
بسم الله الرحمن الرحیم

Laboratory Monitoring of Anticoagulant medication

Peyman Eshghi MD.

Prof. of Pediatric Hematology and Oncology

Mofid Children`s Hospital, SBMU

Pediatric Congenital Hematologic Disorders Research Center

مرکز تحقیقات بیماری های خونی مادرزادی کودکان

IRSTH

Iranian Society of Thrombosis and Hemostasis

انجمن ترومبوز هموستاز ایران

AIMs of presentation

- **When testing is indicated,**
- **What should be measured,**
- **Which assay should be used, and**
- **How results should be interpreted**
- **Special clinical situations requiring consideration**

<u>“Will measuring the drug change clinical management?”</u>

Classification of Anticoagulants

- **Indirect anticoagulants (antithrombin-dependent)**
  - UFH
  - LMWH
  - Fondaparinux
- **Direct anticoagulants**
  - **Direct thrombin inhibitors:**
    - Oral: Dabigatran
    - Parentral:Argatroban, Bivalirudin,
  - **Direct factor Xa inhibitors:** Apixaban, Rivaroxaban, Edoxaban, Betrixaban
- **Vitamin K antagonists**
  - Warfarin
  - Acenocoumarol
  - Phenprocoumon

Direct Oral Anticoagulants(DOAC)

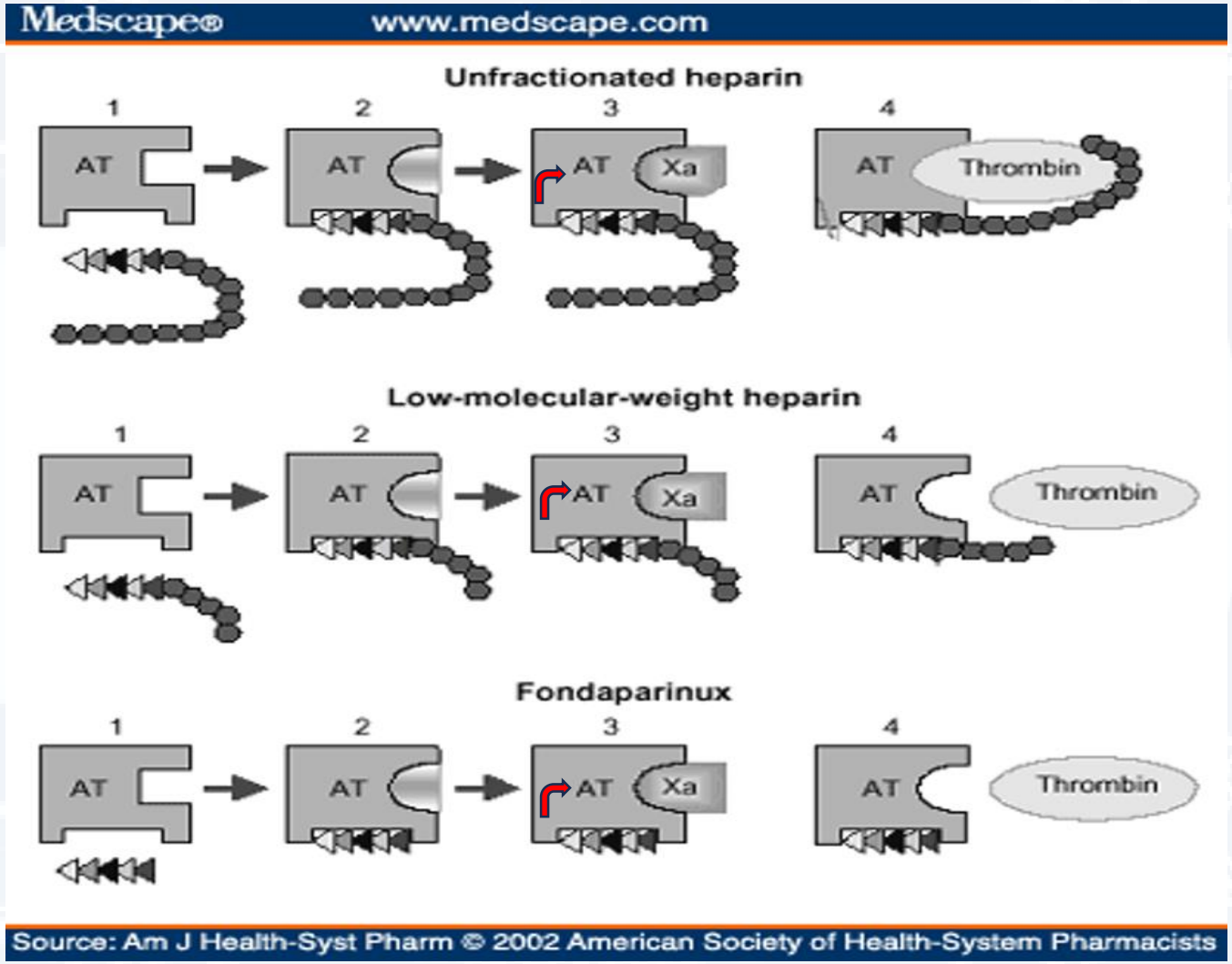
Indirect anticoagulants (antithrombin-dependent)

