# A Potpourri of Coagulation

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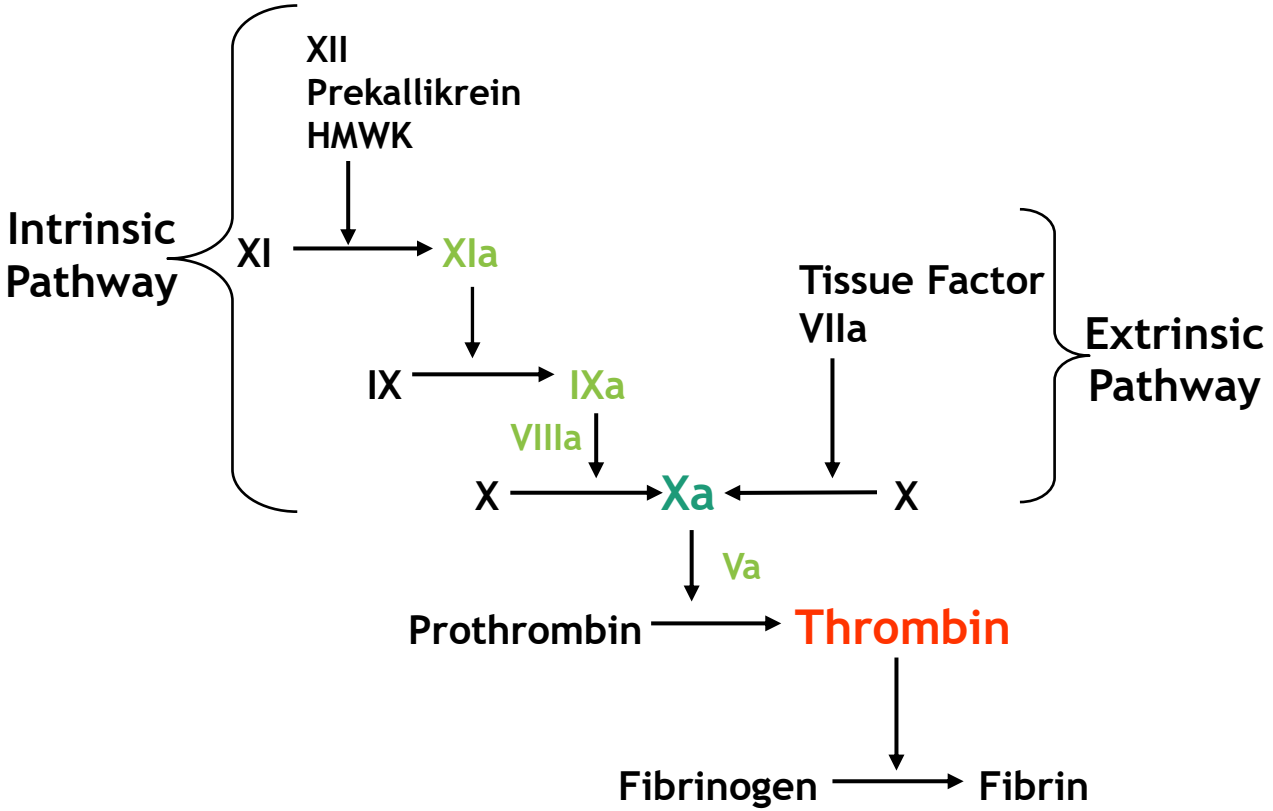
# Objectives:

| | |
|---|---|
| Identify | Identify issues in coagulation that can impact patient outcomes. |
| Analyze | Analyze data and correlate possible clinical conditions. |
| Enhance | Enhance problem solving skills. |

# OVERVIEW OF COAGULATION

- PT-VII; monitor warfarin, Vitamin K, Liver disease
- aPTT- VIII, IX, XI and XII;monitor heparin, lupus, replacement
- Fibrinogen- Factor I – quantity of fibrinogen; acute phase reactant, DIC
- TT: Factor IIa  looks at the quality of fibrinogen; DIC, anti IIa drugs
- Platelets: Prim:ary Hemostasis; not measured in PT/aPTT

# The Coagulation “Cascade”



# Pre-analytical Variables

Up to 65% of errors in the coagulation laboratory are due to pre-analytical errors

Due to the better precision and reproducibility of the analyzers less errors are made in the analytical phase

Results automatically going to LIS really have minimized transcription errors

# Most common errors:

- Improperly filled tube- 90%
- Hemolysis
- Platelet poor plasma

# Case Study

- A 42 year old male presented with the following symptoms:

  fatigue

  difficulty breathing

  tingling in the hands and feet

- Coagulation results:

  PT= 25.6 seconds (11.5-13.9 sec)

  aPTT=77.5 seconds (25.0-35.7 sec)

