

The background of the slide features a large, circular seal of the Ruprecht-Karls University of Heidelberg. The seal is rendered in a dark blue color and contains intricate heraldic designs, including a central figure, a cross, and various symbols. The Latin motto "SIGILLUM UNIVERSITATIS RUPRECHTI CAROLI HEIDELBERGENSIS" is visible around the perimeter of the seal.

# Clinical dosing of UFH & neutralization of heparin

3rd USP-Workshop  
USP Headquarters, Rockville, USA  
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# Background (1)

Heparin is routinely monitored by activated thromboplastin time (aPTT) methods

After withdrawal blood is prevented from coagulation by addition of 9/1 v/v blood/sodium-citrate (3.1% in US and 3.8% in Europe). Calcium in blood is bound by citrate, sodium is liberated, blood does not clot without calcium.

Blood is centrifuged to obtain plasma free of blood cells, specially free or poor of platelets (platelet poor plasma, PPP).

Plasma is used to determine the activity of UFH on aPTT.

## Background (2)

aPTT reagent is added, including calcium. Time until coagulation is measured in a coagulometer-machine with incubation at human body temperature. Coagulation takes normally 25 to 40 seconds, depending on the aPTT reagent.

The prolongation of the coagulation time of plasma determined by aPTT is routinely used to monitor the amount and the effect of UFH as well as the antagonization of UFH by protamine.

aPTT reagents differ widely in their normal and therapeutic ranges. A 2-3 fold prolongation of the normal aPTT is defined as therapeutic range.

This has been recently seen that impurities in some UFH and LMWH preparations lead to serious problems in patients. Impurities have an impact on the aPTT results.

# Aim of the presentation

- Significance of aPTT for outcome of patients
- Effects of protamine on UFH measured by aPTT
- Impact of impurities in Heparin preparation
- Dosing problems resulting from impurified UFH preparations
- Guidelines for Biosimilar Heparins of the Scientific SubCommittee on Anticoagulation of the International Society of Thrombosis and Haemostasis

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# Relation Between the Time to Achieve the Lower Limit of the APTT Therapeutic Range and Recurrent Events During Heparin Treatment for Deep Vein Thrombosis