Anti Xa/Anti IIa activity

UFH : 1/1

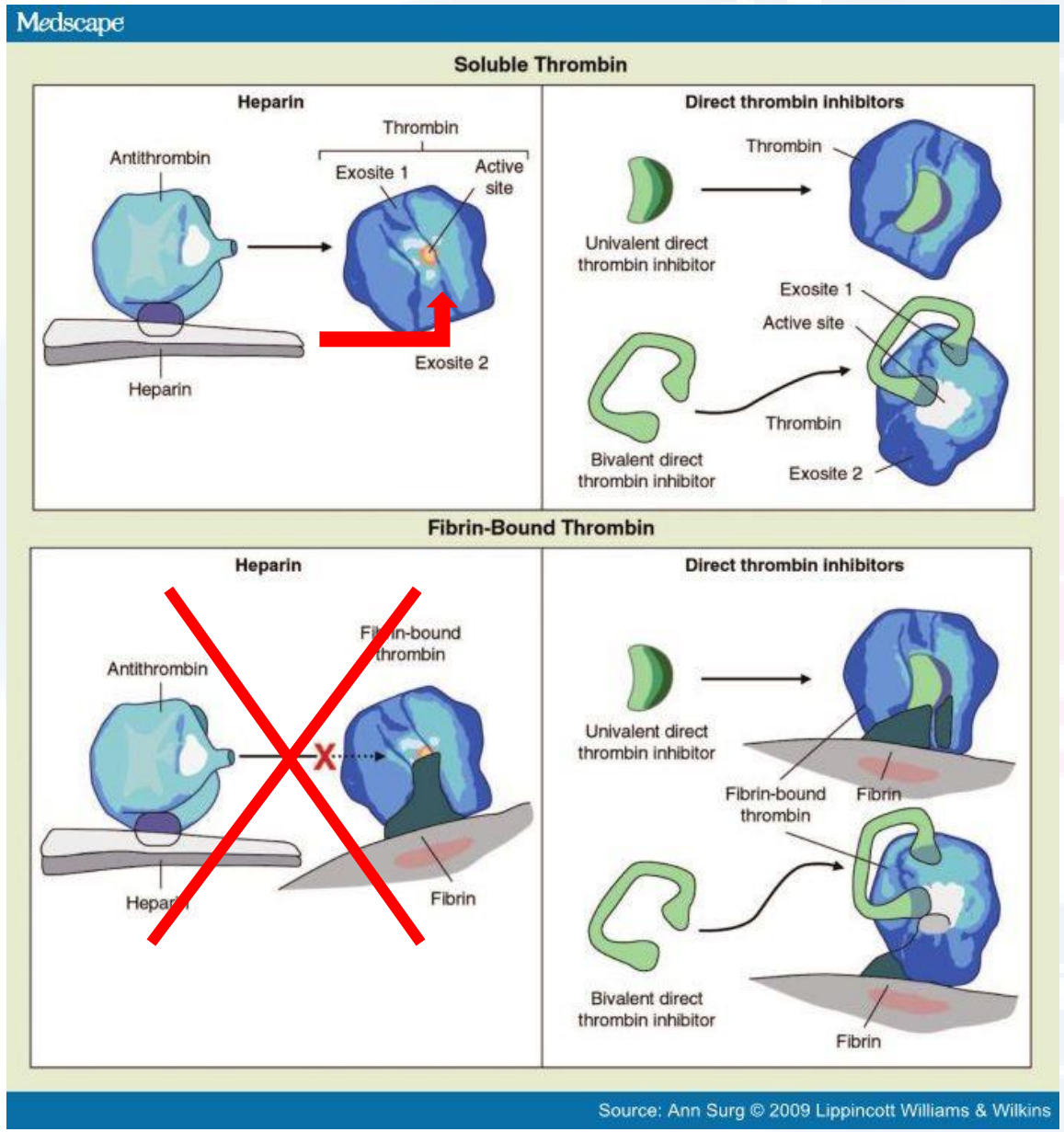
MWH : 3/1

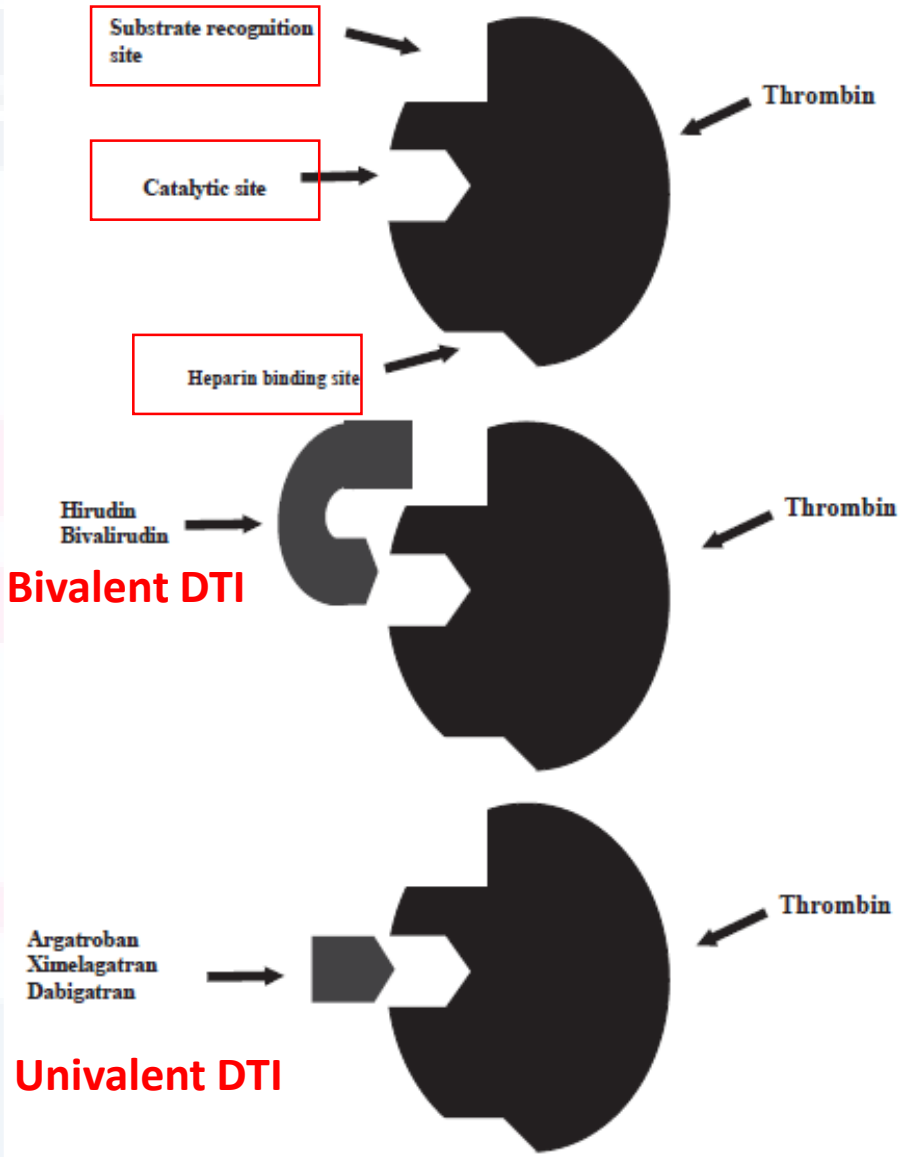