Clinician questioned the results, patient had no bleeding symptoms

# Prior to additional testing:

- Reviewed additional results:
- HB= 23.5 gm/dL
- HCT= 76.2%

- High HCT = hemoconcentrated -
- CLSI guideline require a 9:1 ratio of blood to anticoagulant
- Results in too much anticoagulant in plasma resulting in a falsely prolonged result
- Adjust HCT if >55%

# SOLUTION:

- C=Volume of NaCitrate mm
- V=Volume of whole blood mm
- H=HCT %
- To collect 2.7 mls of blood from a patient with a HCT of 76%
- C=(1.85x10)-3 X(100-76) x 2.7
- 0.00185 x 24x 2.7 = 0.12mls citrate

# Repeat sample

- RESULTS:  Collected in a new adjusted citrate volume tube:

PT= 13.7

aPTT= 35.1

Both results are within normal limits

# High hemoglobin and hematocrit

Additional Tests:

Thrombocytosis, platelet count =481,000/µL

Leukocytosis =13,200/µL

Increased leukocyte alkaline phosphatase (LAP) = 207

Serum vitamin B-12 concentration 988 pg/mL(200-500pg/mL)

# Diagnosis

Polycythemia vera

occurs more often in men than in women, and rarely in anyone under 40.

PV is usually associated with a JAK2 gene mutation (JAK2V617F) which is responsible for RBC production.

Scott LM, Tong W, Levine RL, et al. *JAK2* exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med.* 2007;356:459-468.

# JAK2 V617F

gain-of-function mutation that leads to clonal proliferation;

it is present in about 95% of PV cases and about half of ET and MF cases.

The JAK2 allele burden decreases with successful therapy, disappears in some patients, and reappears during relapse

# Case Study

Patient presents to the outpatient clinic for INR testing

Testing is performed on the ROCHE

CoaguCheck

INR= 4

Previous result from last month INR=2.9

Which one is correct?  Should patient be treated based on an INR 4.0

# Laboratory results

- Blood sample sent to Core Hematology Laboratory
- Results run PT-34.5 sec.
- INR= 3.0
- Where is the discrepancy?

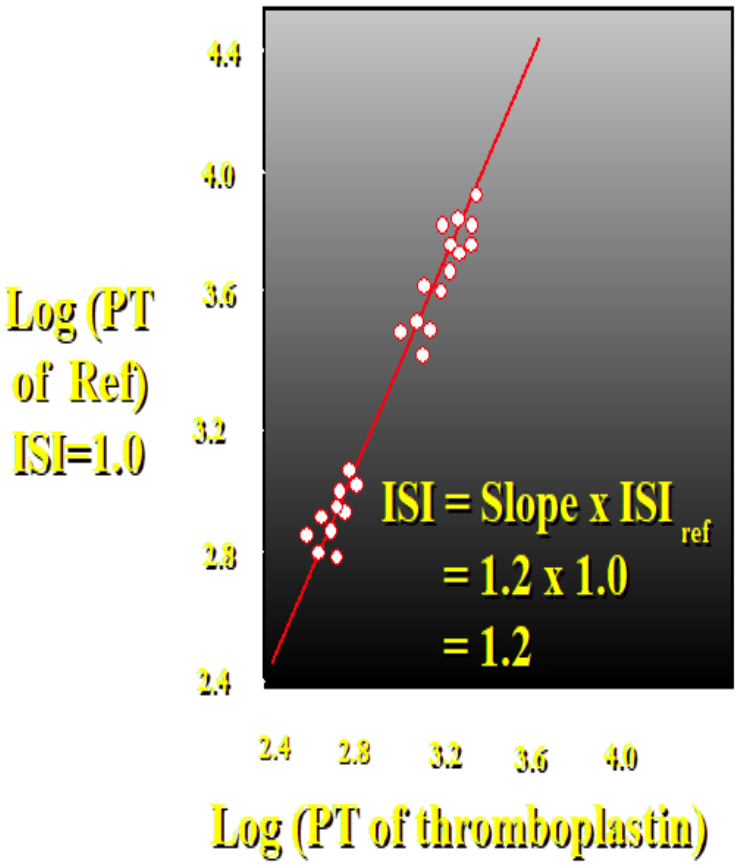
# Action of Warfarin

- Works by making the Vitamin K dependent factors non-functional-
- Factors  II, VII, IX and X, Protein C and S
- Impacts factors based on their half life:  VII has the shortest ( 4 hours) and II the longest (2 days)
- Effected by food
- Oral anticoagulation:  non-compliance
- Patients are only in the therapeutic range 57% of the time

# Difference in results

International Normalized Ratio (INR) was developed to standardize the results regardless of instrument/reagents

