폐색전증을 진료하는 의사가 알아야할 thrombophilia test

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1. Questions for coagulation tests
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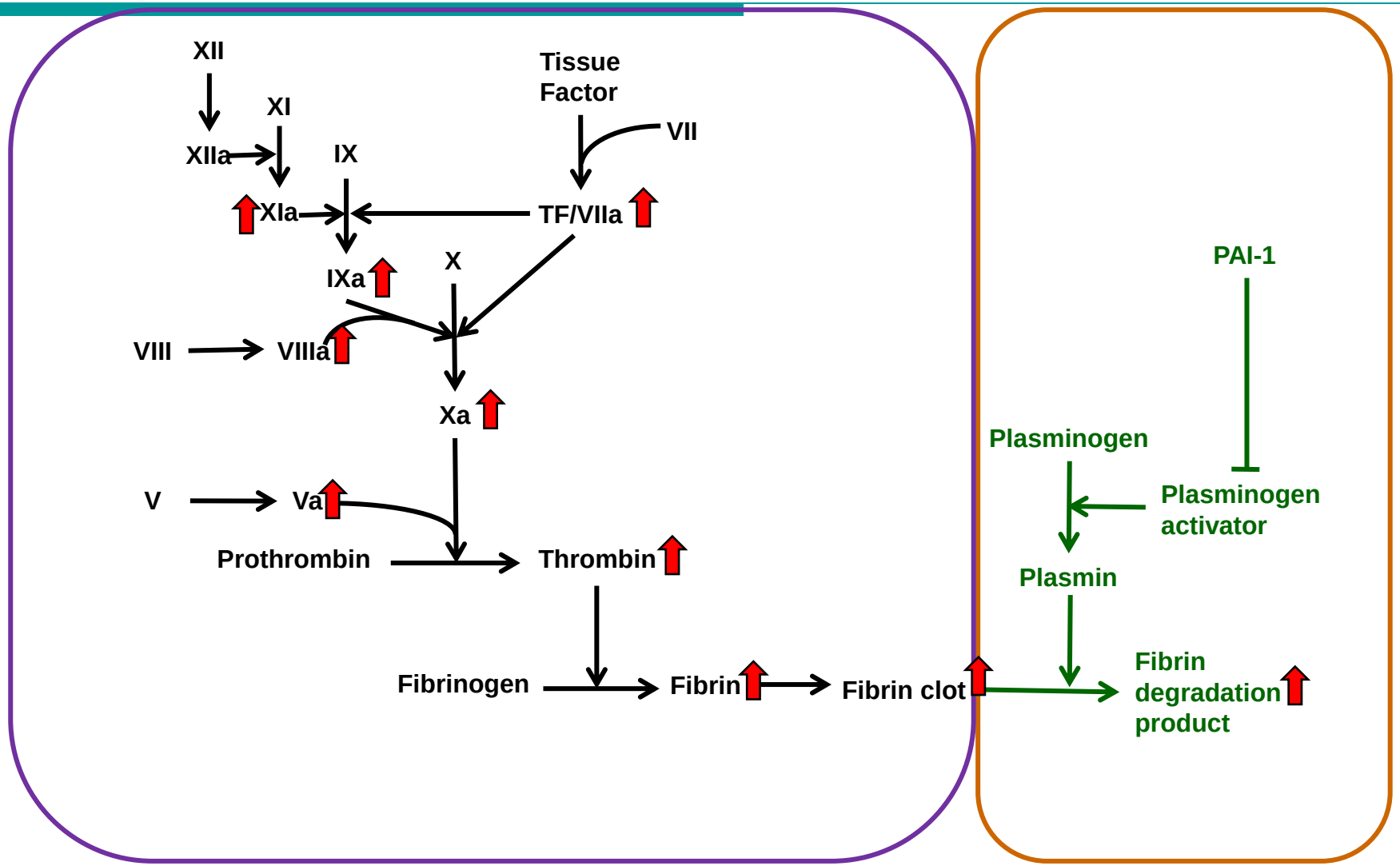
Anticoagulation, coagulation tests…

