Hirsh J, et al. Continuous intravenous heparin compared with intermittent subcutaneous heparin in the initial treatment of proximal-vein thrombosis. N Engl J Med 1986; 315:1109–1114
39 Hirsh J, van Aken WG, Gallus AS, et al. Heparin kinetics in venous thrombosis and pulmonary embolism. Circulation 1976; 53:691–695
40 Young E, Prins MH, Levine MN, et al. Heparin binding to plasma proteins, an important mechanism for heparin resistance. Thromb Haemost 1992; 67:639–643
41 Barzu T, Molho P, Tobelem G, et al. Binding and endocytosis of heparin by human endothelial cells in culture. Biochem Biophys Acta 1985; 845:196–203
42 de Swart CAM, Nijmeyer B, Roelofs JMM, et al. Kinetics of intravenously administered heparin in normal humans. Blood 1982; 60:1251–1258

43 Olsson P, Lagergren H, Ek S. The elimination from plasma of intravenous heparin: an experimental study on dogs and humans. Acta Med Scand 1963; 173:619–630
44 Bjornsson TO, Wolfram BS, Kitchell BB. Heparin kinetics determined by three assay methods. Clin Pharmacol Ther 1982; 31:104–113
45 Glimelius B, Busch C, Hook M. Binding of heparin on the surface of cultured human endothelial cells. Thromb Res 1978; 12:773–782
46 Mahadoo J, Hiebert L, Jaques LB. Vascular sequestration of heparin. Thromb Res 1977; 12:79–90
47 Friedman Y, Arsenis C. Studies on the heparin sulphamidase activity from rat spleen: intracellular distribution and characterization of the enzyme. Biochem J 1974; 139:699–708
48 Dawes J, Pepper DS. Catabolism of low-dose heparin in man. Thromb Res 1979; 14:845–860
49 McAllister BM, Demis DJ. Heparin metabolism: isolation and characterization of uroheparin. Nature 1966; 212:293–294
50 Bara L, Billaud E, Gramond G, et al. Comparative pharmacokinetics of low molecular weight heparin (PK 10169) and unfractionated heparin after intravenous and subcutaneous administration. Thromb Res 1985; 39:631–636
51 Turpie AGG, Robinson JG, Doyle DJ, et al. Comparison of high-dose with low-dose subcutaneous heparin to prevent left ventricular mural thrombosis in patients with acute transmural anterior myocardial infarction. N Engl J Med 1989; 320:352–357
52 Pini M, Pattacini C, Quintavalla R, et al. Subcutaneous vs intravenous heparin in the treatment of deep venous thrombosis: a randomized clinical trial. Thromb Haemost 1990; 64:222–226
53 Raschke RA, Gollihare B, Peirce JC. The effectiveness of implementing the weight-based heparin nomogram as a practice guideline. Arch Intern Med 1996; 156:1645–1649
54 EPIC Investigators. Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. N Engl J Med 1994; 330:956–961
55 EPILOG Investigators. Platelet glycoprotein IIb/IIIa receptor blockade and low-dose heparin during percutaneous coronary revascularization. N Engl J Med 1997; 336:1689–1696
56 Cruickshank MK, Levine MN, Hirsh J, et al. A standard heparin nomogram for the management of heparin therapy. Arch Intern Med 1991; 151:333–337
57 Camilleri JF, Bonnet JL, Bouvier JL, et al. Thrombolyse intraveineuse dans l'infarctus du myocarde. Influence de la qualite de l'antiocoagulation sur le taux de recidives precoces d'angor ou d'infarctus. Arch Mal Coeur 1988; 81:1037–1041
58 Kaplan K, Davison R, Parker M, et al. Role of heparin after intravenous thrombolytic therapy for acute myocardial infarction. Am J Cardiol 1987; 59:241–244
59 de Bono DP, Simoons ML, Tijssen J, et al. Early intravenous heparin improves coronary patency in thrombolysis with recombinant human tissue-type plasminogen activator: European Cooperative Study Group. Br Heart J 1992;67:122–128
60 Arnout J, Simoons ML, de Bono D, et al. Correlation between the intensity of heparinization and patency of the infarct-related coronary artery after treatment of acute myocardial infarction with alteplase (rt-PA). J Am Coll Cardiol 1992; 20:513–519
61 Basu D, Gallus AS, Hirsh J, et al. A prospective study of value of monitoring heparin treatment with the activated partial thromboplastin time. N Engl J Med 1972; 287:324–327
62 Levine MN, Hirsh J, Gent M, et al. A randomized trial comparing activated thromboplastin time with heparin assay in patients with acute venous thromboembolism requiring large daily doses of heparin. Arch Intern Med 1994; 154: 49–56
63 Anand S, Ginsberg JS, Kearon C, et al. The relation between the activated partial thromboplastin time response and recurrence in patients with venous thrombosis treated with continuous intravenous heparin. Arch Intern Med 1996; 156:1677–1681
64 Anand SS, Bates S, Ginsberg JS, et al. Recurrent venous thrombosis and heparin therapy: an evaluation of the importance of early activated partial thromboplastin times. Arch Intern Med 1999; 159:2029–2032
65 GUSTO Investigators. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. N Engl J Med 1993; 329:673–682
66 Brill-Edwards P, Ginsberg JS, Johnston M, et al. Establishing a therapeutic range for heparin therapy. Ann Intern Med 1993; 119:104–109
67 Levine MN, Hirsh J, Kelton JG. Heparin-induced bleeding. In: Lane DA, Lindahl U, eds. Heparin: chemical and biological properties clinical applications. London, England: Edward Arnold, 1989; 517–532
68 Morabia A. Heparin doses and major bleedings. Lancet 1986; 1:1278–1279
69 GUSTO IIA Investigators. Randomized trial of intravenous heparin versus recombinant hirudin for acute coronary syndromes. Circulation 1994; 90:1631–1637
70 Antman E. Hirudin in acute myocardial infarction: safety report from the Thrombolysis and Thrombin Inhibition in Myocardial Infarction (TIMI) 9A Trial. Circulation 1994; 90:1624–1630
71 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; 335:775–782
72 Antman E. Hirudin in acute myocardial infarction: Thrombolysis and Thrombin Inhibition in Myocardial Infarction (TIMI) 9B trial. Circulation 1996; 94:911–921
73 Landefeld S, Cook F, Flatley M, et al. Identification and preliminary validation of predictors of major bleeding in hospitalized patients starting anticoagulant therapy. Am J Med 1987; 82:703–723
74 Fennerty AG, Thomas P, Backhouse G, et al. Audit of control of heparin treatment. BMJ 1985; 290:27–28
75 Reilly B, Raschke R, Sandhya S, et al. Intravenous heparin dosing: patterns and variations in internist's practices. J Gen Intern Med 1993; 8:536–542
76 Wheeler AP, Jacquiss RD, Newman JH. Physician practices in the treatment of pulmonary embolism and deep venous thrombosis. Arch Intern Med 1988; 148:1321–1325
77 LeBras P, Halfon P. Standardization of heparin therapy improves efficacy [letter]. Arch Intern Med 1992; 152:2140–2143
78 Raschke RA, Reilly BM, Guidry JR, et al. The weight-based heparin dosing nomogram compared with a "standard care" nomogram. Ann Intern Med 1993; 119:874–881
79 Hull RD, Raskob GE, Rosenbloom D, et al. Optimal therapeutic level of heparin therapy in patients with venous thrombosis. Arch Intern Med 1992; 152:1589–1595
80 Elliot GC, Hiltunen SJ, Suchyta M, et al. Physician-guided treatment compared with a heparin protocol for deep vein thrombosis. Arch Intern Med 1994; 154:999–1004
81 Flaker GC, Bartolozzi J, Davis V, et al. Use of a standardized heparin nomogram to achieve therapeutic anticoagulation after thrombolytic therapy in myocardial infarction. Arch Intern Med 1994; 154:1492–1496
82 Gunnarsson PS, Sawyer WT, Montague D, et al. Appropriate use of heparin: empiric vs nomogram-based dosing. Arch Intern Med 1995; 155:526–532
83 Schaefer DC, Hufnagle J, Williams L. Rapid heparin anti-

coagulation: use of a weight-based nomogram. Am Fam Physician 1996; 54:2517–2521
84 Kershaw B, White RH, Mungall DM, et al. Computer-assisted dosing of heparin. Arch Intern Med 1994; 154: 1005–1011