Russel Hull et al,



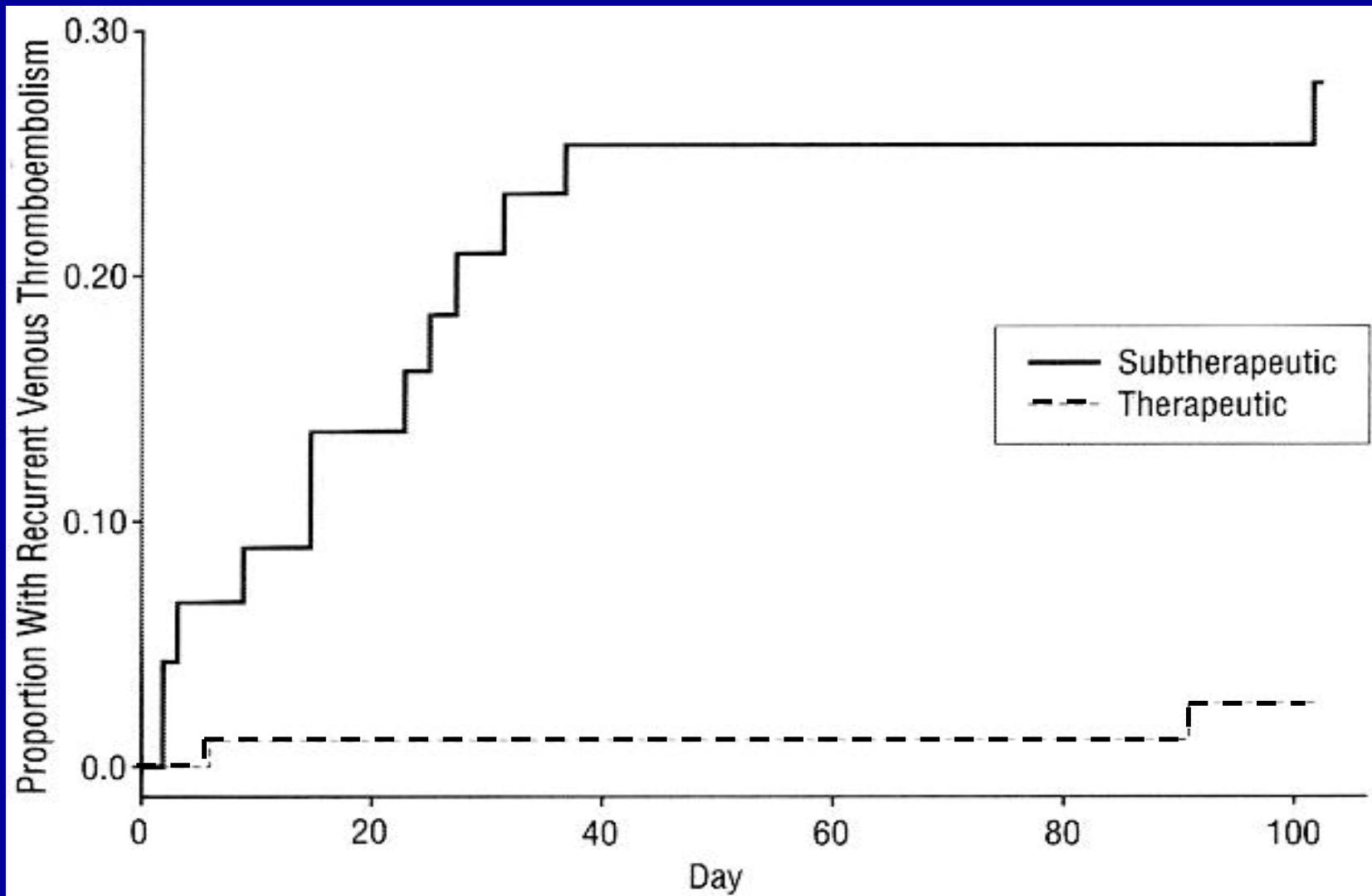
ARCHIVES  
OF  
INTERNAL MEDICINE

1997, 157: 2562-2568

## Hypothesis

risk of recurrent venous thromboembolism is critically dependent on achieving a therapeutic aPTT result (2.3 fold prolongation) at 24 to 48 hours.

Time-to-event analysis of the frequency of recurrent VTE of those patients who were subtherapeutic vs therapeutic by 24 hours ( $P=.002$ ).



# Significance of aPTT for outcome of patients

Russel Hull et al 1997, 157: 2562-2568

Time-to-event analysis shows the increased frequency of recurrent venous thromboembolic events during the period of study in patients who were subtherapeutic for 24 hours compared with those who were therapeutic ( $P=.002$ ).

Failure to achieve a therapeutic aPTT by 24 hours was associated with a 23.3% frequency of venous thromboembolism vs 4% to 6% for those whose aPTT exceeded the therapeutic threshold by 24 hours ( $P=.02$ ).

# Parenteral Anticoagulants

American College of Chest Physicians Evidence-Based Clinical Practice Guidelines  
(8th Edition)

Jack Hirsh, et al *Chest* 2008;133;141S-159S

## Varibales

## Adjustment

Initial dose 80 U/kg bolus,	then 18 U/kg/h as conituous infusion
APTT, 35 s 80 U/kg bolus,	then increase 4 U/kg/h
APTT, 35–45 s 40 U/kg bolus,	then increase 2 U/kg/h
APTT, 46–70 s (2-3 fold prolonged)	No change
APTT, 71–90 s	Decrease infusion rate by 2 U/kg/h
APTT, 90 s	Hold infusion 1 h, then decrease infusion rate by 3 U/kg/h

# Conclusion 1

- The results of aPTT are very important for the beneficial outcome of patients receiving UFH
- The speed of achieving a therapeutic aPTT during initial therapy with UFH are relevant
- Overtherapeutic aPTT are related with bleeding complications (data not shown and not so evident as for benefit of UFH therapy)

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# Use of Protamine to antagonize UFH

- Protamine is a polyarginine protein from fish sperm. Positively charged Arginine binds to negatively charged UFH groups
- Binding is quite specific for heparin, less specific for LMWH and other heparin like polysaccharides
- 100 Units of protamine (1mg) bind 100 units of UFH (0.7 mg)
- The neutralization is measured by aPTT and other assays.
- The amount of iv administered Protamine is calculated by the amount and the time since last UFH administration.
- Protamine has some relevant side effects