Fondaparinox : 4/0



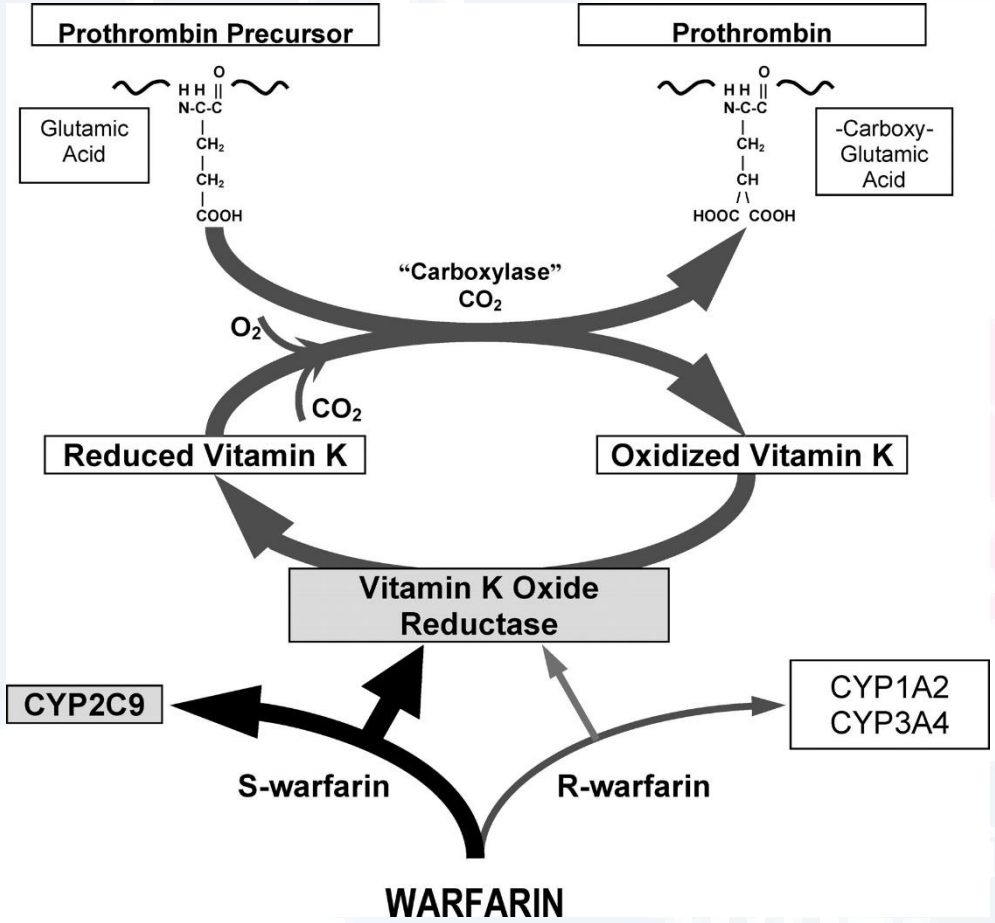
the smaller molecules are unable to form the requisite ternary complex with IIa