85 Mungall DR, Anbe D, Forrester PL, et al. A prospective randomized comparison of the accuracy of computer-assisted versus GUSTO nomogram-directed heparin therapy. Clin Pharmacol Ther 1994; 55:591–596
86 Braunwald E, Jones RH, Mark DB, et al. Diagnosing and managing unstable angina: Agency for Health Care Policy and Research. Circulation 1994; 90:613–622
87 US Agency for Health Care Policy and Research. Unstable angina: diagnosis and management; clinical practice guideline No. 10. May 1994 (amended). Bethesda, MD: National Library of Medicine. DOCLINE Information: CAT/9422862. AHCPR Publication No. 94–0602
88 Berry BB, Geary DL, Jaff MR. A model for collaboration in quality improvement projects: implementing a weight-based heparin dosing nomogram across an integrated health care delivery system. Joint Comm J Qual Improv 1998; 24:459–469
89 Hochman JS, Wali AU, Gavrila D, et al. A new regimen for heparin use in acute coronary syndromes. Am Heart J 1999; 138:313–318
90 Schlicht JR, Sunyecz L, Weber RJ, et al. Reevaluation of a weight-based heparin dosing nomogram: is institution-specific modification necessary? Ann Pharmacother 1997; 31: 1454–1459
91 Olson JD, Arkin CA, Brandt JT, et al. College of American Pathologists Conference XXXI on Laboratory Monitoring of Anticoagulant Therapy: laboratory monitoring of unfractionated heparin therapy. Arch Pathol Lab Med 1998; 122:782–798
92 Hollingsworth JA, Rowe BH, Brisebois FJ, et al. The successful application of a heparin nomogram in a community hospital. Arch Intern Med 1995; 155:2095–2100
93 Brown G, Dodek P. An evaluation of empiric vs nomogram-based dosing of heparin in an intensive care unit. Crit Care Med 1997; 25:1534–1538
94 Raschke RA, Gollihare B, Peirce JC. The effectiveness of implementing the weight-based heparin nomogram as a practice guideline. Arch Intern Med 1996; 156:1645–1649
95 OASIS-2 Investigators. Effect of recombinant hirudin (lepirudin) compared with heparin on death, myocardial infarction, refractory angina, and revascularisation procedures in patients with acute myocardial ischemia without ST elevation: a randomised trial. Lancet 1999; 353:429–438
96 Antman E, McCabe C, Gurfinkel E, et al. Enoxaparin prevents death and cardiac ischemic events in unstable angina/non-Q wave myocardial infarction. Circulation 1999; 100:1593–1601
97 Raschke RA, Hertel G. Clinical use of the heparin nomogram [letter]. Arch Intern Med 1991; 151:2318–2321
98 Levine MN. Clinical use of the heparin nomogram [letter]. Arch Intern Med 1991; 151:2321
99 Anand SS, Brimble S, Ginsberg JS. Management of iliofemoral thrombosis in a pregnant patient with heparin resistance. Arch Intern Med 1997; 157:815–816
100 Hirsh J, Salzman EW, Marder VJ. Treatment of venous thromboembolism. In: Colman RW, Hirsh J, Marder VJ, et al, eds. Hemostasis and thrombosis: basic principles and clinical practice. 3rd ed. Philadelphia, PA: Lippincott, 1994; 1346–1366
101 Doyle DJ, Turpie AGG, Hirsh J, et al. Adjusted subcutaneous heparin or continuous intravenous heparin in patients with acute deep venous thrombosis: a randomized trial. Ann Intern Med 1987; 107:441–445
102 Prandoni P, Lensing AW, Buller HR, et al. Comparison of subcutaneous low molecular weight heparin with intravenous standard heparin in proximal deep vein thrombosis. Lancet 1992; 339:441–445
103 Hull RD, Raskob GE, Rosenbloom D, et al. Heparin for five days as compared with ten days in the initial treatment of proximal venous thrombosis. N Engl J Med 1990; 322:1260–1264
104 Whitfield LR, Lele AS, Levy G. Effect of pregnancy on the relationship between concentration and anticoagulant action of heparin. Clin Pharmacol Ther 1983; 34:23–28
105 Lijnen HR, Hoylaerts M, Collen D. Heparin binding properties of human histidine-rich glycoprotein: mechanism and role in the neutralization of heparin in plasma. J Biol Chem 1983; 258:3803–3808
106 Marci CD, Prager D. A review of the clinical indications for the plasma heparin assay. Am J Clin Pathol 1993; 99:546–550
107 Edson JR, Krivit W, White JG. Kaolin partial thromboplastin time: high levels of procoagulants producing short clotting times or masking deficiencies of other procoagulants or low concentrations of anticoagulants. J Lab Clin Med 1967; 70:463–470
108 Levine SP, Sorenson RR, Harris MA, et al. The effect of platelet factor 4 on assays of plasma heparin. Br J Hematol 1984; 57:585–596
109 Fisher AR, Bailey CR, Shannon CN, et al. Heparin resistance after aprotinin. Lancet 1992; 340:1230–1231
110 Becker RC, Corrao JM, Bovill EG, et al. Intravenous nitroglycerin-induced heparin resistance: a qualitative antithrombin III abnormality. Am Heart J 1990; 119:1254–1261
111 Raschke R, Guidry J, Laufer N. Heparin-nitroglycerin interaction [letter]. Am Heart J 1991; 121:1849
112 Cirisano FD, Lee S, Greenspoon JS. Apparent heparin resistance from elevated factor VIII in a patient with postoperative deep venous thrombosis: a case report. J Reprod Med 1996; 41:191–194
113 Stirling Y, Woolf L, North WR, et al. Haemostasis in normal pregnancy. Thromb Haemost 1984; 52:176–182
114 Chiu HM, Hirsh J, Yung WL, et al. Relationship between the anticoagulant and antithrombotic effects of heparin in experimental venous thrombosis. Blood 1977; 49:171–184
115 Gallus AS, Goodall KT, Tillet J, et al. The relative contributions of antithrombin III during heparin treatment and of clinically recognizable risk factors to early recurrence of venous thromboembolism. Thromb Res 1987; 46:539–553
116 Marciniak E, Gockemen JP. Heparin-induced decrease in circulating antithrombin III. Lancet 1977; 2:581–584
117 Hirsh J, Warkentin TE, Raschke R, et al. Heparin and low-molecular weight heparin: mechanisms of action, pharmacokinetics, dosing considerations, monitoring, efficacy and safety. Chest 1998; 114:489S–510S
118 Young E, Wells P, Holloway S, et al. *Ex-vivo* and *in-vitro* evidence that low molecular weight heparins exhibit less binding to plasma proteins than unfractionated heparin. Thromb Haemost 1994; 71:300–304
119 Lane DA. Heparin binding and neutralizing protein. In: Lane DA, Lindahl U, eds. Heparin, chemical and biological properties, clinical applications. London, UK: Edward Arnold, 1989;363–374
120 Visentin GP, Ford SE, Scott JP, et al. Antibodies from patients with heparin-induced thrombocytopenia/thrombosis are specific for platelet factor 4 complexed with heparin or bound to endothelial cells. J Clin Invest 1994; 93:81–88
121 Greinacher A, Potzsch B, Amiral J, et al. Heparin-associated thrombocytopenia: isolation of the antibody and character-

ization of a multimolecular PF4-heparin complex as the major antigen. Thromb Haemost 1994; 71:247–251
122 Hirsh J, Levine MN. Low molecular weight heparin. Blood 1992; 79:1–17
123 Hirsh J, Weitz J. New antithrombotic agents. Lancet 1999; 353:1431–1436
124 Marciniak E. Factor X_a inactivation by antithrombin III: Evidence for biological stabilization of factor X_a by factor V-phospholipid complex. Br J Haematol 1973; 24:391–400
125 Walker FJ, Esmon CT. The effects of phospholipid and factor V_a on the inhibition of factor X_a by antithrombin III. Biochem Biophys Res Commun 1979; 90:641–647
126 Lane DA, Pejler G, Flynn AM, et al. Neutralization of heparin-related saccharides by histidine-rich glycoprotein and platelet factor 4. J Biol Chem 1986; 261:3980–3986
127 Weitz JI, Hudoba M, Massel D, et al. Clot-bound thrombin is protected from inhibition by heparin-antithrombin but is susceptible to inactivation by antithrombin III-independent inhibitors. J Clin Invest 1990; 86:385–391
128 Hogg PJ, Jackson CM. Fibrin monomer protects thrombin from inactivation by heparin-antithrombin III: implications for heparin efficacy. Proc Natl Acad Sci U S A 1989; 86:3619–3623