# Monitoring OAT



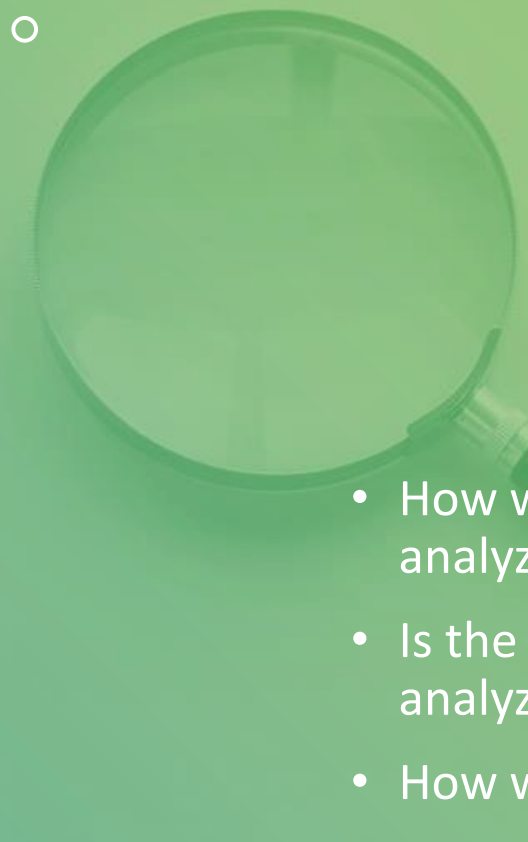

- **INR developed by WHO using an IRP to which all other thromboplastins can be compared**
  - **ISI = measure of the sensitivity and responsiveness of a particular thromboplastin reagent to warfarin-induced reduction of the VKDF's**
  - **ISI of the IRP = 1.0**
  - **Recommended that a PT value be expressed as a ratio by normalizing it to the IRP**

$$INR = \left[\frac{\text{Patient's PT}}{\text{GM Normal PT}}\right]^{(IS)}$$

***Advantages*: INR for monitoring patients receiving OAT**

1. **Minimizes the variation in the PT assay**
2. **Allows comparability of PT results among different laboratories**

# ISSUES



- How was your INR on  your POC analyzer determined?
- Is the reagent similar to laboratory analyzer?
- How well do they correlate?
- Does the laboratory/POC/Clinician understand that?

# Understand your reagents and analyzers

- Both laboratory and POC PT/INR systems demonstrate variability as the INRs increase.
- This is further confounded when thromboplastins are derived from different sources (human versus rabbit). (6)
- POC analyzers also have different methods to determine clot formation including optical, electro-mechanical and electrochemical clot detection.
- It is important for the POC analyzers to be compared to the laboratory analyzers across the measuring range to understand at what point the INR correlate with the analyzer and the patient's clinical condition.
- In order to minimize discrepancies between the POC analyzer and the laboratory analyzer, ideally both analyzers should be standardized using the same type of thromboplastin reagent (eg. Recombinant to recombinant)I POC