# INHIBITION OF UFH and LMWH BY PROTAMINE IN VIVO

J. Harenberg. Et al, THROMBOSIS RESEARCH 38; 11-20, 1985

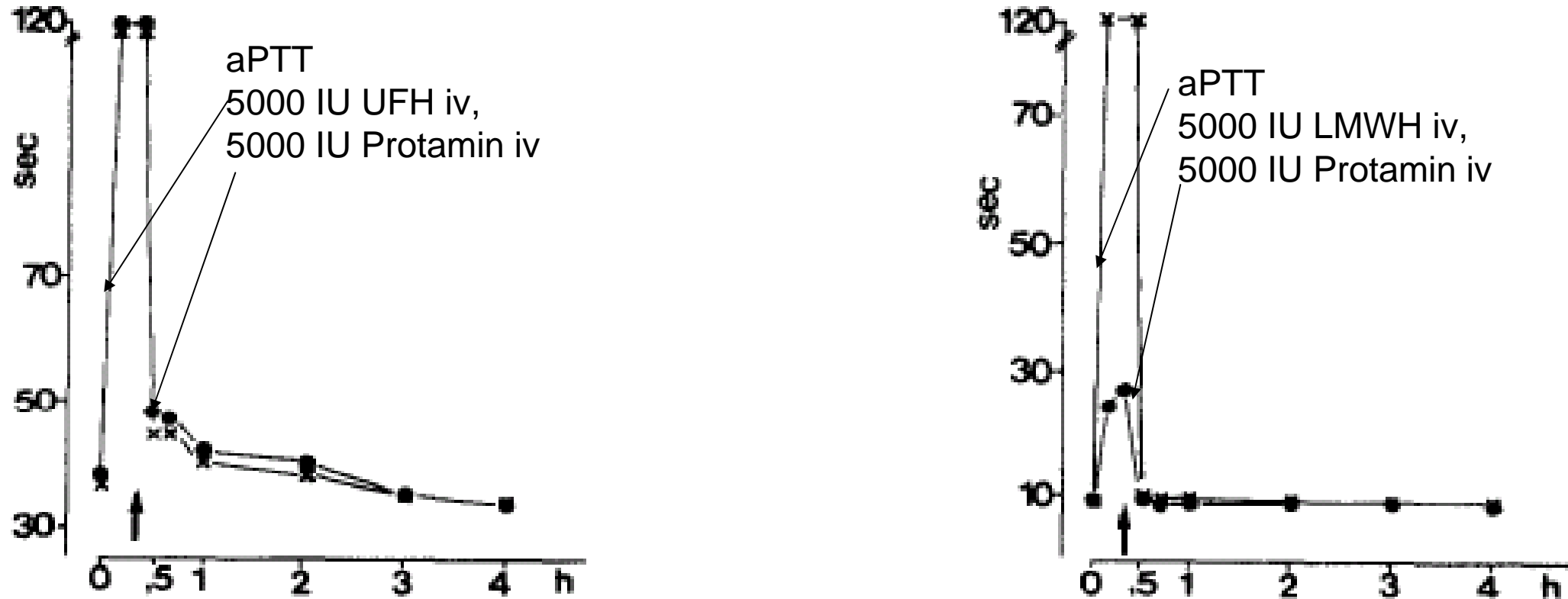


Figure 2 : Effect of protamine chloride on the aPTT after administration of UFH (left) or LMWH (right) (mean values).

# Partial In-Vivo Neutralization of Plasma Anticoagulant Effects of Danaparoid (ORG 10172) by Protamine Chloride

J.C.J. Stiekema, J. Harenberg et al, Thromb Res 1991, 63: 157-67

## Org 10172

Mixture of heparan sulphate, of which a fraction has high affinity for anti-thrombin III (AT-III)

dermatan sulphate

minor amount of chondroitin sulphate.

PT and APTT The PT was slightly and the APTT was moderately prolonged after the intravenous administration of Org 10172. Both were not influenced by the administration of protamine chloride (data not shown).