129 Becker DL, Fredenburgh JC, Stafford AR, et al. Exosites 1 and 2 are essential for protection of fibrin-bound thrombin from heparin-catalyst inhibition by antithrombin and heparin cofactor II. J Biol Chem 1999; 274:6226–6233
130 Bar-Shavit R, Eldor A, Vlodavsky I. Binding of thrombin to subendothelial extracellular matrix: protection and expression of functional properties. J Clin Invest 1989; 84:1096–2004
131 Heras M, Chesebro JH, Penny WJ, et al. Effects of thrombin inhibition on the development of acute platelet-thrombus deposition during angioplasty in pigs: heparin versus recombinant hirudin, a specific thrombin inhibitor. Circulation 1989; 79:657–665
132 Agnelli G, Pascucci C, Cosmi B, et al. The comparative effects of recombinant hirudin (CGP 39393) and standard heparin on thrombus growth in rabbits. Thromb Haemost 1990; 63:204–207
133 Barritt DW, Jordon SC. Anticoagulant drugs in the treatment of pulmonary embolism: a controlled trial. Lancet 1960; 1:1309–1313
134 Brandjes DPM, Heijboer H, Buller HR, et al. Acenocoumarol and heparin compared with acenocoumarol alone in the initial treatment of proximal vein thrombosis. N Engl J Med 1992; 327:1485–1489
135 Salzman EW, Deykin D, Shapiro RM, et al. Management of heparin therapy. N Engl J Med 1975; 292:1046–1050
136 Glazier RL, Corwell EB. Randomized prospective trial of continuous vs intermittent heparin therapy. JAMA 1976; 236:1365–1367
137 Mant MJ, O'Brien BD, Thong KL, et al. Haemorrhagic complications of heparin therapy. Lancet 1977; 1:1133–1135
138 Wilson JR, Lampman J. Heparin therapy: a randomized prospective study. Am Heart J 1979; 97:155–158
139 Fagher B, Lundh B. Heparin treatment of deep vein thrombosis. Acta Med Scand 1981; 210:357–361
140 Wilson JE, Bynum LJ, Parkey RW. Heparin therapy in venous thromboembolism. Am J Med 1981; 70:808–816
141 Krahenbuehl B, Simon CA, Bouvier CA, et al. Traitement heparinique: comparaison entre les voies d'admistration intraveineuse et souscutanee. Schweiz Med Wochenschr 1979; 109:1322–1325
142 Bentley PG, Kakkar VV, Scully MF, et al. An objective study of alternative methods of heparin administration. Thromb Res 1980; 18:1771–1787
143 Andersson G, Fagrell B, Holmgren K, et al. Subcutaneous administration of heparin: a randomised comparison with intravenous administration of heparin to patients with deep vein thrombosis. Thromb Res 1982; 27:631–639
144 Walker MG, Shaw JW, Thomson GJL, et al. Subcutaneous calcium heparin versus intravenous sodium heparin in treatment of established acute deep vein thrombosis of the legs: a multicenter prospective randomized trial. Br J Med 1987; 294:1189–1192
145 Gould MK, Dembitzer AD, Doyle RL, et al. Low molecular weight heparins compared with unfractionated heparin for the treatment of acute deep venous thrombosis: a meta-analysis of randomized controlled trials. Ann Intern Med 1999; 130:800–809
146 Gallus AS, Jackaman J, Tillett J, et al. Safety and efficacy of warfarin started early after submassive venous thrombosis or pulmonary embolism. Lancet 1986; 2:1293–1296
147 Clagett GP, Reisch JS. Prevention of venous thromboembolism in general surgical patients. Ann Surg 1988; 208:227–240
148 Collins R, Scrimgeour A, Yusuf S, et al. Reduction in fatal pulmonary embolism and venous thrombosis by perioperative administration of subcutaneous heparin. N Engl J Med 1988; 318:1162–1173
149 Kakkar VV, Corrigan TP, Fossard DP. Prevention of fatal postoperative pulmonary embolism by low doses of heparin. Lancet 1975; 2:45–51
150 Gallus AS. Overview of the management of thrombotic disorders. Semin Thromb Hemost 1989; 15:99–109
151 Halkin H, Goldberg J, Modan M, et al. Reduction of mortality in general medical inpatients by low-dose heparin prophylaxis. Ann Intern Med 1982; 96:561–565
152 Leyvraz PF, Richard J, Bachmann F. Adjusted versus fixed dose subcutaneous heparin in the prevention of deep vein thrombosis after total hip replacement. N Engl J Med 1983; 309:154–157
153 Powers PJ, Gent M, Jay RM, et al. A randomized trial of less intense postoperative warfarin or aspirin therapy in the prevention of venous thromboembolism after surgery for fractured hip. Arch Intern Med 1989; 149:771–774
154 Francis CW, Marder VJ, Evarts CM, et al. Two-step warfarin therapy: prevention of postoperative venous thrombosis without excessive bleeding. JAMA 1983; 249:374–378
155 Oler A, Whooley MA, Oler J, et al. Adding heparin to aspirin reduces the incidence of myocardial infarction and death in patients with unstable angina: a meta-analysis. JAMA 1996; 276:811–815
156 PRISM PLUS Study Investigators. Inhibition of the platelet glycoprotein IIb/IIIa receptor with tirofiban in unstable angina and non-q-wave myocardial infarction. N Engl J Med 1998; 338:1488–1497
157 CAPTURE Investigators. Randomised placebo-controlled trial of abciximab before and during coronary intervention in refractory unstable angina: the CAPTURE Study. Lancet 1997; 349:1429–1435
158 Collins R, Peto R, Baigent C, et al. Aspirin, heparin, and fibrinolytic therapy in suspected acute myocardial infarction. N Engl J Med 1997; 336:847–860
159 The RISC Group. Risk of myocardial infarction and death during treatment with low dose aspirin and intravenous heparin in men with unstable coronary artery disease. Lancet 1990; 336:827–830
160 Theroux P, Ouimet H, McCans J, et al. Aspirin, heparin, or both to treat acute unstable angina. N Engl J Med 1988; 319:1105–1111
161 Theroux P, Waters D, Lam J, et al. Reactivation of unstable

angina after the discontinuation of heparin. N Engl J Med 1992; 327:141–145
162 Serneri GGN, Gensini GF, Poggesi L, et al. Effect of heparin, aspirin, or alteplase in reduction of myocardial ischemia in refractory unstable angina. Lancet 1990; 335: 615–618
163 Theroux P, Waters D, Qiu S, et al. Aspirin versus heparin to prevent myocardial infarction during the acute phase of unstable angina. Circulation 1993; 99:2045–2048
164 MacMahon S, Collins R, Knight C, et al. Reduction in major morbidity and mortality by heparin in acute myocardial infarction [abstract]. Circulation 1988; 78(suppl II):98
165 Serneri GGN, Roveli F, Gensini GF, et al. Effectiveness of low-dose heparin in prevention of myocardial reinfarction. Lancet 1987; 1:937–942
166 The SCATI (Studio sulla Calciparina nell'Angina e nella Thrombosi Ventricolare nell'Infarto) Group: randomised controlled trial of subcutaneous calcium-heparin in acute myocardial infarction. Lancet 1989; 2:182–186
167 Folts JD, Crowell EB Jr, Rowe GG. Platelet aggregation in partially obstructed vessels and its elimination with aspirin. Circulation 1976; 54:365–370
168 ISIS-3 Collaborative Group. ISIS-3: a randomized comparison of streptokinase vs tissue plasminogen activator vs anistreplase and of aspirin plus heparin vs aspirin alone among 41,299 cases of suspected acute myocardial infarction. Lancet 1992; 339:753–770
169 O'Connor CM, Meese R, Carney R, et al. A randomized trial of intravenous heparin in conjunction with anistreplase (anisolyated plasminogen streptokinase activator complex) in acute myocardial infarction: the Duke University Clinical Cardiology Study (DUCCS). J Am Coll Cardiol 1994; 29:11–18
170 Collins R, MacMahon S, Flather M, et al. Clinical effects of anticoagulant therapy in suspected acute myocardial infarction: systematic overview of randomized trials. BMJ 1996; 313:652–659
171 Kruse KR, Califf RM, Ohman EM. Adjunctive therapies: thrombolytic therapy; a critical review of clinical trials. New York, NY: Chapman and Hall, 1996; 459–471