# POC INR RESULTS

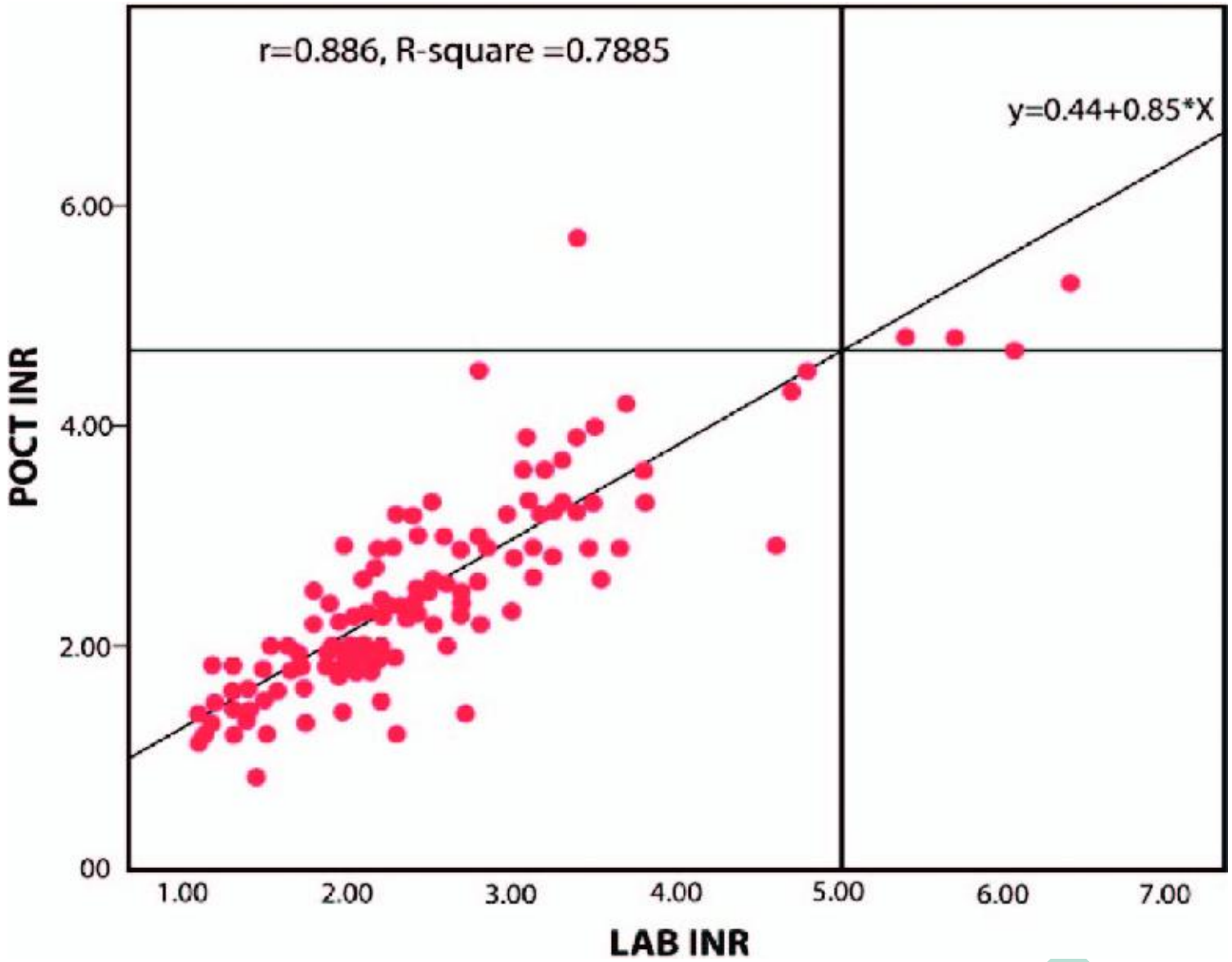
- Several factors will increase the POC INR results. These include hematocrit and protein induced vitamin K absence (PIVKA).
- INRs may also differ if a patient is not in a steady state of anticoagulation, which usually occurs with 5 weeks from the time the patient started.
- When a patient has a low hematocrit there will be excess plasma for the amount of cartridge in the POC analyzer. This results in slowing down clot formation resulting in a higher INR.
- The presence of PIVKA does not inhibit clotting in the body, but they do inhibit the thromboplastin used in the INR test making the INR appear elevated.

Wool, GD., Benefits and Pitfalls of Point-of-Care Coagulation Testing for Anticoagulation Management: An ACLPS Critical Review, *American Journal of Clinical Pathology*, 2019,151:1-17

# DISCREPANT RESULTS

- There are inherent bias between the laboratory INR and the POC INR when the INR measurement are elevated above 3.
- It is important to know at what INR the POC analyzer occurs.  When levels of VKA are supra therapeutic (>5) or when the anticoagulation therapy is not stable, INR discrepancies are exacerbated.
- In guidelines for patient INR self-monitoring it is recommended that any INR between 4-8 should be repeated on a laboratory analyzer.

1. CLSI. Point-of-Care Coagulation Testing and Anticoagulation Monitoring. 2nd ed. CLSI guideline POCT14. Clinical and Laboratory Standards Institute; 2020.


r=0.886, R-square =0.7885
y=0.44+0.85*X
POCT INR
6.00
4.00
2.00
00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
LAB INR

# GUIDELINES: RECOMMENDATIONS

**The following guidelines provide recommendations for POC coagulation testing**.

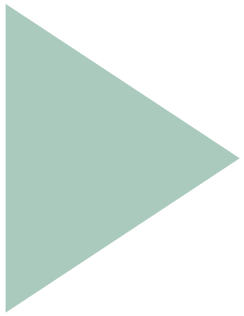
1. ***A new point of care (POC) device should be assessed for reproducibility against a central laboratory analyser or other established technology***
2. ***Users should be aware of the method employed by a point of care testing (POCT) device to measure parameters and the possible limitations of the method***
3. ***International normalized ratio POCT methods should be assessed for comparability with central laboratory analyzers using warfarin samples in order to establish a reflex testing algorithm for confirmation of supra-therapeutic levels***

***RECOMMENDATION; KNOW AT WHAT LEVEL YOUR POC ANALYZER NEEDS TO BE CONFIRMED BY THE LABORATORY ANALYZER***

, British Journal of Haematology, 2019, 187, 296–306.