- UFH 투여를 시작한 환자인데, 투여량을 늘려도 aPTT가 충분히 연장되지 않습니다…
- APTT 외에 UFH의 다른 모니터링 방법은?…
- Heparin resistance가 의심되는데 다음에는 무엇을 …
- ICU 입실후 지속적으로 thrombocytopenia 발생하여, anti-heparin/PF4 검사를 했는데 양성이 나왔습니다. HIT…?
- HIT가 의심되어 heparin 대신 argatroban으로 바꾸었습니다. 모니터링은 어떻게…
- 와파린을 쓰다가 rivaroxaban으로 바꾸었는데 INR 이 5가 넘어요…
- A. fib이 있어 와파린을 쓰는 환자에서 원인을 찾기 위해 PC, PS, AT 검사를 하고 싶습니다…
- PE이 있어 rivaroxaban 쓰는 환자에서 LA 검사를 했는데, 양성이 나왔습니다. APS…?
- 와파린 복용중인 환자가 약 용량 변화 없이 갑자기 INR이 올라갔습니다…

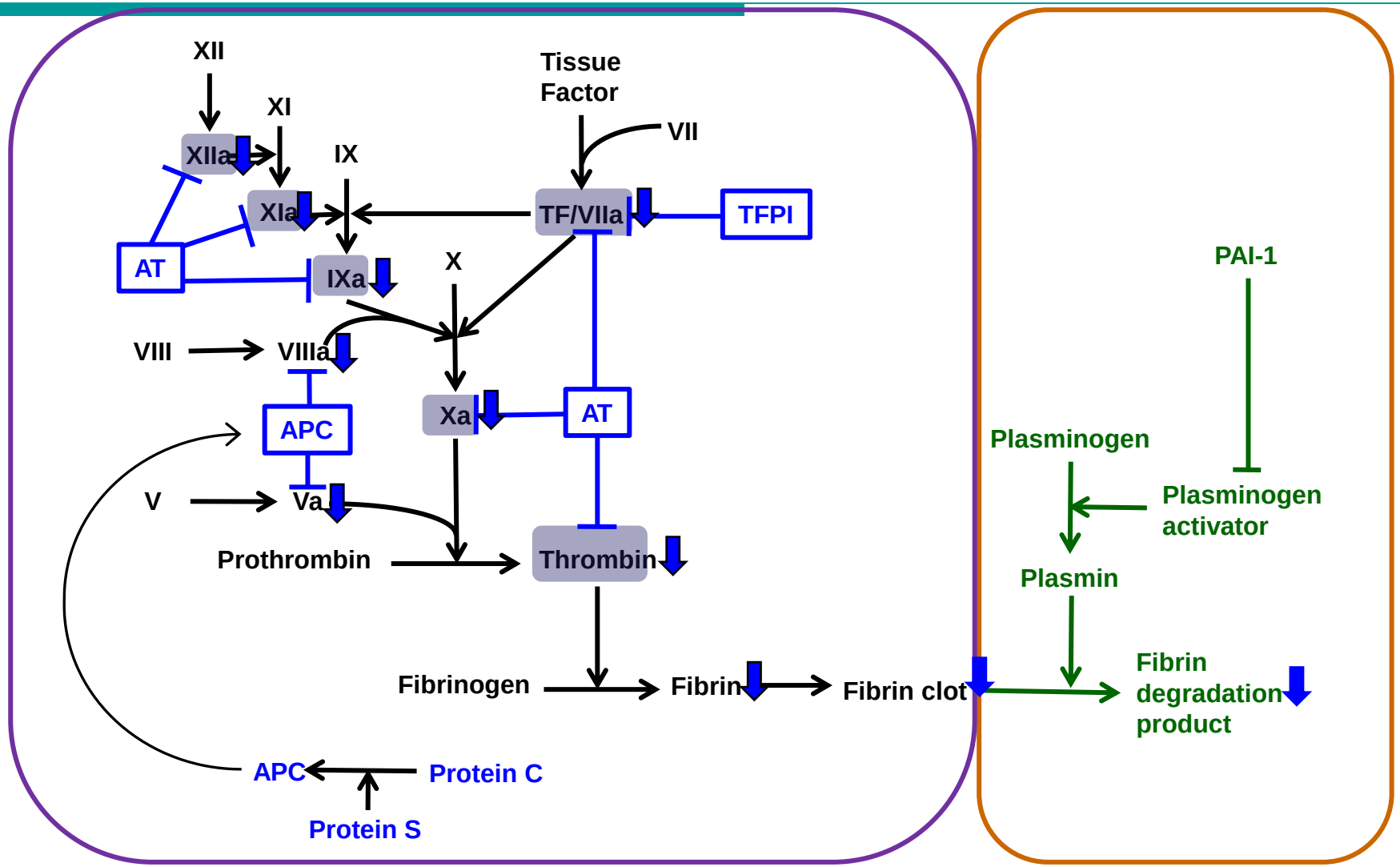
COAGULATION CASCADE

Coagulation cascade

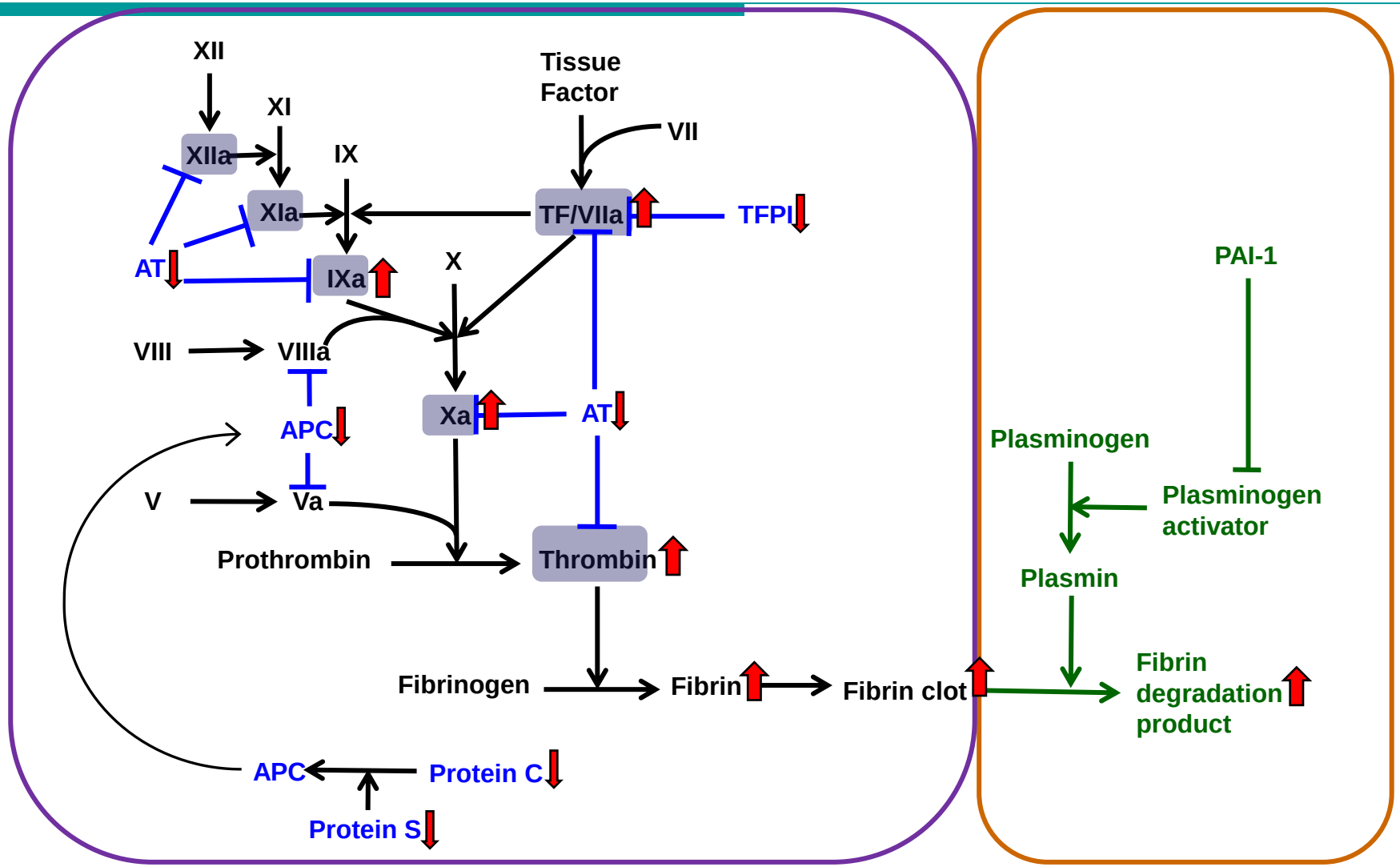
Stimuli to produce
Tissue Factor



Natural anticoagulants



Primary thrombophilia



THROMBOPHILIA AND LABORATORY TESTS

Thrombophilia

- **Primary**
  - Natural anticoagulant deficiency
    - Protein C (PC) deficiency
    - Protein S (PS) deficiency
    - Antithrombin (AT) deficiency
  - Increased prothrombotic factors
    - Factor V Leiden mutation
    - Prothrombin G20210A mutation