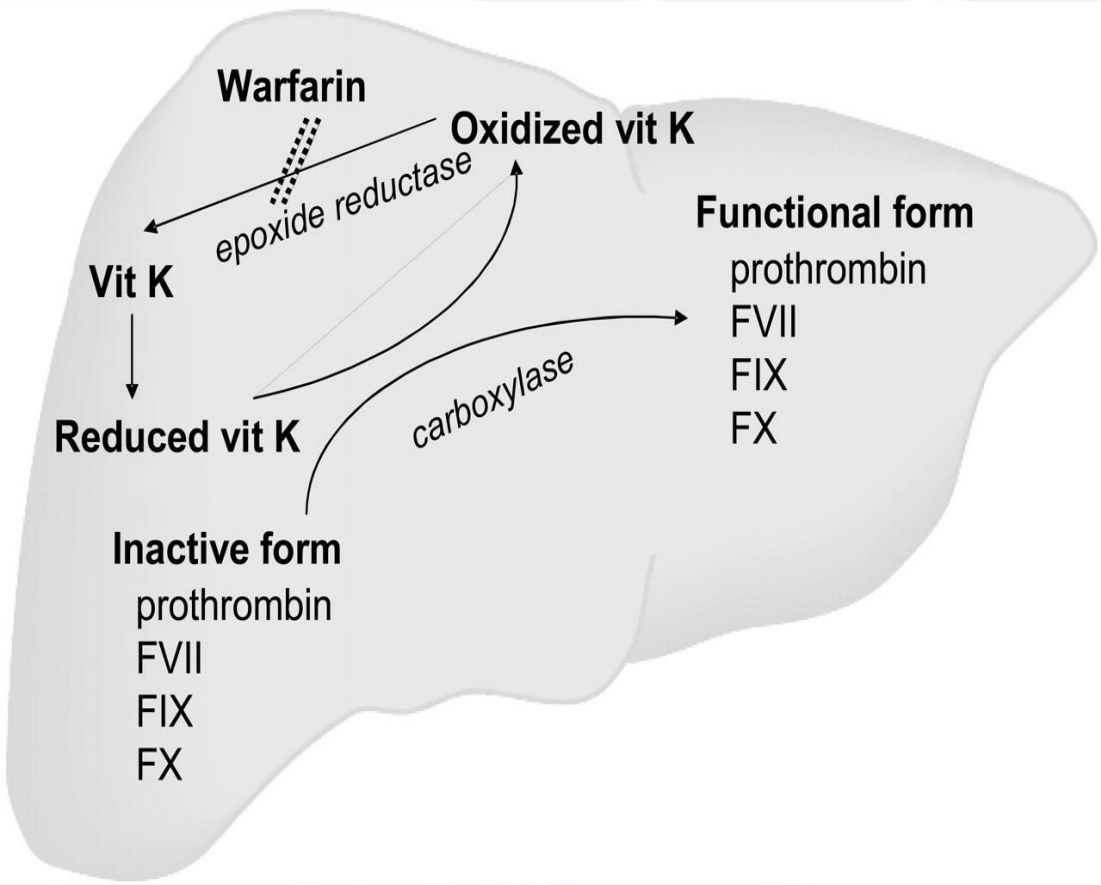
Direct anticoagulants





Vitamin K antagonists

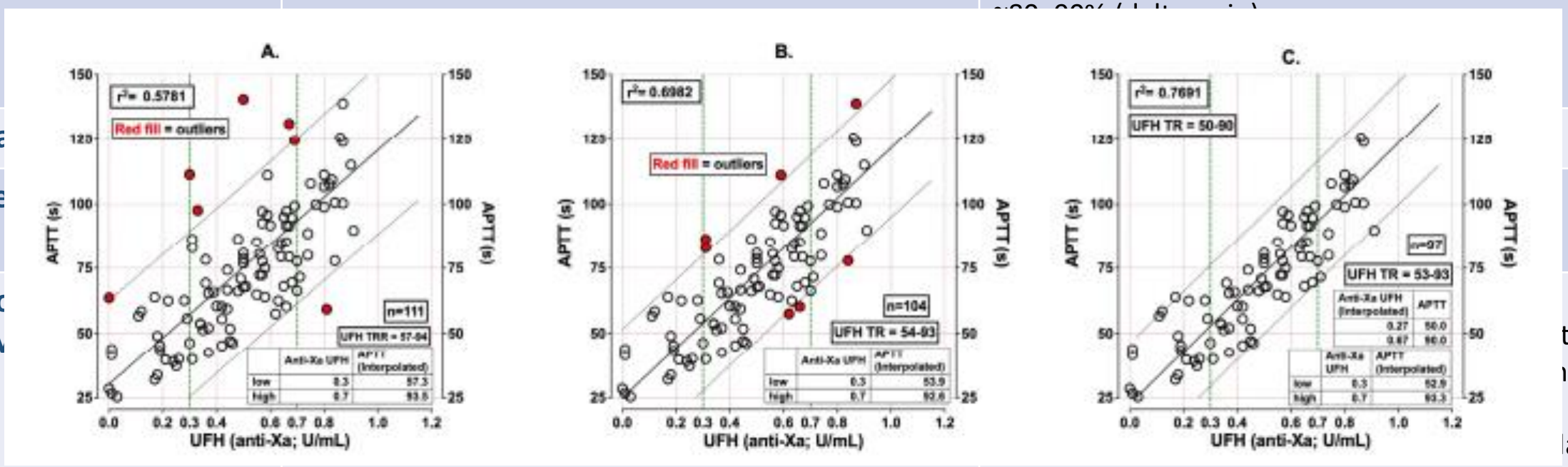




Anticoagulant main indications

Class	Examples	Main clinical uses
Vitamin K Antagonists (VKAs)	Warfarin, Acenocoumarol, Phenprocoumon	AF, **mechanical heart valves**, VTE
Unfractionated Heparin (UFH)	Heparin	VTE, ACS, bridging, ECMO, dialysis
Low-Molecular-Weight Heparins (LMWHs)	Enoxaparin, Dalteparin, Tinzaparin	VTE treatment/prophylaxis, cancer-associated thrombosis
Indirect Factor Xa Inhibitors	Fondaparinux	HIT (off-label in many countries), VTE treatment/prophylaxis
Direct Oral Anticoagulants (DOACs) **[Direct factor Xa inhibitors]**	Factor Xa inhibitors: Apixaban, Rivaroxaban, Edoxaban, Betrixaban*	AF, VTE treatment/prophylaxis
Direct thrombin inhibitor (Oral)	Dabigatran	AF, VTE treatment/prophylaxis
Direct Thrombin Inhibitors (Parenteral)	Argatroban, Bivalirudin	HIT, PCI, cardiac surgery

	UFH	LMWH
Pharmacokinetics	**High protein/endothelial binding** leading to nonlinear, variable kinetics **with ~30% bioavailability**.	**Less non-specific binding** leading to **more predictable PK/PD** with ~90–100% bioavailability (enoxaparin),
Ha		
Be		
Do /M		tremes d renal aily.
	An APTT ratio of 2.0–3.5× the laboratory control is often ,but NOT always associated with therapeutic UFH	***Anti-Xa assay calibrated specifically for the LMWH being used** (e.g., enoxaparin, dalteparin). *PT and aPTT are **not reliable** for monitoring LMWH.
Dosing (Prophylaxis)	**SC**; fixed (2500 IU or 5000 IU BID–TID); OR weight-adjusted; **No routine monitoring**	**SC**; fixed 40 m once daily OR weight-adjusted 0.5 mg/kg once daily. **No routine monitoring**



Benjamin Reardon,et al. Biomolecules 2026, 16, 425

UFH monitoring: aPTT vs anti-Xa

aPTT

- Advantages:
  - Widely available
  - Relatively inexpensive
  - Familiar to clinicians
- Limitations:
  - Acute-phase response
  - Factor VIII elevation
  - Lupus anticoagulant
  - Factor deficiencies
  - Consumptive coagulopathy
  - Liver disease
  - Fibrinogen abnormalities

Anti-Xa

- Advantages:
  - More directly reflects heparin activity
  - Less influenced by many biological variables affecting aPTT
- Limitations:
  - Requires specialized assay
  - **Antithrombin dependence**
  - Preanalytical problems
  - **Assay/reagent variability:** Therapeutic ranges are assay/institution specific and **should be locally validated.**

Anti-factor Xa **assay principle** (chromogenic)

- **Heparin + antithrombin**

↓

- inhibits **Factor Xa**

↓

- Residual Xa acts on chromogenic substrate

↓

- **Color generation inversely correlates with heparin activity :**
  - **More heparin → less residual Xa → less color**
  - **Less heparin → more residual Xa → more color**

- **IU/mL anti Xa actually mean** The **IU of the reference heparin per mL**. **concentration/activity** in that plasma
- **Therapeutic ranges are different because of :**
  - **Assay/institution specific and should be locally validated.**
  - **UFH vs LMWH**
  - **Age specific:(neonates, children and adults)**

When is anti-Xa monitoring preferred over aPTT for UFH?