# Case Study

A 20 year old male presents to the ED after an excessive bleed from a fall. His past medical history includes a diagnosis of mild hemophilia.



PT = 19.3 sec (11.5-13.5)

aPTT= 76.5 sec (25.9-37.2 sec)

FVIII = 18%

# Additional Testing

- Ristocetin cofactor (R: CoF): 112% ( nr= 50-150%)
- von Willebrand antigen (vWFAg): 120% (nr= 50-150%)
- Normal von Willebrand testing
- Need to perform this testing to rule out vWD, can also have a decreased FVIII

**Confirmed Diagnosis: Hemophilia A**

# ISTH Classification of Hemophilia

- Severe hemophilia FVIII < 1%
- Moderate hemophilia FVIII 1-5%
- Mild hemophilia FVIII >5 <40%
- Severe hemophilia – bleed occurs within first 18 months of life- CNS bleed, umbilical bleed, hemotharsis
- Moderate will be discovered in early childhood, after a fall or cut
- **Mild hemophilia discovered** when screened for surgery

White, GC et al: Thrombosis & Hemostasis 2001

# Factor VIII (FVIII)

- FVIII is not an enzyme but a cofactor for cleavage of FX to FXa by IXa.
- FVIII protein is bound to a carrier protein, von Willebrand factor (vWF)
- VWF is an adhesive glycoprotein that facilitates adhesion of platelets to injured vessels
- FVIII circulates with VWF which essential for maintaining stable levels of FVIII in circulation.
- The half life of FVIII in the circulation is from 12-16 hours
- Clearance is proposed to occur in the liver and possibly the spleen

# Factor VIII deficiency- Hemophilia

- Hemophilia A is caused by a defect in clotting protein FVIII
- Inherited disorder identified 1500 years ago, in Jewish law, if 2 older brothers died after circumcision, the third was not allowed to have the procedure.
- Sex-linked recessive, found on the X chromosome, disorder is almost exclusively in males
- One in 5,000 males born has hemophilia. All races and economic groups are affected equally.
- Present with joint bleeds, intracranial bleeds, intramuscular bleeds

# Screening tests

- Factor VIII =18%
- PT was prolonged
- 1:1 Mix- corrected for both PT and aPTT

PT = 19.3 sec (11.5-13.5)

1:1 mix= 12.1

aPTT= 76.5 sec (25.9-37.2 sec)

1:1 mix = 34.7

Known hemophiliac- however PT is also prolonged and corrects

# Additional family history

- episodes of prolonged dental bleeding, a GI bleed
- absence of hemarthrosis
- a sister with some bleeding episodes
- incidence of consanguinity

# Tested sister

- 15 yr old female presents with heavy periods
- Her HGB is 9.0 g/dl
- She has had intermittent period of nose bleeding as a child
- An evaluation for a bleeding work-up was ordered

PT= 17.1 sec

APTT=  42.1 sec

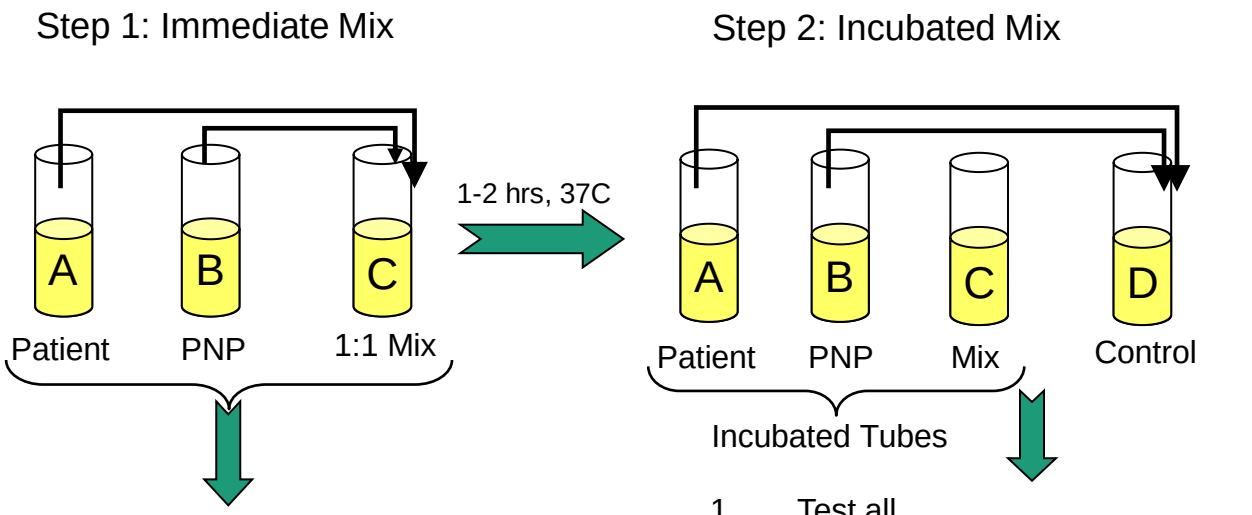
Mixing study: APTT 1:1 mix = 31.5 sec
Correction