    - Factor VIII, vWF, …
- **Secondary**
  - Antiphospholipid syndrome (APS)
  - Myeloproliferative neoplasm (MPN)
  - Paroxysmal nocturnal hemoglobinuria (PNH)
  - Heparin induced thrombocytopenia (HIT)
  - Cancer, Pregnancy, OCs, HRT,…

Useful Coagulation tests

- For anticoagulants monitoring
  - **aPTT : heparin, argatroban**
  - **PT (INR) : Warfarin**
  - **Anti-Xa : Heparin, LMWH**
  - ACT, TEG : heparin
- For anti-platelet drug
  - Platelet aggregation test, VerifyNow, PFA-100
- For HIT
  - Anti-PF4 EIA test
- *For DOACs*
  - **Prothrombin time modification?**
  - ***Anti-Xa test** : Rivaroxaban, Apixaban, Edoxaban*
  - ***Ecarin clotting assay, diluted TT** : Dabigatran*
- For bleeding disease
  - Factor assay, vWF antigen, Ristocetin cofactor assay
- For thrombosis
  - D-dimer/FDP
  - Protein C, Protein S, AT
  - LA, b2GPI, ACA
- For DIC
  - Platelet, PT, D-dimer/FDP, fibrinogen
- For inflammation
  - CRP, fibrinogen, factor VIII, vWF

Diagnostic tests for thrombophilia