172 Mahaffey KW, Granger CB, Collins R, et al. Overview of randomized trials of intravenous heparin in patients with acute myocardial infarction treated with thrombolytic therapy. Am J Cardiol 1996; 77:551–556
173 Ryan, TJ Anderson JL, Antman EM, et al. ACC/AHA guidelines for the management of patients with acute myocardial infarction. J Am Coll Cardiol 1996; 28:1328–1428
174 Nairns CR, Hillegass WBJ, Nelson CL, et al. Relation between activated clotting time during angioplasty and abrupt closure. Circulation 1996; 93:667–671
175 Johnson EA, Kirkwood TBL, Stirling Y, et al. Four heparin preparations: anti-Xa potentiating effect of heparin after subcutaneous injection. Thromb Haemost 1976; 35:586–591
176 Carter CJ, Kelton JG, Hirsh J, et al. The relationship between the hemorrhagic and antithrombotic properties of low molecular weight heparins and heparin. Blood 1982; 59:1239–1245
177 Esquivel CO, Bergqvist D, Bjork C-G, et al. Comparison between commercial heparin, low-molecular weight heparin and pentosan polysulphate on hemostasis and platelets in vivo. Thromb Res 1982; 28:389–399
178 Cade JF, Buchanan MR, Boneu B, et al. A comparison of the antithrombotic and hemorrhagic effects of low molecular weight heparin fractions: the influence of the method of preparation. Thromb Res 1984; 35:613–625
179 Holmer E, Matsson C, Nilsson S. Anticoagulant and antithrombotic effects of low molecular weight heparin fragments in rabbits. Thromb Res 1982; 25:475–485
180 Andriuoli G, Mastacchi R, Barnti M, et al. Comparison of the antithrombotic and hemorrhagic effects of heparin and a new low molecular weight heparin in the rat. Haemostasis 1985; 15:324–330
181 Bergqvist D, Nilsson B, Hedner U, et al. The effects of heparin fragments of different molecular weight in experimental thrombosis and hemostasis. Thromb Res 1985; 38: 589–601
182 Frydman A, Bara L, Leroux Y, et al. The antithrombotic activity and pharmacokinetics of enoxaparin, a low molecular weight heparin, in man given single subcutaneous doses of 20 up to 80 mg. J Clin Pharmacol 1988; 28:609–618
183 Briant L, Caranobe C, Saivin S, et al. Unfractionated heparin and CY216: pharmacokinetics and bioavailabilities of the anti-factor Xa and IIa; effects of intravenous and subcutaneous injection in rabbits. Thromb Haemost 1989; 61:348–353
184 Bratt G, Tornebohm E, Widlund L, et al. Low molecular weight heparin (KABI 2165, Fragmin): pharmacokinetics after intravenous and subcutaneous administration in human volunteers. Thromb Res 1986; 42:613–620
185 Matzsch T, Bergqvist D, Hedner U, et al. Effect of an enzymatically depolymerized heparin as compared with conventional heparin in healthy volunteers. Thromb Haemost 1987; 57:97–101
186 Bara L, Samama MM. Pharmacokinetics of low molecular weight heparins. Acta Chir Scand 1988; 543:65–72
187 Bradbrook ID, Magnani HN, Moelker HC, et al. ORG 10172: a low molecular weight heparinoid anticoagulant with a long half life in man. Br J Clin Pharmacol 1987; 23:667–675
188 Weitz JI. Low-molecular-weight heparins. N Engl J Med 1997; 337:688–698
189 Jordan RE, Oosta GM, Gardner WT, et al. The kinetics of hemostatic enzyme-antithrombin interactions in the presence of low molecular weight heparin. J Biol Chem 1980; 255:10081–10090
190 Holmer E, Kurachi K, Soderstrom G. The molecular-weight dependence of the rate-enhancing effect of heparin on the inhibition of thrombin, factor Xa, factor IXa, factor XIa, factor XIIa and kallikrein by antithrombin. Biochem J 1981; 193:395–400
191 Holmer E, Soderberg K, Bergqvist D, et al. Heparin and its low molecular weight derivatives: anticoagulant and antithrombotic properties. Haemostasis 1986; 16(suppl 2):1–7
192 Griffith MJ. Heparin-catalyzed inhibitor/protease reactions: kinetic evidence for a common mechanism of action of heparin. Proc Natl Acad Sci U S A 1983; 80:5460–5464
193 Pletcher CH, Nelsestuen GL. Two-substrate reaction models for the heparin-catalyzed bovine antithrombin/protease reaction. J Biol Chem 1983; 258:1086–1091
194 Rosenberg RD, Jordon RE, Favreau LV, et al. Highly active heparin species with multiple binding sites for antithrombin. Biochem Biophys Res Commun 1979; 86:1319–1324
195 Danielsson A, Raub E, Lindahl U, et al. Role of ternary complexes in which heparin binds both antithrombin and proteinase, in the acceleration of the reactions between antithrombin and thrombin or factor Xa. J Biol Chem 1986; 261:15467–15473
196 Jordan RE, Favreau LV, Braswell EH, et al. Heparin with two binding sites for antithrombin or platelet factor 4. J Biol Chem 1982; 257:400–406
197 Boneu B, Caranobe C, Cadroy Y, et al. Pharmacokinetic studies of standard UFH, and low molecular weight heparins in the rabbit. Semin Thromb Hemost 1988; 14:18–27
198 Caranobe C, Barret A, Gabaig AM, et al. Disappearance of circulating anti-Xa activity after intravenous injection of unfractionated heparin and of low molecular weight heparin

(CY216) in normal and nephrectomized rabbits. Thromb Res 1985; 40:129–133
199 Palm M, Mattsson CH. Pharmacokinetics of heparin and low molecular weight heparin fragment (Fragmin) in rabbits with impaired renal or metabolic clearance. Thromb Haemost 1987; 58:932–935
200 Handeland GF, Abidgaard GF, Holm U, et al. Dose adjusted heparin treatment of deep venous thrombosis: a comparison of unfractionated and low molecular weight heparin. Eur J Clin Pharmacol 1990; 39:107–112
201 Choay J, Petitou M, Lormeau JC, et al. Structure-activity relationship in heparin: a synthetic pentasaccharide with high affinity for antithrombin and eliciting high anti-factor Xa activity. Biochem Biophys Res Commun 1983; 116:492–499
202 Litin SC, Heit JA, Mees KA. Use of low-molecular-weight heparin in the treatment of venous thromboembolic disease: answers to frequently asked questions. Mayo Clin Proc 1998; 73:545–551
203 Kessler CM. Low molecular weight heparins: practical considerations. Semin Hematol 1997; 34:35–42
204 Abbate R, Gori AM, Farsi A, et al. Monitoring of low-molecular-weight heparins. Am J Cardiol 1998; 82:33L–36L
205 Samama MM. Contemporary laboratory monitoring of low molecular weight heparins. Clin Lab Med 1995; 15:119–123
206 College of American Pathologists. Coagulation (Comprehensive) Survey. Set CG2-B. Northfield, IL: College of American Pathologists, 1999
207 Kessler CM, Esparraguera IM, Jacobs HM, et al. Monitoring the anticoagulant effects of a low molecular weight heparin preparation. Am J Clin Pathol 1995; 103:642–648
208 Laposata M, Green K, Elizabeth MVC, et al. The clinical use and laboratory monitoring of low-molecular-weight heparin, danaparoid, hirudin and related compounds, and argatroban: College of American Pathologists Conference XXXI on Laboratory Monitoring of Anticoagulant Therapy. Arch Pathol Lab Med 1998; 122:799–807
209 Alhenc-Gelas M, Jestin-Le Guernic C, Vitoux JF, et al. Adjusted versus fixed doses of the low-molecular-weight heparin Fragmin in the treatment of deep vein thrombosis. Thromb Haemost 1994; 71:698–702
210 Levine MN, Planes A, Hirsh J, et al. The relationship between antifactor Xa level and clinical outcome in patients receiving enoxaparin low molecular weight heparin to prevent deep vein thrombosis after hip replacement. Thromb Haemost 1989; 62:940–944
211 Hettiarachchi RJK, Smorenburg SM, Ginsberg J, et al. Do heparins do more than just treat thrombosis? The influence of heparins on cancer spread. Thromb Haemost 1999; 82:947–952
212 Scuimbatta T, Caretto P, Pirovano P, et al. Treatment with modified heparins inhibits experimental metastasis formation and leads, in some animals, to long term survival. Invasion Metastasis 1996; 16:132–143