FVIII= 38%

von Willebrand work-up- normal

Female- carrier of hemophilia

# Classical 1:1 Mixing Study




1. Test all.
2. Compare C to A & B. Did C correct?
3. Incubate all.

1. Test all.
2. Compare incubated C to D. Is it prolonged? Yes, possible time dependent inhibitor.
3. Interpret final results

# Interpretation of a Mixing Study

- Based on PT or aPTT normal range
  - Within limits @ 2SD or 3SD
  - Within 5 seconds of the 2SD upper limit
- Rosner Index
  - 1:1 mix
  - Index = $\frac{\text{CT of 1:1 mix} - \text{CT of NPP}}{\text{CT of patient}} \times 100$ <15 = FD >15 = Inhibitor
  - NPP tested with Mixing Study
  - Within 5 seconds of NPP value
  - 10% of NPP value
  - Lack of correction @ >15% of NPP
- *No recommendations or guidelines from ISTH Scientific & Standardization Committee CAP Coagulation Resource Committee*

# Pt workup

Both patients correct on mix:

Factor Assays:

| | Factor II | Factor V | Factor VII | Factor X |
|---|---|---|---|---|
| Brother | 98% | **15%** | 112% | 87% |
| Sister | 105% | **19%** | 92% | 108% |

**FV Deficiency**

# Factor V

- Only 150 cases of congenital factor V deficiency have been reported worldwide since 1943. Homozygous factor V deficiency is rare, occurring in approximately 1 per million population
- severity varies from bruising to lethal hemorrhage.
- **Race:** No apparent racial predilection for factor V deficiency exists.
- **Sex:** affects males and females with equal frequency.
- **Age:** Factor V deficiency affects all ages. The age at presentation indirectly varies with the severity of disease.
- inheritance is autosomal recessive
- other modes of inheritance have been described. Heterozygotes have lowered levels of factor V but probably never bleed abnormally.
- Consanguinity has been observed in families with factor V deficiency
- Factor V deficiency has also been called parahemophilia, since hemarthrosis can occur with severe deficiencies.
- Heterozygous deficiency states are generally unrecognized because of a lack of significant clotting time prolongation or bleeding risk.

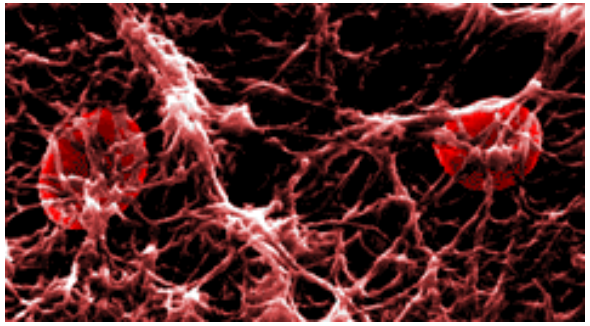
diagnosis:

**Brother: simultaneous FV and FVIII deficiency.**

**Sister:  FV deficiency- carrier for hemophilia.**

# *General Background*
# D-dimer is …

… a marker of fibrinolysis



- … a marker of coagulation activation (hypercoagulability → hyperfibrinolysis)

- Fibrinolysis is a vital mechanism of the organism
  - Dissolves clots after the bleeding is stopped
  - Limits clot formation
  - Prevents dissemination
- Under physiological conditions the fibrinolytic system is
  <u>continuously</u> activated
- Under certain pathological conditions the fibrinolytic system is considerably enhanded

# Outcome

- D-dimer is a product of 2 mechanisms
- Addresses both thrombin and plasmin generation
    - thrombin resulting in a cross linked fibrin clots
    - plasmin resulting in the lysis of cross-linked fibrin clot
- Plasma d-dimers significantly advanced over  FDP's in that it is **specific** for cross-linked fibrin derivatives
- no interference from fibrinogen and non-cross linked fibrin
- Half-life is 6 hours
- Increased in patients with more extensive thrombosis

# Clinical Utility of D-dimer

Disseminated Intravascular Coagulation (DIC)

Simultaneous formation of thrombin and plasmin

Sensitive, but not specific marker for DIC

Venous Thromboembolism (VTE)

- High negative predictive value for exclusion
- *Potential* positive predictive value (?)