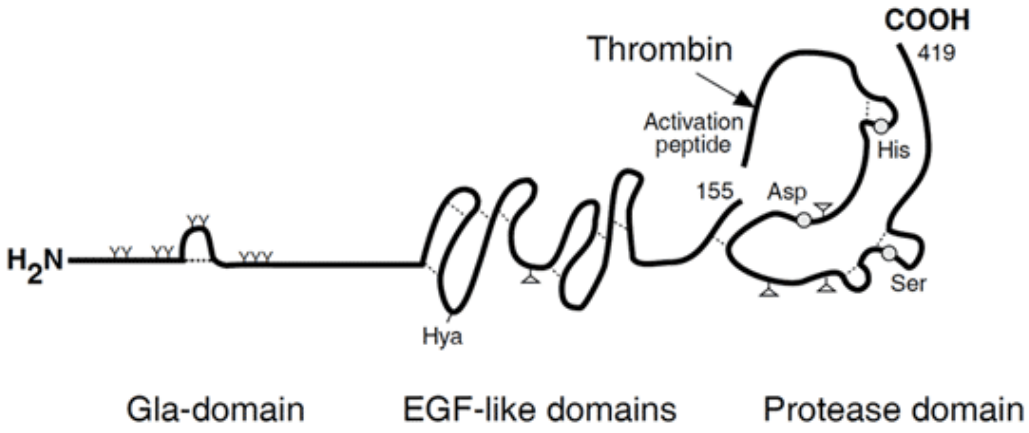
- Routine tests
  - PT/aPTT, D-dimer/FDP, CBC,
  - Fibrinogen, Factor VIII, vWF, CRP…
- For primary (natural anticoagulant deficiency)
  - PC, PS, AT functional and/or antigenic level
  - Mutation study, if needed
- For secondary
  - APS - Lupus anticoagulant, ACA, ß2-GPI
  - MPN – CBC, *JAK2* and/or *CALR* mutation
  - PNH - LDH, CBC, flowcytometry (CD55, CD59)
  - HIT - Heparin Hx (+), PF4 antibody, functional assay

Considering points for lab tests

- Past history
  - **Anticoagulants therapy**
    - Warfarin treatment vs protein C, protein S deficiency
      - Stopping anticoagulation? (for protein C, protein S deficiency)
    - Direct oral anticoagulants and LA, protein S (functional), …
    - Heparin Hx for LA test and for HIT
  - **Time point of event**
    - Consumptive decreased?
- Laboratory tests
  - Acute phase reactants : fibrinogen, prothrombin, factor VIII, vWF
- Careful interpretation is needed!