213 Vlodavsky I, Mohsen M, Lider O, et al. Inhibition of tumour metastasis by heparanase inhibiting species of heparin. Invasion Metastasis 1994; 14:290–302
214 Albada J, Nieuwenhuis HK, Sixma JJ. Treatment of acute venous thromboembolism with low molecular weight heparin (Fragmin). Circulation 1989; 80:935–940
215 Nieuwenhuis HK, Albada J, Banga JD, et al. Identification of risk factors for bleeding during treatment of acute venous thromboembolism with heparin or low molecular weight heparin. Blood 1991; 78:2337–2343
216 Boneu B. Low molecular weight heparin therapy: is monitoring needed? Thromb Haemost 1994; 72:330–334
217 Simonneau G, Sors H, Charbonnier B, et al, for the THESEE Study Group. A comparison of low-molecular-weight heparin with unfractionated heparin for acute pulmonary embolism. N Engl J Med 1997; 337:663–669
218 Levine M, Gent M, Hirsh J, et al. A comparison of low molecular weight heparin administered primarily at home with UFH administered in the hospital for proximal deep vein thrombosis. N Engl J Med 1996; 334:677–681
219 Decousus H, Leizorovicz A, Parent F, et al. A clinical trial of vena caval filters in the prevention of pulmonary embolism in patients with proximal deep-vein thrombosis: Prevention du Risque d'Embolie Pulmonaire par Interruption Ca e Study Group. N Engl J Med 1998; 338:409–415
220 Hull RD, Raskob GE, Pineo GF, et al. Subcutaneous low-molecular-weight heparin compared with continuous intravenous heparin in the treatment of proximal-vein thrombosis. N Engl J Med 1992; 326:975–982
221 Spiro A. Multicenter clinical trial comparing once daily and twice daily subcutaneous enoxaparin and intravenous heparin in the treatment of acute deep vein thrombosis. Thromb Haemost 1997; (suppl):373–374
222 Gurfinkel EP, Manos EJ, Mejail RI, et al. Low molecular weight heparin versus regular heparin or aspirin in the treatment of unstable angina and silent ischemia. J Am Coll Cardiol 1995; 26:313–318
223 Klein W, Buchwald A, Hillis S, et al. Comparison of low molecular weight heparin with UFH acutely and with placebo for 6 weeks in the management of unstable coronary artery disease: the Fragmin in Unstable Coronary Artery Disease study (FRIC). Circulation 1997; 96:61–68
224 Cohen M, Demers C, Gurfinkel E, for the ESSENCE Trial. A comparison of low-molecular-weight heparin with unfractionated heparin for unstable coronary artery disease. N Engl J Med 1997; 337:447–452
225 Koopman MMW, Prandoni P, Piovella F, et al, for the TASMAN Study Group. Treatment of venous thrombosis with intravenous UFH administered in the hospital as compared with subcutaneous low-molecular-weight heparin administered at home. N Engl J Med 1996; 334:682–687
226 The COLUMBUS Investigators. Low-molecular-weight heparin in the treatment of patients with venous thromboembolism. N Engl J Med 1997; 337:657–662
227 Charbonier BA, Fiessinger J-N, Banga JD, et al, for the FRAXODI Group. Comparison of a once daily with a twice daily subcutaneous low molecular weight heparin regimen in the treatment of deep vein thrombosis. Thromb Haemost 1998; 79:897–901
228 FRISC Study Group. Low-molecular-weight heparin during instability in coronary artery disease: Fragmin During Instability in Coronary Artery Disease (FRISC) Study Group. Lancet 1996; 347:561–568
229 Dalpozzo A, Acquasaliente M, Geron MR. New heparin complexes active by intestinal absorption: I. Multiple ion pairs with basic organic compounds. Thromb Res 1989; 56:119–124
230 Brayden D, Creed E, O'Connell A, et al. Heparin absorption across the intestine: effects of sodium N-8-(2-hydroxybenzoyl)amino caprylate in rat *in situ* intestinal installations and in caco-2 monolayers. Pharm Res 1997; 14:1772–1779
231 Leone-Bay A, Paton DR, Variano B, et al. Acylated non-α-amino acids as novel agents for the oral delivery of heparin sodium, USP. J Control Release 1998; 50:41–49
232 Salartash K, Gonze MD, Leone-Bay A, et al. Oral low-molecular weight heparin and delivery agent prevent jugular venous thrombosis in the rat. J Vasc Surg 1999; 30:526–531
233 Baughman RA, Kapoor SC, Agarwal RK, et al. Oral delivery of anticoagulant doses of heparin: a randomized, double-blind, controlled study in humans. Circulation 1998; 98: 1610–1615

234 Norrby K. Heparin and angiogenesis: a low molecular weight fraction inhibits and a high molecular weight fraction stimulates angiogenesis systemically. Haemostasis 1993; 23:141–149
235 Lepri A, Benelli U, Bernardini N, et al. Effect of low molecular weight heparin sulphate on angiogenesis in the rat cornea after chemical cauterization. J Ocul Pharmacol 1994; 10:273–280
236 Pezzuoli G, Serneri GGN, Settembrini P, et al, for the STEP-Study Group. Prophylaxis of fatal pulmonary embolism in general surgery using low molecular weight heparin Cy216: a multicenter double-blind randomized controlled clinical trial versus placebo. Int Surg 1989; 74:205–210
237 Nurmohamed MT, Rosendaal FR, Buller HR, et al. Low-molecular-weight heparin versus standard heparin in general and orthopedic surgery: a meta-analysis. Lancet 1992; 340: 152–156
238 Leclerc J, Geerts W, Desjardins L, 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; 67:417–423
239 Turpie AGG, Levine MN, Hirsh J, et al. A randomized controlled trial of a low molecular weight heparin (enoxaparin) to prevent deep vein thrombosis in patients undergoing elective hip surgery. N Engl J Med 1986; 315:925–929
240 Hoek J, Nurmohamed MT, Hamelynck KJ, et al. Prevention of deep vein thrombosis (DVT) following total hip replacement by a low molecular weight heparinoid. Thromb Haemost 1992; 67:28–32
241 Levine MN, Hirsh J, Gent M, et al. Prevention of deep vein thrombosis after elective hip surgery: a randomized trial comparing low molecular weight heparin with standard unfractionated heparin. Ann Intern Med 1991; 114:545–551
242 Planes A, Vochelle N, Mazas F, et al. Prevention of postoperative venous thrombosis: a randomized trial comparing unfractionated heparin with low molecular weight heparin in patients undergoing total hip replacement. Thromb Haemost 1988; 60:407–410
243 Eriksson BI, Kalebo P, Anthmyr BA, et al. Prevention of deep vein thrombosis and pulmonary embolism after total hip replacement: comparison of low-molecular-weight heparin and unfractionated heparin. J Bone Joint Surg Am 1991; 73:484–493
244 Leyvraz PF, Bachmann F, Hoek J, et al. Prevention of deep vein thrombosis after hip replacement: randomized comparison between unfractionated heparin and low molecular weight heparin. BMJ 1991; 303:543–548
245 Dechavanne M, Ville D, Berruyer M, et al. Randomized trial of 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
246 Bergqvist D, Kettunen K, Fredin H, et al. Thromboprophylaxis in hip fracture patients: a prospective randomised comparative study between ORG 10172 and dextran. Surgery 1991; 109:617–622
247 Borris LC, Hauch O, Jorgensen LN, et al, for the Danish Enoxaparin Study Group. Low-molecular-weight heparin (enoxaparin) vs dextran 70: the prevention of postoperative deep vein thrombosis after total hip replacement. Arch Intern Med 1991; 151:1621–1624
248 Heit J, Kessler C, Mammen E, et al, for the RD Heparin Study Group. Efficacy of RD heparin (a LMWH) and warfarin for prevention of deep-vein thrombosis after hip or knee replacement [abstract]. Blood 1991; 778:187A
249 Anderson DR, O'Brien BJ, Levine MN, et al. Efficacy and cost of low-molecular-weight heparin compared with standard heparin for the prevention of deep vein thrombosis after total hip arthroplasty. Ann Intern Med 1993; 119:1105–1112