- Anti-Xa monitoring is particularly useful when:
  - **Baseline aPTT is prolonged** (e.g., lupus anticoagulant, factor deficiency).
  - **Acute-phase reactants** (especially elevated factor VIII or fibrinogen) make aPTT unreliable.
  - There is **heparin resistance** or suspected altered heparin responsiveness.
  - Patients are **critically ill**, on **ECMO**, or have **major inflammatory conditions.**
  - **Pediatric patients**, where anti-Xa often correlates better with heparin effect than aPTT.

Lab monitoring for UFH usage in children and neonates

- **WHEN should we monitor UFH?**
  - For children and neonates, laboratory monitoring should be considered standard whenever UFH is given as a **therapeutic continuous infusion**—UFH is titrated rather than dosed solely by weight(Grade 2C):
  - There is not any **recommended laboratory monitoring for prophylactic UFH**.
- **Which test?**
  - aPTT and anti-Xa correlate **poorly** in children, AND **neither** has been proven superior for predicting bleeding or recurrent thrombosis, therefore: **One assay should be chosen and used consistently** rather than alternating between tests
  - Threshold level:
    - **anti-Xa target of 0.35–0.70 IU/mL**,
    - aPTT range is the institution-specific value and validated against that anti-Xa range(usually but not always about **2-3.5× the laboratory control)**
    - a protamine-titration target of **0.2–0.4 IU/mL**.
  - ***Use a lower threshold for close monitoring in neonates and young infants**, critically ill children, children after cardiac surgery, and patients on ECMO or with major inflammation, consumptive coagulopathy, liver dysfunction, antithrombin deficiency, or rapidly changing physiology.
  - **Infants may require higher UFH doses than older children to reach an anti-Xa goal**.
  - A pediatric cohort found that **aPTT frequently overestimated heparin activity relative to anti-Xa**
- **Monitoring frequency?**
  - After **starting** or **changing** the infusion repeat **every 4 hours** until therapeutic.
  - Once **two consecutive therapeutic values** are obtained repeat **the following day**.

P. Monagle, et al. ASH/ISTH 2024 updated guidelines for treatment of venous thromboembolism in pediatric patients. Blood Advances,27 MAY 2025 • VOLUME 9, NUMBER 10.

P. Monagle, et al. Antithrombotic Therapy And Prevention Of Thrombosis, 9th Ed: ACCP Guidelines. CHEST / 141 / 2 / FEBRUARY, 2012 SUPPLEMENT

Lab monitoring for LMWH usage in children and neonates

- UNLIKE ADULTS, the recent guidelines recommends **routine anti-Xa monitoring in children**
- **Therapeutic-dose LMWH in any child: I**n a **first time** sample taken **4 h** (acceptable range **3.5–6 hours**) **after** the **2nd or 3rd** subcutaneous LMWH dose injection ,should reach to **0.5 to 1.0 units/mL (Grade 2C) .**
  - the ideal therapeutic anti-Xa range has not been conclusively validated in pediatrics,
  - anti-Xa assays show significant interlaboratory variability,

Frequency of monitoring

NOTE:

Always specify whether the assay is calibrated for LMWH AND
which dosing regimen is being used , (once daily vs twice daily) AND
timing of level in relation to last administered dose **(peak or trough levels).**

- **Renal impairment**
- **Extremely BW:Obesity , sever malnourished ,** Very low body weight
- Criticaly ill and **High bleeding risk OR unexpected bleeding events OR Recurrent thrombosis despite therapy**
- **Patients receiving interacting therapies : ECMO,CRRT,etc.**

- Routine monitoring in **prophylaxis** is **less consistently required**, except in some of the above situation.
  - **Stable older children on standard prophylactic LMWH with normal renal function often do not require routine anti-Xa monitoring.**

P. Monagle, et al. ASH/ISTH 2024 updated guidelines for treatment of venous thromboembolism in pediatric patients. Blood Advances,27 MAY 2025 • VOLUME 9, NUMBER 10.

P. Monagle, et al. Antithrombotic Therapy And Prevention Of Thrombosis, 9th Ed: ACCP Guidelines. CHEST / 141 / 2 / FEBRUARY, 2012 SUPPLEMENT

Heparin Resistance

Definitions:

- Minimal changes in **APTT** or **ACT,** or subtherapeutic **anti-Xa levels,** despite standard or increased UFH dosing (e.g., **35,000 or more** units per day**)** within **24 h** of heparin infusion. **[although the threshold dose required is not well defined]**
- Cardio-Pulmonary Bypass (CPB): the need for a dose of **over 500 U/kg** to achieve an **ACT of 400 to 480 s**

Table 4. Causes of heparin resistance and management considerations.