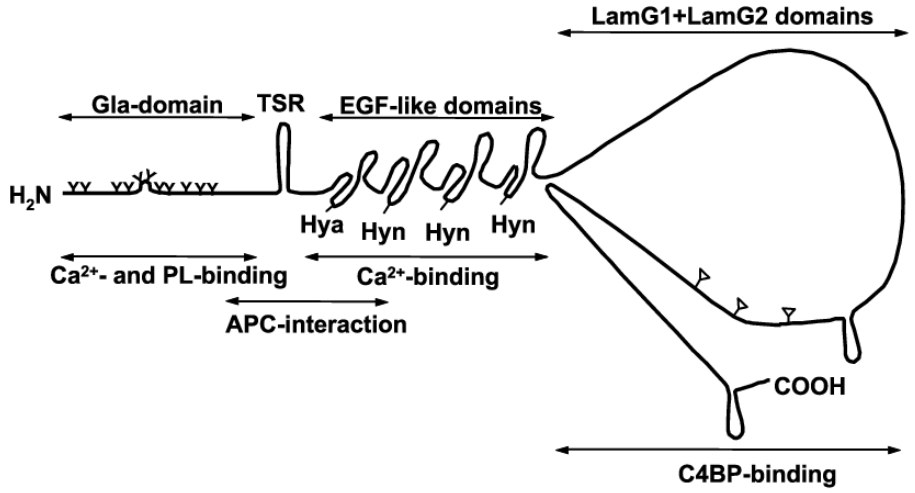
Protein C deficiency

- Protein C
  - Laboratory tests
    - Protein C activity – recommended
    - Protein C antigen
    - Influenced by warfarin!
  - Plasma level – difficult to establish
    - Normal : 70-140%, Borderline : 55-70%
    - increase 4% per decade
    - Newborn : 20-40%
  - Mutation study
    - *PROC*



Protein S deficiency

- Protein S deficiency
  - Laboratory tests
    - Total protein S
    - Free protein S - recommended
    - Functional protein S – recommended
    - Influenced by warfarin!
  - Plasma level
    - Normal : 65-140%, increase with age
    - Newborn : 35%
  - Mutation study : *PROS1*



Antithrombin deficiency

- Antithrombin deficiency
  - Laboratory tests
    - Antithrombin functional and/or antigenic
  - Mutation study : *SERPINC1*
  - AT replacement therapy
    - For heparin treatment (target:80-120%)
  - Type
    - Type I - both decreased activity and antigen (most freq.)
    - Type II - decreased activity and normal antigen

Natural anticoagulant tests during anticoagulation

- Warfarin
  - Potent vitamin K antagonist
  - Inhibit production of factor II, VII, IX, X, protein C, protein S
  - May influence laboratory tests
    - Protein C – false (+), decreased but normal protein C individual
    - Protein S – false (+)

    ⇒ Discordantly reduced level compared with coagulation factors (similar half life)

    ⇒ factor VII vs protein C, factor II vs protein S

    ⇒ Switch to LMWH or DOACs and than tests (more than 1week (PC) or 4-6 weeks (PS))
- Heparin
  - No effects on protein C and protein S
  - Long term heparin Tx – may influence decreasing AT level
- DOACs
  - May influence functional assays – false (-), normal activity but deficient plasma

Revised classification criteria for the APS (2006)

APS is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met*

Clinical criteria

1. Vascular thrombosis_

   One or more clinical episodes_ of arterial, venous, or small vessel thrombosis §, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2. Pregnancy morbidity

   (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or

   (b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions [11], or (ii) recognized features of placental insufficiency–, or

   (c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

   In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.

Laboratory criteria**

1**. Lupus anticoagulant (LA)** present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies).

2. **Anticardiolipin (aCL**) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA .

3**. Anti-b2 glycoprotein-I antibody** of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

Lupus anticoagulant test

- LA test
  - LA test choice (**2 tests**)
    - DRVVT
      - Russell's Viper Venom (Activate factor X with PL, Ca^{++})
      - Diluted specimen is sensitive to LAC
    - LA sensitive aPTT or SCT
  - Mixing test with PNP
  - Confirmatory test with phospholipid
- APS laboratory criteria
  - lab test (+) on two or more occasions at least 12 weeks apart

LA tests during anticoagulation

- Warfarin
  - Screening test : false (+)
  - Confirm test : false (-), not corrected with PL adding
  - For tests : Stop warfarin 1-2 weeks and <INR 1.5
- Heparin
  - Screening test : false (+) ?

  => But, commercially available kits containing neutralizer (up to 0.4-1.0 IU)
- DOACs
  - False (+) on screening and confirm tests of DRVVT
  - Less influenced to SCT

    ⇒ Stop DOACs more than 2 days

    (5-7 times half life of drug is considered to be negligible effect)

CASES

PE Case

◈ 20-year-old man

◈ C.C : Lt. leg pain (10DA)

◈P.I : # h/o pulmonary TE (2010.3)

s/p warfarin (2010.3.3-2011.9.18)

◈ 내원 1년 10개월 전 1개월 동안 **하루 8시간동안 앉아서 컴퓨터게임을 한 후** 발생한

Lt. pleuritic chest pain으로 본원 내원하여 pulmonary embolism으로 진단,

w/u 진행하였으나 뚜렷한 원인 찾지 못하고 warfarin 복용하며 퇴원하였음.

'11.9.18까지 warfarin 복용 후 호전되어 warfarin 복용 중단하였고, 이후 특별한 event 없었음

◈ 내원 1주일전부터 Lt. medial thigh의 pain이 발생하였고, ER 내원후 시행한 PECT,

L/Ex CT에서 DVT, PE 발견되어 heparinization 시작한 상태로 전동됨

◈Subject

보호자] 원인이 뭔지 지난번에도 잘 못찾았었는데 이번에 또 생겨서 ...

이런 경우가 있을 수도 있나요?

환자] 2주전부터 허벅지가 아파서 운동하다 다친 줄 알고 동네병원에 갔더니

기브스를 해줬었어요. 그래도 계속 아팠어요.

◈Object

혈압 128/ 95mmHg; 맥박 78회/min; 호흡 20회/min; 체온 36.3℃ 2012/01/21 15:57

◈Lab Finding & Study

[2012/01/20 - Factor V Leiden 유전자검사]