250 Colwell CW, Spiro TE, Trowbridge AA, et al. Efficacy and safety of enoxaparin versus unfractionated heparin for prevention of deep venous thrombosis after elective knee arthroplasty. Clin Orthop 1995; 321:19–27
251 Fauno P, Suomalainen O, Rehnberg V, 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; 76:1814–1818
252 RD Heparin Arthroplasty Group. RD heparin compared with warfarin for prevention of venous thromboembolic disease following total hip or knee arthroplasty. J Bone Joint Surg Am 1994; 76:1174–1185
253 Spiro TE, Johnson GJ, Christie MJ, for the Enoxaparin Clinical Trial Group. Efficacy and safety of enoxaparin to prevent deep venous thrombosis after hip replacement surgery. Ann Intern Med 1994; 121:81–89
254 Heit JA, Berkowitz SD, Rona R, for the Ardeparin Athroplasty Study Group. Efficacy and safety of low molecular weight heparin (ardeparin sodium) compared to warfarin for the prevention of venous thromboembolism after total knee replacement surgery: a double-blind dose-ranging study. Thromb Haemost 1997; 77:32–38
255 Hull R, Raskob G, Pineo G, 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; 19:1370–1376
256 Leclerc JR, Geerts WH, Desjardins L, et al. Prevention of venous thromboembolism (VTE) after knee arthroplasty: a randomized double-blind trial comparing enoxaparin with warfarin sodium. Ann Intern Med 1996; 124:619–626
257 Leclerc JR, Geerts WH, Desjardins L, et al. Prevention of venous thromboembolism (VTE) after knee arthroplasty: a randomized, double-blind trial, comparing enoxaparin to warfarin sodium [abstract]. Haemostasis 1994; 24(suppl 1):232
258 Deleted in proof
259 Hamulyak K, Lensing AWA, van der Meer J, et al, for the Fraxiparine Oral Anticoagulant Study Group. Subcutaneous low-molecular weight heparin or oral anticoagulants for the prevention of deep-vein thrombosis in elective hip and knee replacement? Thromb Haemost 1994; 74:1428–1431
260 Gent M, Hirsh J, Ginsberg J, et al. Low molecular weight heparinoid orgaran is more effective than aspirin in the prevention of venous thromboembolism after surgery for hip fracture. Circulation 1996; 93:80–84
261 Geerts WH, Jay R, Cade K, et al. A comparison of low-dose heparin with low-molecular-weight heparin as prophylaxes against venous thromboembolism after major trauma. N Engl J Med 1996; 335:701–707
262 Agnelli G, Piovella F, Buoncristiani P, et al. Enoxaparin plus compression after elective neurosurgery. N Engl J Med 1998; 339:80–85
263 Prins MH, Den Ottolander GJH, Gelsema R, et al. Deep vein thrombosis prophylaxis with a low molecular weight heparin (Kabi 2165) in stroke patients [abstract]. Thromb Haemost 1987; 58(suppl):117
264 Turpie AGG, Levine MN, Hirsh J, et al. A double-blind randomized trial of ORG 10172 low molecular weight heparinoid in the prevention of deep vein thrombosis in thrombotic stroke. Lancet 1987; 1:523–526
265 Green D, Lee MY, Lim AC, et al. Prevention of thromboembolism after spinal cord injury using low molecular weight heparin. Ann Intern Med 1990; 113:571–574
266 Turpie AGG, Gent M, Cote R, et al. A low molecular weight heparinoid compared with unfractionated heparin in the

prevention of deep vein thrombosis in patients with acute ischemic stroke. Ann Intern Med 1992; 117:353–357
267 Kuijer PMM, Prins MH, Buller HR. Low-molecular-weight heparins: treatment of venous thromboembolism. In: Sasahara AA, Loscalzo J, eds. Advances in therapeutic agents in thrombosis and thrombolysis. New York, NY: Marcel Dekker, 1997:129–147
268 Patrono C, Coller B, Dalen JE, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. Chest 1998; 114(suppl):470S–488S
269 Goodman SG, Cohen M, Bigonzi F, et al, for the Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q-Wave Coronary Events Study Group. Randomized trial of low molecular weight heparin (enoxaparin) versus unfractionated heparin for unstable coronary artery disease: one year results of the ESSENCE study. J Am Coll Cardiol 2000; 36:693–698
270 Leizorivicz A. The FRAXIS Investigators. XXth Congress of the European Society of Cardiology; August 22–26, 1998; Vienna, Austria
271 FRISC II Investigators. Long-term low-molecular-mass heparin in unstable coronary-artery disease: FRISC II prospective randomised multicentre study: Fragmin and fast revascularisation during instability in coronary artery disease. Lancet 2000; 356:9–16
272 Kontny F, Dale J, Abildgaard U, et al. Randomized trial of low molecular weight heparin (dalteparin) in prevention of left ventricular thrombus formation and arterial embolism after acute anterior myocardial infarction: the Fragmin in Acute Myocardial Infarction (FRAMI) Study. J Am Coll Cardiol 1997; 39:962–969
273 Glick A, Kornowski R, Michowich Y, et al. Reduction of reinfarction and angina with use of low-molecular-weight heparin therapy after streptokinase (and heparin) in acute myocardial infarction. Am J Cardiol 1996; 77:1145–1148
274 Hanke H, Oberhoff M, Hanke S, et al. Inhibition of cellular proliferation after experimental balloon angioplasty by low-molecular-weight heparin. Circulation 1992; 85:1548–1556
275 Buchwald AB, Unterberg C, Nebendahl K, et al. Low-molecular-weight heparin reduces neointimal proliferation after coronary stent implantaton in hypercholesterolemic minipigs. Circulation 1992; 86:531–537
276 Faxon DP, Spiro TE, Minor S, et al. Low molecular weight heparin in prevention of restenosis after angioplasty. Circulation 1994; 90:908–914
277 Cairns JA, Gill J, Morton B, et al, for the EMPAR Collaborators. Fish oils and low-molecular-weight heparin for the reduction of restenosis after percutaneous transluminal coronary angioplasty. Circulation 1996; 94:1553–1560
278 Protamine sulfate antiheparin agents 20:12.08. In: McEvoy GK, Litvak K, Welsh OH, et al, eds. AHFS Drug Information 1999. Bethesda, MD: American Society of Health-System Pharmacists, 1999; 1265–1267
279 Horrow JC. Protamine: a review of its toxicity. Anesth Analg 1985; 64:348–361
280 Caplan SN, Berkman EM. Protamine sulfate and fish allergy [letter]. N Engl J Med 1976; 295:172
281 Stewart WJ, McSweeney SM, Kellett MR, et al. Increased risk of severe protamine reactions in NPH insulin-dependent diabetics undergoing cardiac catheterization. Circulation 1984; 70:788–792
282 Cooney A, Mann TJ. Recent experiences with hexadimethrine for neutralizing heparin after cardiopulmonary bypass. Anesth Intensive Care Med 1999; 27:298–300
283 Kikura M, Lee MK, Levy JH. Heparin neutralization with methylene blue, hexadimethrine, or vancomycin after cardiopulmonary bypass. Anesth Analg 1996; 83:223–227
284 Despotis GJ, Summerfield AL, Joist JH, et al. *In vitro* reversal of heparin effect with heparinase: evaluation with whole blood prothrombin time and activated partial thromboplastin time in cardiac surgical patients. Anesth Analg 1994; 79:670–674
285 Dehmer GJ, Fisher M, Tate DA, et al. Reversal of heparin anticoagulation by recombinant platelet factor 4 in humans. Circulation 1995; 91:2188–2194
286 D'Ambra M. Restoration of the normal coagulation process: advances in therapies to antagonize heparin. J Cardiovasc Pharmacol 1996; 27(suppl):S58–S62
287 Hendrikx M, Leunens V, Vandezande E, et al. The use of a heparin removal device: a valid alternative to protamine. Int J Artif Organs 1997; 20:166–174
288 Tao W, Deyo DJ, Brunston RL Jr, et al. Extracorporeal heparin adsorption following cardiopulmonary bypass with a heparin removal device: an alternative to protamine. Crit Care Med 1998; 26:1096–1102
289 Hulin MS, Wakefield TW, Andrews PC, et al. A novel protamine variant reversal of heparin anticoagulation in human blood *in vitro*. J Vasc Surg 1997; 26:1043–1048