  Positive predictive of recurrent MI ?

  Independent predictor of ischemic heart disease ?

  Differentiate between a traumatic spinal tap or a subarachnoid bleed ?

Predictor of VTE recurrence

- Elevated levels following discontinuation of anticoagulant therapy associated with recurrence

# Quantifying the D-dimer

- D-dimer concentration is dependent on clot size and age
- Half life of D dimer is about 6 hours
- Larger more proximal clots are associated with higher d-dimer levels
- Highest levels seen early in the disease process, also levels higher in elderly
- Elevated in most critically ill patients with high APACHE II scores

  Acute Physiological and Chronic Health Evaluation – indices of severity
- Assess neurological, respiratory, cardiovascular, renal & hematologic systems
- Considers age, chronic ill health, in ICU, elective or emergency surgery

# D-dimer & VTE

- Almost all patients with acute disease (DVT or PE) have elevated D-dimer
  - A positive D-dimer is not specific
  - Negative D-dimer: highly unlikely VTE

***The greatest utility of D-dimer is its negative predictive value***

# What is Deep Vein Thrombosis (DVT)?

- DVT is a single disease that manifests itself as a blood clot in the leg which can migrate to the lung as a pulmonary embolism (PE)
- Mortality rates are 6% DVT, 12% PE
- Recurrent rate 3-10%/year
- 10% develop post thrombotic syndrome
- Serious & potentially fatal
- Frequency increases with age
- Increases with injury to the leg, prolonged stasis, trauma, pregnancy, tumors
- Inpatients
- Positive family history

# Pulmonary Embolism (PE)

- Can occur after a DVT when a clot breaks  loose from a leg and migrates to the lung
- PE is potentially fatal
- 95% of all PE’s are from DVT’s of the lower extremities
- 15-20% die prior to intervention
- Ventilation/perfusion scan is the best test for a PE, however it can be non-diagnostic in 70% of patients
- Costly. not available 24/7

# Who is at risk?

- Increasing age- 10% increase/decade
- Prolonged stasis – inpatients
- Stroke & paralysis
- Cancer patients
- Surgical patients- pelvis abdomen, legs
- Trauma-fractures
- Respiratory failure

- Obesity
- Varicose veins
- Heart failure & MI
- Indwelling catheters
- Nephritic syndrome
- Pregnancy, hormones, oral contraceptives
- Prolonged travel
- Inherited predisposition for clotting

# Clinical Model for Predicting DVT

| Pre-Test Probability Questionnaire | Score |
|---|---|
| Active cancer | 1 |
| Paralysis, paresis or recent plaster immobilization of lower extremities | 1 |
| Recently bedridden > 3d or major surgery w/in 4 wk | 1 |
| Localized tenderness along the distribution of deep venous system | 1 |
| Entire leg swollen | 1 |
| Calf swelling 3 cm > asymptomatic side (measured 10 cm below tibial tuberosity) | 1 |
| Pitting edema confined to the symptomatic leg | 1 |
| Collateral superficial veins (nonvaricose) | 1 |
| Alternative diagnosis as likely or greater than that of DVT | -2 |

- *Wells, et al. Lancet, 1997;350:1795-1798*

# Case Study:

- A 76 year old male patient has been admitted into the ED for an infection
- After performing an APACHE assessment, it is determined that he is critically ill, possibly septic
- He has a history of DVT so a D-dimer is ordered
- Has a high PTP with the Wells score
- D-dimer is positive  6.4 mg/L
- Does the patient have a blood clot?

# Clues:

- Older patient, septic, previous history of DVT
- Wells scoring results in a high PTP
- What is the utility of an elevated D-dimer?
- What are you looking for?

# Case study

- This patient has all the reasons to have a positive D-dimer
- Older, septic with previous history of DVT
- We don’t know specifically why it is increased
- There are many reasons
- Not the patient population to be using the D-dimer test to determine a DVT
- The patient may be in DIC

- RECOMMEND: Imaging

# Case study

- 42 year old male enters the ED complaining of rapid onset, shortness of breath, pain in chest, numbness in arm
- Wells score is mod PTP
- Should this patient be imaged for a PE?
- D-dimer is normal 0.411mg/L
- Additionally testing included an elevated troponin and BNP
- Does this person have a PE?

# Case study

- Patient test results appear to be more consistent with an MI, versus a PE
- Imaging doesn’t seem to be indicated at this time
- Patient is then admitted
- Follow up with imaging if symptoms still persist several days post admission
- D-dimer will most likely be elevated as an inpatient, and imaging would be the more diagnostic approach.