Mechanism	Pathophysiology	Diagnostic Clues	Management Considerations
Antithrombin deficiency	Reduced AT-dependent UFH effect	Anti-Xa low despite high dose of heparin; low AT activity	AT concentrate or FFP; continue UFH or switch to direct thrombin inhibitor (DTI)
Acute-phase proteins (increasing heparin binding)	Neutralization by FVIII, fibrinogen, VWF	APTT discordant with anti-Xa; high levels of acute-phase reactants	Titrate UFH by anti-Xa
PF4 neutralization	PF4 binds heparin, reducing activity	High platelet counts; clinical context	Manage underlying condition; consider alternative agents
Andexanet alfa exposure	Alters Xa pathway; assay interference	Clinical context/recent DOAC reversal	Use APTT temporally; delay anti-Xa-based UFH titration
Pseudo-resistance	Line/phlebotomy issues; sampling errors	Inconsistent laboratory tests vs. clinical picture	Re-collection from fresh venipuncture site; check quality control

APTT, activated partial thromboplastin time; AT, antithrombin; DOAC, direct oral anticoagulant; FFP, fresh-frozen plasma; PF4, platelet factor 4; VWF, von Willebrand factor; UFH, unfractionated heparin.

Warfarin: the classic monitored anticoagulant

- VKAs **have a very narrow therapeutic index**, and patients require frequent blood monitoring to ensure therapeutic anticoagulation and to minimize the risk of bleeding.
- The blood monitoring test for warfarin therapy is the **INR** ,because thromboplastin reagents differ in sensitivity
  - **INR = (Patient PT / Mean Normal PT)^ISI** (CLSI guidance validation and local calibration)
- The age group found to be most susceptible to fluctuations in INR are **children aged** :Infants and small children require very regular INR tests (on average, **2 tests per week**) compared with **once-a-month testing in most adult patients.**
- **INR is highly useful for warfarin and** reflects the effect of vitamin K antagonism primarily through vitamin K-dependent clotting factors.**—but not for DOAC quantification**
- In patients with **APS on warfarin**, the INR may not be representative of true anticoagulation intensity due to an **interaction between LA and the thromboplastin reagent** used in the INR determination
- **Point-Of-Care (POC) tests** are reliable, accurate INRs versus standard venous INRs in **general population** ,however Interpret with **caution in APS patients** due to discordance with venous INRs

DOACs:
role of anti-Xa and drug level monitoring

Siraj Mithoowani1 and Deb o rah Siegal. Hematology 2024 | ASH Education Program

- Unlike warfarin, DOACs have relatively **predictable** pharmacokinetics, **short-half** lives, and a **wide therapeutic index**, and they are prescribed in fixed doses **without the need for routine monitoring**.
- **o**

	MOA target	Oral bioavailability (%)	T Max (h)	Half-life (h)	Renal excretion (%)	Documented pediatric interactions
Apixaban	Xa	50	3–4	9–14	26	P-gp substrates, CYP 3A4[b]
Dabigatran	Thrombin	7	0.5–2	12–17	80	P-gp substrates
Edoxaban	Xa	62	1–2	10–14	50	Rifampin
Rivaroxaban	Xa	66–100	2–4	5–11	66	P-gp substrates, CYP 3A4

- **Drug + dose + time of last dose + renal function + time of blood sampling** is much more informative for Lab. Interpretation than drug concentration level:
  - For example: Apixaban 5 mg BID / Last dose: 08:00 / Blood sample: 14:00 / CrCl: 35 mL/min is more clinically informative than “Apixaban level = 85 ng/mL”
    - **Drug concentration and anticoagulant effect are not necessarily interchangeable.**

DOACs:
role of anti-Xa and drug level monitoring

Siraj Mithoowani1 and Deb o rah Siegal. Hematology 2024 | ASH Education Program

- **“No routine monitoring” ≠ “No needed laboratory testing.”**

- **The biggest mistake: using PT/aPTT to measure DOAC effect:**
  - **Normal PT/aPTT** does **NOT** reliably **EXCLUDE** clinically relevant DOAC activity**.**
  - **Abnormal PT/aPTT** does **NOT** reliably **QUANTIFY** DOAC concentration**.**
- **Drug-specific calibrated chromogenic anti-Xa assay is** Preferred quantitative method:
  - Report as **ng/mL** rather than simply: **IU/mL**
  - **The anti-Xa assay is a method/platform—not a single universal test- which needs specific calibration.**
  - A conventional **LMWH-calibrated anti-Xa** result:
    - should not automatically be interpreted **as an apixaban or rivaroxaban concentration.**
    - **MAY BE USEFUL AS EMERGENCY SCREENING when drug-specific calibration is unavailable**, but this is not equivalent to validated **QUANTITATIVE** DOAC measurement**.**
- **Sample time:**
  - **Peak sample** Usually approximately: **2–4 h after dose**
  - **Trough sample :**Immediately before next dose

How to interpret coagulation tests in patients treated with DOACs

Table 2. Practical approach to interpreting lab tests in the presence of DOACs

Test	Result	Interpretation: is clinically significant anticoagulant effect present?			
		Dabigatran	Apixaban	Rivaroxaban	Edoxaban
Routine coagulation tests – qualitative assessment					
PT/INR	Normal	← POSSIBLY PRESENT →			
	Abnormal	← LIKELY PRESENT →			
aPTT	Normal	← POSSIBLY PRESENT →			
	Abnormal	← LIKELY PRESENT →			
Thrombin time	Normal	NOT PRESENT	← Not relevant →		
	Abnormal	PRESENT			
Heparin or LMWH	<0.1 IU/mL	Not relevant	← LIKELY ABSENT →		
Anti-Xa assay	>0.1 IU/mL		← LIKELY PRESENT →		
Specialized DOAC assays—quantitative assessment					
Drug-specific quantitative assay	Concentration	dTT (Hemoclot®), ECT	Drug-specific anti-Xa activity assay		
	<50 ng/mL	← LIKELY ABSENT →			
	≥50 ng/mL	← LIKELY PRESENT →			

anti-Xa, anti-Xa activity assay; dTT, dilute thrombin time; ECT, ecarin clotting time.

Siraj Mithoowani1 and Deb o rah Siegal. Hematology 2024 | ASH Education Program

Why and When to measure DOACs drug level?