290 Racanelli A, Fareed J, Walenga JM, et al. Biochemical and pharmacologic studies on the protamine interactions with heparin: its fractions and fragments. Semin Thromb Hemost 1985; 11:176–189
291 Lindblad B, Borgstrom A, Wakefield TW, et al. Protamine reversal of anticoagulation achieved with a low molecular weight heparin: the effects on eicosanoids, clotting and complement factors. Thromb Res 1987; 48:31–40
292 Massonnet-Castel S, Pelissier E, Bara L, et al. Partial reversal of low molecular weight heparin (PK 10169) anti-Xa activity by protamine sulfate: *in vitro* and *in vivo* study during cardiac surgery with extracorporeal circulation. Haemostasis 1986; 16:139–146
293 Hirsh J, Buchanan MR. Comparative effects of heparin and LMW heparin on hemostasis. Thromb Res 1991; 14:11–17
294 Woltz M, Weltermann A, Nieszpaur-Los M, et al. Studies on the neutralizing effects of protamine on unfractionated and low molecular weight heparin (Fragmin) at the site of activation of the coagulation system in man. Thromb Haemost 1995; 73:439–443
295 Gram J, Mercker S, Bruhn HD. Does protamine chloride neutralize low molecular weight heparin sufficiently? Thromb Res 1988; 52:353–359
296 Sugiyama T, Itoh M, Ohtawa M, et al. Study on neutralization of low molecular weight heparin by protamine sulfate and its neutralization characteristics. Thromb Res 1992; 68:119–129
297 Harenberg J, Gnasso A, de Vries JX, et al. Inhibition of low molecular weight heparin by protamine chloride *in vivo*. Thromb Res 1985; 38:11–20
298 Racanelli A, Fareed J, Huan XQ. Low molecular weight heparin induced bleeding can be neutralized by protamine. Haemostasis 1988; 18(suppl 2):163–164
299 Van Ryn-McKenna J, Cai L, Ofosu FA, et al. Neutralization of enoxaparine-induced bleeding by protamine sulfate. Thromb Haemost 1990; 63:271–274
300 Doutremepuich C, Bonini F, Toulemonde F, et al. *In vivo* neutralization of low-molecular weight heparin fraction CY 216 by protamine. Semin Thromb Hemost 1985; 11:318–322
301 Wakefield TW, Andrews PC, Wrobleski SK, et al. Effective and less toxic reversal of low-molecular weight heparin anticoagulation by a designer variant of protamine. J Vasc Surg 1995; 21:839–850
302 Byun Y, Singh VK, Yang VC. Low molecular weight protamine: a potential nontoxic heparin antagonist. Thromb Res 1999; 94:53–61

303 Hulin MS, Wakefield TW, Andrews PC, et al. Comparison of the hemodynamic and hematologic toxicity of a protamine variant after reversal of low-molecular-weight heparin anticoagulation in a canine model. Lab Animal Sci 1997; 47:153–160
304 Wakefield TW, Andrews PC, Wrobleski SK, et al. A [+18RGD] protamine variant for nontoxic and effective reversal of conventional heparin and low-molecular-weight heparin anticoagulation. J Surg Res 1996; 63:280–286
305 Dietrich CP, Shinjo SK, Fabio AM, et al. Structural features and bleeding activity of commercial low molecular weight heparins: neutralization by ATP and protamine. Semin Thromb Hemost 1999; 25(suppl 3):43–50
306 Shenoy S, Sobel M, Harris RB. Development of heparin antagonists with focused biological activity. Curr Pharm Design 1999; 5:965–986
307 Warkentin TE, Chong BH, Greinacher A. Heparin-induced thrombocytopenia: towards consensus. Thromb Haemost 1998; 79:1–7
308 Warkentin TE. Heparin-induced thrombocytopenia: a clinicopathologic syndrome. Thromb Haemost 1999; 82(suppl): 439–447
309 Warkentin TE, Levine MN, Hirsh J, et al. Formation of heparin-induced thrombocytopenia IgG without thrombocytopenia: analysis of a clinical trial [abstract]. Blood 1995; 86(suppl 1):537a
310 Warkentin TE. Heparin-induced skin lesions. Br J Haematol 1996; 92:494–497
311 Amiral J, Bridey F, Dreyfus M, et al. Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia [letter]. Thromb Haemost 1992; 68:95–96
312 Amiral J, Meyer D. Heparin-dependent antigens in heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia. New York, NY: Marcel Dekker, 2000; 127–138
313 Visentin GP, Bacsi S, Aster RH. Molecular immunopathogenesis of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia. New York, NY: Marcel Dekker, 2000; 139–154
314 Greinacher A, Michels I, Schäfer M, et al. Heparin-associated thrombocytopenia in a patient treated with polysulphated chondroitin sulphate: evidence for immunological crossreactivity between heparin and polysulphated glycosaminoglycan. Br J Haematol 1992; 81:252–254
315 Tardy-Poncet B, Tardy B, Grelac F, et al. Pentosan polysulfate-induced thrombocytopenia and thrombosis. Am J Hematol 1994; 45:252–257
316 Collins JL, Aster RH, Moghaddam M, et al. Diagnostic testing for heparin-induced thrombocytopenia (HIT): an enhanced platelet factor 4 complex enzyme linked immunosorbent assay (PF ELISA) [abstract]. Blood 1997; 90(suppl 1):461a
317 Funk S, Eichler P, Albrecht D, et al. Heparin-induced thrombocytopenia (HIT) in orthopedic patients: a prospective cohort trial comparing UFH and LMWH [abstract]. Ann Hematol 2000; 79(suppl 1):A92
318 Warkentin TE, Levine MN, Hirsh J, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. N Engl J Med 1995; 332:1330–1335
319 Amiral J, Lormeau JC, Marfaing-Koka A, et al. Absence of cross-reactivity of SR90107A/ORG31540 pentasaccharide with antibodies to heparin-PF4 complexes developed in heparin-induced thrombocytopenia. Blood Coagul Fibrinolysis 1997; 8:114–117
320 Petitou M, Herault JP, Bernat A, et al. Synthesis of thrombin-inhibiting heparin mimetics without side effects. Nature 1999; 398:417–422
321 Warkentin TE, Kelton JG. Thrombocytopenia due to platelet destruction and hypersplenism. In: Hoffman R, Benz EJ Jr, Shattil SJ, et al. Hematology: basic principles and practice. 3rd ed. New York, NY: Churchill Livingstone, 1999; 2138–2154
322 Chong BH, Murray B, Berndt MC, et al. Plasma P-selectin is increased in thrombotic consumptive platelet disorders. Blood 1994; 83:1535–1541
323 Warkentin TE, Hayward CPM, Boshkov LK, et al. Sera from patients with heparin-induced thrombocytopenia generate platelet-derived microparticles with procoagulant activity: an explanation for the thrombotic complications of heparin-induced thrombocytopenia. Blood 1994; 84:3691–3699
324 Kelton JG, Sheridan D, Santos A, et al. Heparin-induced thrombocytopenia: laboratory studies. Blood 1988; 72:925–930
325 Warkentin TE, Elavathil LJ, Hayward CPM, et al. The pathogenesis of venous limb gangrene associated with heparin-induced thrombocytopenia. Ann Intern Med 1997; 127:804–812
326 Greinacher A, Eichler P, Lubenow N, et al. Heparin-induced thrombocytopenia with thromboembolic complications: meta-analysis of two prospective trials to assess the value of parenteral treatment with lepirudin and its therapeutic aPTT range. Blood 2000; 96:846–851
327 Warkentin TE, Sheppard JI. Generation of platelet-derived microparticles and procoagulant activity by heparin-induced thrombocytopenia IgG/serum and other IgG platelet agonists: a comparison with standard platelet agonists. Platelets 1999; 10:319–326
328 Warkentin TE. Clinical picture of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia. New York, NY: Marcel Dekker, 2000; 43–80
329 Warkentin TE, Sikov WM, Lillicrap DP. Multicentric warfarin-induced skin necrosis complicating heparin-induced thrombocytopenia. Am J Hematol 1999; 62:44–48
330 Warkentin TE. Heparin-induced thrombocytopenia: IgG-mediated platelet activation, platelet microparticle generation, and altered procoagulant/anticoagulant balance in the pathogenesis of thrombosis and venous limb gangrene complicating heparin-induced thrombocytopenia. Transfus Med Rev 1996; 10:249–258
331 Warkentin TE. Venous limb gangrene (VLG) complicating warfarin treatment of deep-vein thrombosis (DVT) in metastatic carcinoma [abstract]. Blood 1999; 94(suppl 1):114b