## Conclusion 2

- aPTT demonstrates the effective antagonization of UFH for patients
- Danapaorid consists of chondrotinisulfates and other heparin-like compounds
- Danaparoid prolongs less effectively aPTT compared to UFH
- Protamine does bind and antagonize only to a small extent if at all chondroitinsulfate and other heparin-like compounds

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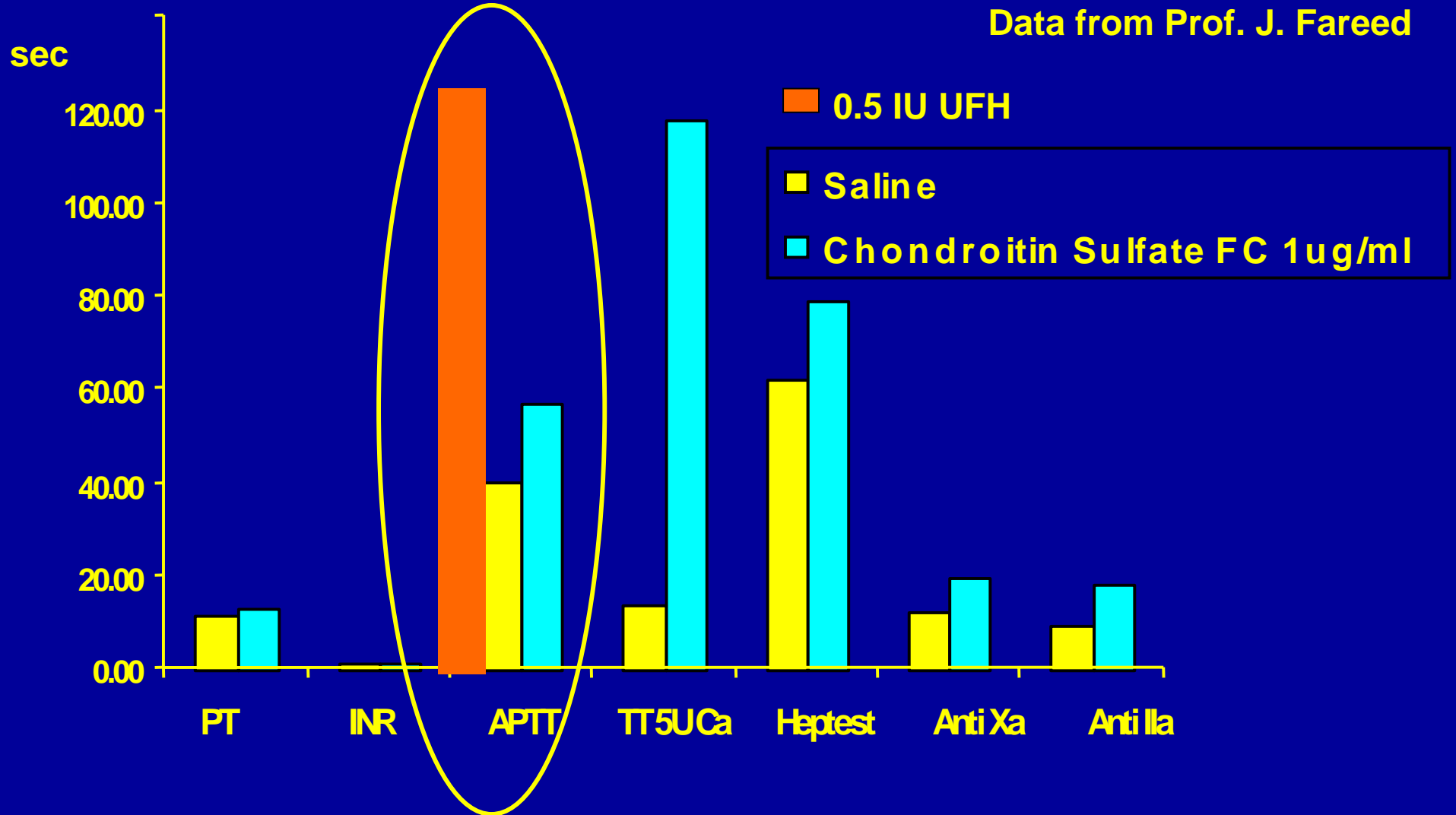
# Characterization of Unfractionated Heparin: Comparison of Materials from the last 50 Years