# Case Study

- A 40 year old male comes into the ED complaining of sudden onset of shortness of breath
- He has had a cold for 2 days, but now feels a heaviness in his chest
- He does have a history of asthma
- The clinician suspects that he might have pneumonia, but wants to make sure he doesn’t have a PE
- Performs a D-dimer, which is negative
- Doesn’t order a VQ scan, treats the patient, gives him medication with orders to follow up with his family physician next week.
- Did he do the right thing?

# TESTING

- The laboratory used an automated immunoturbidimetric D-dimer assay
- This assay has been FDA approved for exclusion for PE
- A prospectively collected clinic trial looking at over 1000 patients suspected of DVT and or PE were tested and imaged and their imaging results were compared to the D-dimer
- A negative predictive value of >99% was determined
- This results in being able to have patients with a low-moderate pre test probability and a negative D-dimer not have to be imaged.
- This patient’s diagnosis was asthmatic bronchitis, did not have to have expensive imaging, D-dimer was able to provide a diagnosis

# Case Study

March 2020- 55 year old female patient presents with cold and flu like symptoms – told possible sinus infection

April 2020- patient presents with severe uticaria on back and limbs

May 2020-patient presents with an enlarged swollen and purple finger, no known injuries- referred to a hematologist

# Hematology workup:

PT= 11.5sec (10.5-13.5 sec)

aPTT= 41.3 sec (24.5-36.5 sec)

Lupus Workup:

DRVVT : screen 48.2

DRVVT: confirm 35.1

Ratio: 1.23 positive for LA

RECOMMENDATION: aspirin for 6 months and retest

# 6 month re-test

- PT= 12.0
- aPTT= 35.0

Patient complained of bruising: von Willebrand workup

VW activity- 100% (nr 50-150%)

Antigen= 92%

What is wrong with this work-up?

# ISSUES:

Initially diagnosed with Lupus in which you are predisposed to clotting- placed on aspirin

6 months: complained of bruising- tested for von Willebrand disease-however the patient was on aspirin

What about the positive lupus testing?

Retested: normal aPTT, DRVV ratio =0.8 (<1.2) normal

Did the patient have lupus?

# REALITY:

- Patient initial diagnosis (March 2020) was most likely COVID- no testing performed to confirm
- Rash from virus, and “COVID Finger”
- What about abnormal aPTT and DRVV?
- The partial thromboplastin time (PTT) has been found to be prolonged in many patients with COVID-19 and may indicate the presence of LA. Most patients with COVID-19 have elevated levels of C-reactive protein (CRP), and CRP is known to interfere with LA PTT-based tests, such as the hexagonal phase phospholipid neutralization assay STACLOT-LA

# Study

- The LA-positive rate by DRVVT in patients who tested negative for COVID-19 was 22% (27 of 119).
- In contrast, the LA-positive rate in patients who tested positive for COVID-19 was 44% (30 of 68) (P = .002).
- Of the 30 COVID-19–positive patients who had a positive LA by DRVVT, 17 (59%) were also positive by hexagonal phospholipid neutralization STACLOT-LA test
- Patient did have an elevated CRP- most likely interfered with the DRVV testing- false positive for lupus!

# Dark chocolate is cardioprotective

- Dark chocolate,(60-70%) derived from cocoa beans, rich in polyphenols, specifically flavonoids exhibit antihypertensive, anti-inflammatory, antithrombotic, and metabolic effects, all of which may contribute to cardioprotection.
- To determine long-term effects; (chocolate every day for 10 years) Australian researchers used statistical modeling techniques (Markov model) on 2013 patients, mean age 53.6
- Outcome: 70 nonfatal cardiovascular events, including nonfatal stroke and nonfatal myocardial infarction per 10 000 population, as well as 15 cardiovascular-related deaths per 10 000 population, could be prevented.
- cost-effectiveness ratio 500 per years of life saved when $42 per person per year was assumed to have been spent on a prevention strategy using dark chocolate.

# WINE AND COAGULATION

- In 1786, an English doctor noted that wine relieved pains of patients suffering from angina pectoris
- In 1970, a cardiologist at the Kaiser Permanente hospital center in Oakland (California) initiated a study on over 100 000 people. Results: risk of death from MI is lower for moderate consumers (1-3 glasses/day)
- School of Public Health of Harvard - Cambridge - Massachusetts calculated that the risk of heart disease is reduced from 25 to 45% for people drinking 1 or 2 glasses of wine a day.

Moderate alcohol intake decreases clot formation by multiple additive mechanisms.

- reduces platelet aggregation,
- decreases fibrinogen levels, plasma viscosity, von Willebrand factor, and factor VII.

# Thank you

- Thank you for your time
- Take away message:

Coagulation is complex

However

A glass of wine and a piece of dark chocolate is not only good for you, but may also decrease the complexity of coagulation and increase your willingness to learn