Scenario	Rationale	Timing of measurement	Potential impact on clinical decision-making
GOAL: To assess whether a clinically significant DOAC level is likely to be present in an emergency requiring normal hemostasis			
Prior to an urgent invasive procedure with high bleeding risk	Based on limited data, a DOAC drug level <30 ng/mL is unlikely to warrant anticoagulant reversal for patients requiring an urgent procedure associated with a high risk of bleeding.[27]	Random sample for drug level drawn immediately prior to the procedure.	• Use of anticoagulant reversal agent. • Timing of surgery/procedure. • Use of neuraxial anesthesia.
Serious bleeding (Ongoing)	Based on limited data, a DOAC drug level <50 ng/mL is unlikely to warrant anticoagulant reversal for patients with severe bleeding.[27]	Random sample for drug level drawn at presentation with serious bleeding.	• Use of anticoagulant reversal agent. (Adnexanate alfa)
Prior to systemic thrombolysis for acute stroke	Observational data suggest a low risk of intracranial hemorrhage among patients treated with systemic thrombolysis and a DOAC drug level <20–50 ng/mL.[30–32] US and European guidelines recommend against use of systemic thrombolysis in patients who ingested a DOAC within the last 48 hours.[56,57]	Random sample for drug level drawn at presentation with acute stroke.	• Use of intravenous thrombolysis or alternatives (mechanical thrombectomy). • Surgical approaches.

Why and When to measure DOACs drug level?

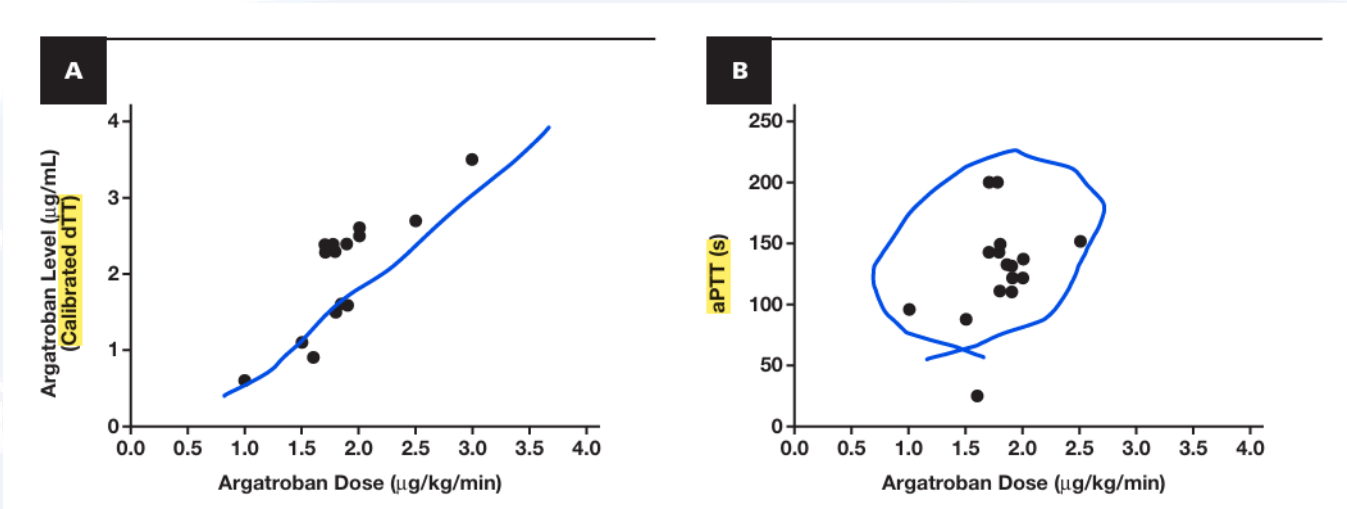
Scenario	Rationale	Timing of measurement	Potential impact on clinical decision-making
GOAL: To assess for excessively low DOAC levels (below on-therapy range)		Non-responsive or Unexpected progressive TE	
Malabsorptive gastrointestinal surgery	DOACs are variably absorbed in the stomach and the proximal gastrointestinal (GI) tract. GI cancer or bariatric surgery may reduce DOAC absorption by altering GI motility, reducing stomach acidity, or disrupting the intestinal absorptive surface.[40] Patients taking dabigatran or who have undergone highly malabsorptive procedures (such as Roux-en-Y gastric bypass) appear to be at greatest risk of malabsorption.[34]	Sample for drug level drawn at peak (1–4 hours after drug ingestion), and/or at trough (just before the next dose is due).[22,40] Drug level should be measured after at least 5 doses to ensure steady state is achieved.	• Engage in shared decision-making with the patient or caregiver on switching DOACs based on site of impaired absorption and remeasuring drug level, or switching to a VKA.[41]
Pharmacokinetic drug-drug interactions	Coadministration of DOACs with strong inducers of p-gp and CYP3A4 (eg, carbamazepine) can decrease DOAC drug levels and may reduce their efficacy[60] (although high quality data regarding clinical outcomes are lacking).	Sample for drug level drawn at peak (1–4 hours after drug ingestion) and after at least 5 doses to ensure drug level is checked at steady state.	• Change treatment based on drug interaction profile.
Suspected therapeutic failure	Nonadherence to long-term anticoagulation therapy is common (impacting around 15% of patients initiated on a DOAC for atrial fibrillation)[61] and associated with a higher risk of thromboembolism. Therefore, checking for adequate drug exposure may be indicated in the workup of a new thromboembolic event sustained while taking a DOAC.	Random sample for drug level drawn at the time of diagnosis of the thromboembolic event.	• Assess and optimize risk factors for nonadherence if applicable (eg, switch from twice daily drug to once daily), or switch anticoagulant (eg, to a VKA with regular INR monitoring).