332 Warkentin TE, Sheppard JA, Horsewood P, et al. The impact of the patient population on the risk of heparin-induced thrombocytopenia. Blood 2000; 96:1703–1708
333 Lee DP, Warkentin TE. Frequency of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia. New York, NY: Marcel Dekker, 2000; 81–112
334 Warkentin TE, Greinacher A. Laboratory testing for heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia. New York, NY: Marcel Dekker, 2000; 211–244
335 Griffiths E, Dzik WH. Assays for heparin-induced thrombocytopenia. Transfus Med 1997; 7:1–11
336 Warkentin TE, Hayward CPM, Smith CA, et al. Determinants of donor platelet variability when testing for heparin-induced thrombocytopenia. J Lab Clin Med 1992; 120:371–379
337 Greinacher A, Amiral J, Dummel V, et al. Laboratory diagnosis of heparin-associated thrombocytopenia and comparison of platelet aggregation test, heparin-induced platelet activation test, and platelet factor 4/heparin enzyme-linked

immunosorbent assay. Transfusion 1994; 34:381–385
338 Eichler P, Budde U, Haas S, et al. First workshop for detection of heparin-induced antibodies: validation of the heparin-induced platelet-activation test (HIPA) in comparison with a PF4/heparin ELISA. Thromb Haemost 1999; 81:625–629
339 Warkentin TE, Kelton JG. A 14-year study of heparin-induced thrombocytopenia. Am J Med 1996; 101:502–507
340 Chong BH. Low molecular weight heparinoid and heparin-induced thrombocytopenia [abstract]. Aust NZ J Med 1996; 26:331
341 Chong BH. Danaparoid for the treatment of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia. New York, NY: Marcel Dekker, 2000; 291–311
342 Newman PM, Swanson RL, Chong BH. Heparin-induced thrombocytopenia: IgG binding to PF4-heparin complexes in the fluid phase and cross-reactivity with low molecular weight heparin and heparinoid. Thromb Haemost 1998; 80:292–297
343 Warkentin TE. Danaparoid (Orgaran®) for the treatment of heparin-induced thrombocytopenia (HIT) and thrombosis: effects on *in vivo* thrombin and cross-linked fibrin generation, and evaluation of the clinical significance of *in vitro* cross-reactivity (XR) of danaparoid for HIT-IgG [abstract]. Blood 1996; 88(suppl 1):626a
344 Greinacher A. Recombinant hirudin for the treatment of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A, eds. Heparin-induced thrombocytopenia. New York, NY: Marcel Dekker, 2000; 313–338
345 Greinacher A, Völpel H, Janssens U, et al, for the HIT Investigators Group. Recombinant hirudin (lepirudin) provides safe and effective anticoagulation in patients with heparin-induced thrombocytopenia: a prospective study. Circulation 1999; 99:73–80
346 Greinacher A, Janssens U, Berg G, et al, for the Heparin-Associated Thrombocytopenia Study (HAT) Investigators. Lepirudin (recombinant hirudin) for parenteral anticoagulation in patients with heparin-induced thrombocytopenia. Circulation 1999; 100:587–593
347 Song X, Huhle G, Wang L, et al. Generation of anti-hirudin antibodies in heparin-induced thrombocytopenic patients treated with r-hirudin. Circulation 1999; 100:1528–1532
348 Lewis BE. Preliminary results of a prospective randomized controlled trial of argatroban versus conventional therapy for heparin-induced thrombocytopenia. Presented at XVI International Congress of the International Society on Thrombosis and Haemostasis. Florence, Italy. June 12, 1997
349 Warkentin TE. Limitations of conventional treatment options for heparin-induced thrombocytopenia. Semin Hematol 1998; 35(suppl 5):17–25
350 Thomas D, Block AJ. Thrombocytopenia, cutaneous necrosis, and gangrene of the upper and lower extremities in a 35-year-old man. Chest 1992; 102:1578–1580
351 Gupta AK, Kovacs MJ, Sauder DN. Heparin-induced thrombocytopenia. Ann Pharmacother 1998; 32:55–59
352 Battey PM, Salam AA. Venous gangrene associated with heparin-induced thrombocytopenia. Surgery 1985; 97:618–620
353 Wallis DE, Quintos R, Wehrmacher W, et al. Safety of warfarin anticoagulation in patients with heparin-induced thrombocytopenia. Chest 1999; 116:1333–1338
354 Greinacher A, Michels I, Mueller-Eckhardt C. Heparin-associated thrombocytopenia: antibody is not heparin-specific. Thromb Haemost 1992; 67:545–549
355 Ranze O, Eichner A, Lubenow N, et al. The use of low-molecular-weight heparins in heparin-induced thrombocytopenia (HIT): a cohort study [abstract]. Ann Hematol 2000; 79(suppl 1):198
356 Contreras M. The appropriate use of platelets: an update from the Edinburgh Consensus Conference. Br J Hematol 1998; 101(suppl 1):10–12
357 Babcock RB, Dumper CW, Scharfman WB. Heparin-induced thrombocytopenia. N Engl J Med 1976; 295:237–241
358 Cimo PL, Moake JL, Weinger RS, et al. Heparin-induced thrombocytopenia: association with a platelet aggregating factor and arterial thromboses. Am J Hematol 1979; 6:125–133
359 Boon DMS, Michiels JJ, Stibbe J, et al. Heparin-induced thrombocytopenia and antithrombotic therapy [letter]. Lancet 1994; 344:1296
360 Wallis DE, Workman DL, Lewis BE, et al. Failure of early heparin cessation as treatment for heparin-induced thrombocytopenia. Am J Med 1999; 106:629–635
361 Tardy B, Tardy-Poncet B, Fournel P, et al. Lower limb veins should be systematically explored in patients with isolated heparin-induced thrombocytopenia [letter]. Thromb Haemost 1999; 82:1199–1200
362 Griffith CC, Nichols G, Asher JD, et al. Heparin osteoporosis. JAMA 1965; 193:85–88
363 Jaffe MD, Willis PW. Multiple fractures associated with long term sodium heparin therapy. JAMA 1965; 193:152–154
364 Miller WE, DeWolfe VG. Osteoporosis resulting from heparin therapy. Cleve Clin Q 1966; 33:31–34
365 Avioli LV. Heparin induced osteoporosis: an appraisal. Adv Exp Med Biol 1975; 52:375–387
366 Squires JW, Pinch LW. Heparin-induced spinal fractures. JAMA 1979; 241:2417–2418
367 Rupp WM, McCarthy HB, Rohde TD, et al. Risk of osteoporosis in patients treated with long-term intravenous heparin. Curr Probl Surg 1982; 39:419–422
368 Monreal M, Olive A, Lafoz E, et al. Heparins, coumarin, and bone density [letter]. Lancet 1991; 338:706
369 Murphy MS, John PR, Mayer AD, et al. Heparin therapy and bone fractures [letter]. Lancet 1992; 340:1098
370 Dahlman TC. Osteoporotic fractures and the recurrence of thromboembolism during pregnancy and the puerperium in 184 women undergoing thromboprophylaxis with heparin. Am J Obstet Gynecol 1993; 168:1265–1270
371 Barbour LA, Kick SD, Steiner JF, et al. A prospective study of heparin-induced osteoporosis in pregnancy using bone densitometry. Am J Obstet Gynecol 1994; 170:862–869
372 Dahlman TC, Sjoberg HE, Ringertz H. Bone mineral density during long-term prophylaxis with heparin in pregnancy. Am J Obstet Gynecol 1994; 170:1315–1320
373 Monreal M, Lafoz E, Olive A, et al. Comparison of subcutaneous unfractionated heparin with a low molecular weight heparin (Fragmin) in patients with venous thromboembolism and contraindications to coumarin. Thromb Haemost 1994; 71:7–11
374 Muir J, Andrew M, Hirsh J, et al. Histomorphometric analysis of the effects of standard heparin on trabecular bone *in vivo*. Blood 1996; 88:1314–1320
375 Shaughnessy SG, Hirsh J, Bhandari M, et al. A histomorphometric evaluation of heparin-induced bone loss after discontinuation of heparin treatment in rats. Blood 1999; 93:1231–1236
376 Muir JM, Hirsh J, Weitz JI, et al. A histomorphometric comparison of the effects of heparin and low molecular-weight heparin on cancellous bone in rats. Blood 1997; 89:3236–3242
377 Bhandari M, Hirsh J, Weitz JI, et al. The effect of heparin and low molecular weight heparin on osteoblastogenesis *in vitro* [abstract]. Blood 1998; 92: No. 1474