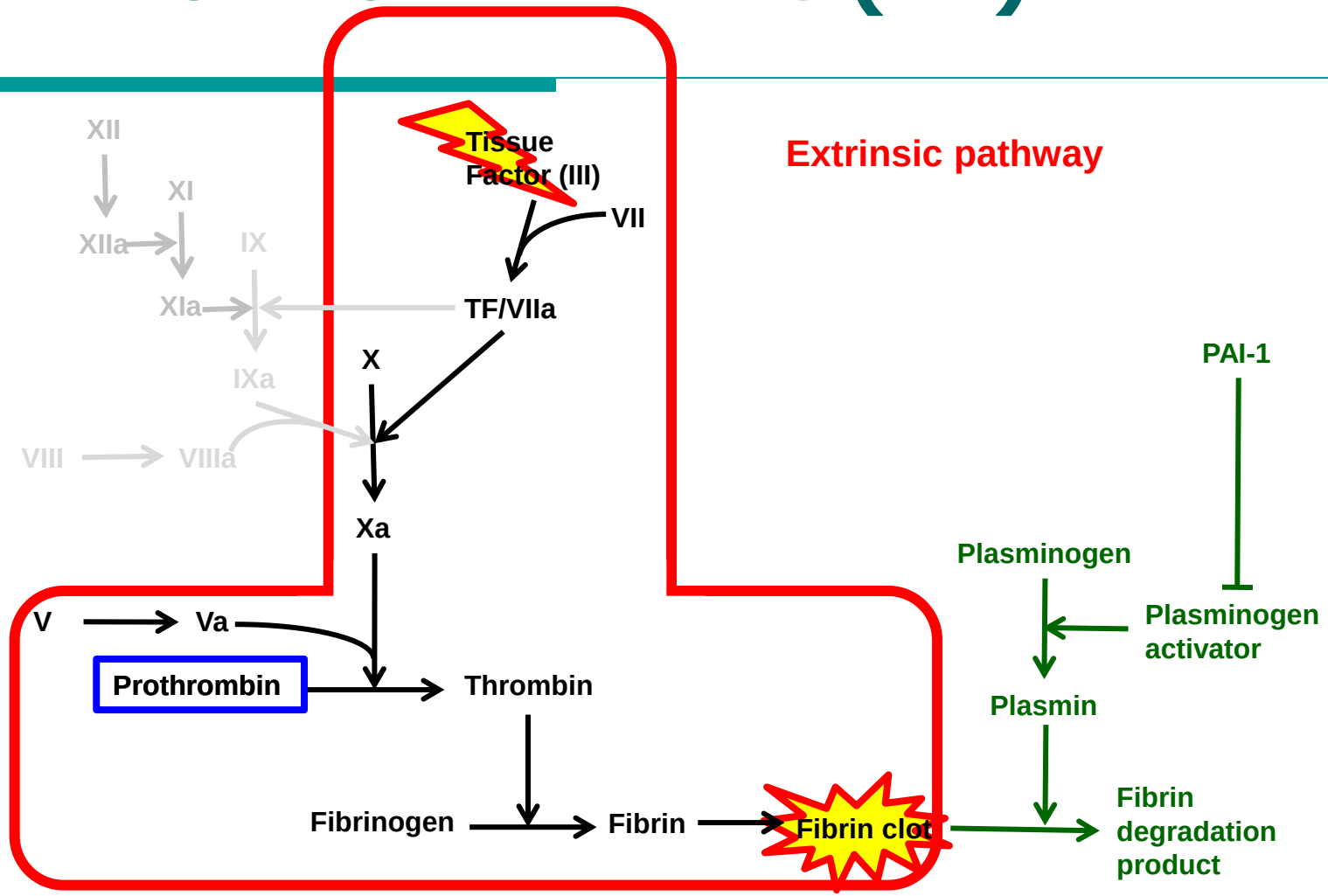
Factor V Leiden PCR --- wild type (174 bp at wild type)

PE, DVT의 원인을 찾기 위해 해야할 검사는?