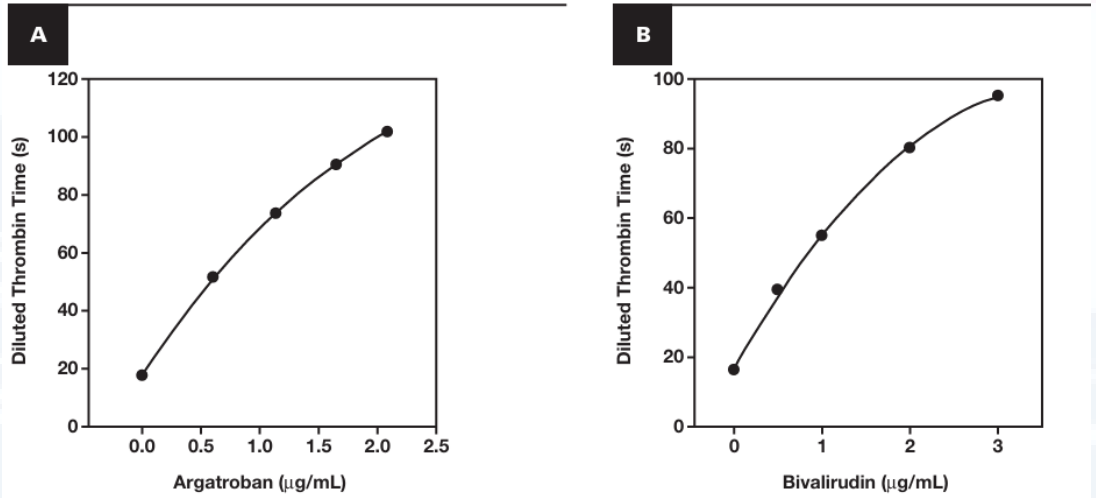
Why and When to measure DOACs drug level?

Scenario	Rationale	Timing of measurement	Potential impact on clinical decision-making
GOAL: To assess for excessively high DOAC levels (above on-therapy range)			
Anticoagulant overdose	The finding of a very high DOAC drug level can confirm a diagnosis of DOAC overdose where the history is unclear.	Random sample for drug level +/− serial drug measurements.	• Reversal agents are not indicated in the absence of bleeding or surgery.[58] • Serial drug level measurements can be used to calculate elimination half-life and inform when the patient is no longer at excess risk of bleeding.
Severe chronic kidney disease (CKD)	There is uncertainty on the appropriate use and dosing of DOACs in severe renal insufficiency. Phase III trials of DOACs for atrial fibrillation and VTE excluded patients with a creatinine clearance (CrCl) <25–30 mL/min.	Sample for drug level drawn at trough, just before the next dose is due, and after at least 5 doses to ensure drug level is checked at steady state.	• Engage in shared decision-making with the patient or caregiver on alternative options, including switching to a VKA • Plan duration of DOAC interruption for elective surgery/procedure.
Pharmacokinetic drug-drug interactions	Apixaban and rivaroxaban are substrates of cytochrome P450 3A4 (CYP3A4), and all DOACs are substrates of p-glycoprotein (p-gp). Strong inhibitors of CYP3A4 and/or p-gp can increase DOAC drug levels and may increase risk of bleeding[59] (although high quality data are lacking regarding clinical outcomes).	Sample for drug level drawn at trough (just before the next dose is due) and after at least 5 doses to ensure drug level is checked at steady state.[22]	• Change therapy based on drug interaction profile.

Monitoring Direct Thrombin Inhibitors

- **Qualitative (aPTT) assays:**
  - Used in most settings due to widespread availability
  - Full anticoagulation target is **1.5 to 3 times** normal aPTT values;
  - The accuracy of aPTT is diminished at higher DTI concentrations, creating a **"plateau" effect** despite increasing doses.
- **Quantitative calibrated assays for DTI concentrations:**
  - The ecarin clotting time (ECT) AND ecarin chromogenic assay (ECA):
    - Expensive and not available standard kits
  - Chromogenic anti–factor IIa:
    - limited by lipemia and elevated bilirubin
  - Diluted thrombin time (dTT) :
    - Reliable in predicting DTI levels for **both intravenous and oral DTIs** even in lupus inhibitors, vitamin K factor deficiencies, and elevated D-dimers (unlike aPTT which overestimate drug concentrations)





Rida A. Hasan, MD, Am J Clin Pathol 2023;159:60-68

Drug/class	Main target	Routine test	Preferred specialized test
UFH	IIa/Xa	aPTT or anti-Xa	Heparin anti-Xa
LMWH	Xa > IIa	Usually none	LMWH anti-Xa
Warfarin	II, VII, IX, X	**PT/INR**	—
Dabigatran	IIa	None	dTT / ECT / TT
Rivaroxaban	Xa	None	Drug-calibrated anti-Xa
Apixaban	Xa	None	Drug-calibrated anti-Xa
Edoxaban	Xa	None	Drug-calibrated anti-Xa
Argatroban	IIa	aPTT	Specialized assays in selected cases
Bivalirudin	IIa	aPTT/ACT	Specialized assays

Take-home messages

- **1. UFH :**Monitor with **aPTT or anti-Xa**, using a validated institutional approach.
- **2. LMWH :**Routine monitoring is unnecessary for most patients, but **anti-Xa can be useful in selected high-risk populations**, particularly in pediatrics.
- **3. Warfarin :INR remains the standard monitoring test.**
- **4. DOACs :No routine monitoring does not mean no laboratory assessment.**
  - **Xa inhibitors :**Use **drug-calibrated anti-Xa assays** when quantitative assessment is clinically required.
  - **Dabigatran :**Use **dTT/ECT or other validated thrombin-based assays** when measurement is necessary.
  - **PT/aPTT:**Useful as screening tests in selected contexts, but **not reliable quantitative measures of DOAC concentration.**
- **5.The laboratory result is only useful if it answers a clinical question.**

- **Why do we need the laboratory?**
  - Warfarin → requires routine laboratory-guided dose adjustment & monitoring
  - UFH → requires laboratory-guided dose adjustment
  - LMWH → usually **more predictable**, but monitoring may be useful in **selected patients**
  - DOACs → **no routine monitoring**, but laboratory assessment may be crucial in **selected situations :** Specialized testing can be useful in emergencies and selected high-risk situations.
  - Newer anticoagulants → increasingly important laboratory challenges
- “No routine monitoring” ≠ “No needed laboratory testing.”
- Drug concentration and anticoagulant effect are not necessarily interchangeable.