Barbara Mulloy, Elaine Gray, Trevor W. Barrowcliffe

**Table 2A** Specific activity of international and Chinese national standard for unfractionated heparin

Samples	Source and counter-ion	Specific Activity IU/mg ( 95% confidence limits)		
		Anti-Xa	Anti-IIa	APTT
1st IS	Bovine lung Sodium	136 128 - 144	145 133 - 157	140 131 - 149
2nd IS	Bovine lung Sodium	135 127 - 144	136 125 - 146	139 131 - 147
3rd IS	Porcine mucosa Sodium	176 161 - 191	178 161 - 195	173 160 - 186
Chinese	Porcine mucosa Sodium	174 159 - 189	156 141 - 171	166 153 - 179

# Prolongation of the aPTT by OSCS supplementation (1 microgramm) /ml plasma



## Conclusion 3

- The origin (country and animal tissue) influences the biological activity of UFH
- Impurities such as chondrotinsulfates (oversulfated) in UFH preparations shorten the aPTT compared to pure UFH
- Other impurities in UFH preparations may influence the aPTT prolongation as well
- Impurities in UFH preparations produce side effects (HIT, mortality)

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# Dosing problems resulting from impurified UFH preparations (1)

- **Impurities with reduced activity** compared to UFH (DS, Chondroitins.)
- The administered dose of UFH will always be lower than that declared by the manufacturer
- Patients receive inadequate low dose of UFH
- Patients are at increased risk of recurrent events and of side effects to the impurity
- Amount of protamine will be too high because of the additive effect of UFH by the impurity on aPTT
- Patients are at risk for side effects of protamine (anaphylactic shock)

# Dosing problems resulting from impurified UFH preparations (2)

- **Impurities which increase the activity** of UFH (EDTA, Ca-Chloride, pentasaccharide)
- The administered dose of UFH to patients will always be lower than that declared by the manufacturer
- Patients are at increased risk of recurrent events and of side effects to the impurity
- Amount of protamine will be too high because of the additive effect of UFH by the impurity on aPTT
- Patients are also at risk for side effects of protamine

## Conclusion 4

- Contaminations of UFH preparations have multiple impacts for the safety of patients
- Impurities with low or high anticoagulant effects in USP-heparin preparations should never occur

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**OFFICIAL COMMUNICATION OF THE SSC**

# Recommendations on biosimilar low-molecular-weight heparins

J. HARENBERG,\* A. KAKKAR,† D. BERGQVIST,‡ T. BARROWCLIFFE,§ B. CASU,¶ J. FAREED,\*\* P. MISMETTI,†† F. A. OFOSU,‡‡ W. RAAKE,§§ M. SAMAMA¶¶ and S. SCHULMAN\*\*\* ON BEHALF OF THE SUBCOMMITTEE ON CONTROL OF ANTICOAGULATION OF THE SSC OF THE ISTH

As a result of expiration or pending expiration of patent protection of the originator LMWHs, many generic or biosimilar LMWHs have been approved in some countries and more are likely to be approved elsewhere.

The Working Party on Requirements for Development of Biosimilar LMWHs of the Subcommittee on Control of Anticoagulation, Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) has reached a consensus on recommendations to ensure the quality of biosimilar LMWHs as compared with the originator LMWHs.

# Definitions (based of EMEA and FDA)

## Generics:

Small synthetic drugs like Aspirin reproduced by chemical synthesis

## Biosimilars:

Proteins which are reproduced by purification from tissues or by recombinant synthesis

## Biosimilar Heparins:

Heparins are biological products. They are polysaccharides not fitting to

## USP-bureaus:

Heparins would fit better in biosimilar bureau of the USP

## Conclusion 5

- Based on the heterogeneity of LMWHs, biosimilar LMWHs have to be prepared according to the monograph of the originator LMWH.
- They have to demonstrate their non-inferiority compared with the originator products in preclinical and clinical investigations.
- Simplified pharmacological, pharmacokinetics and clinical studies may be required for biosimilar LMWHs whose compositional profiles and physicochemical properties are similar to those of the originator.