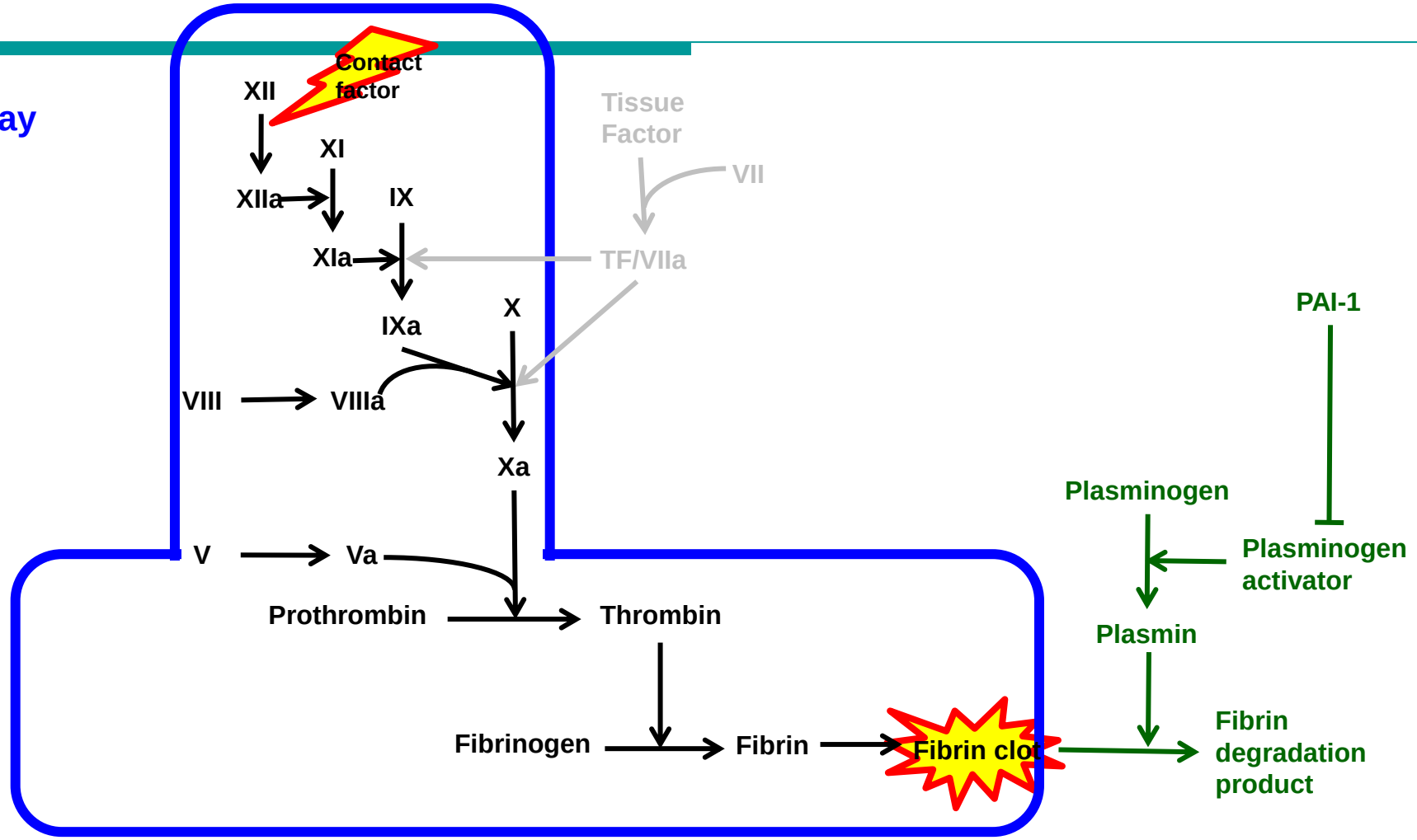
항응고제 치료를 하고 있는데…

MISCONCEPTIONS ON ROUTINE COAGULATION TESTS

Prothrombin time (PT)



Partial thromboplastin time (PTT)



Contact factors : Kaolin, silica, ellagic acids,…

Misconceptions in laboratory tests (1)

- Misconception 1 : *“Prothrombin time” is the test for prothrombin*
  - Reagent for prothrombin time does not include prothrombin
  - Target variable of prothrombin time is not only for “prothrombin”
- Misconception 2: *Heparin cannot cause a prolongation of the PT*
  - Heparinase in PT reagent
- Misconception 3: D-dimer is more specific than FDP
  - Uusally, but not always
  - Rarely heterophil antibodies making error!

Misconceptions in laboratory tests (2)

- Misconception 4: *Anti-Xa is better for heparin monitoring than PTT*
  - Anti-Xa test is accurate and is not influenced by inflammation (i.e., Acute phase reactant)
  - But, Low antithrombin decreases the responsiveness of anti-Xa test for heparin
- Misconception 5: *Increased PTT means bleeding tendency*
  - Presence of LA increases the thrombotic risk
  - Factor XII deficiency does not cause bleeding
- Misconception 6 : *Established aPTT-based heparin therapeutic range is well used by clinicians*
  - There are so many unverified targets of heparin therapeutic ranges are used in clinics

SUMMARY

Diagnostic tests for thrombophilia

- 1st line tests
  - PT/aPTT, CBC, Chemistry tests with LDH, D-dimer/FDP
  - PC, PS free and functional, AT
  - Lupus anticoagulant, ACA, ß2-GPI
- 2nd line tests
  - Decreased PC, PS or AT => mutation study
  - Positive APS lab => one or more tests 12 weeks apart
  - R/O MPN with or without abnormal CBC => *JAK2* and/or *CALR* mutation
  - R/O PNH with increased LDH => flowcytometry (CD55, CD59)
  - R/O HIT with heparin Hx (+) => PF4 antibody
  - Fibrinogen, Factor assay (VIII, II, VII, IX, XI), vWF, CRP, homocysteine, *MTHFR*, folate…

Thrombophilia tests with anticoagulation

- Warfarin
  - Consider changing anticoagulant to DOACs or LMWH
  - Or carefully tests and interpretation
- Heparin
  - Mostly not influence LA tests and other coagulation tests
  - Samples should be drawn at least 12 hours after last LMWH dosing
- DOACs
  - Consider influences of DOACs to coagulation tests
  - Sampling education for patient is important
  - At least 2 days stopping drugs before sampling

THANK YOU FOR YOUR TIME AND ATTENTION!