DDIMER history, measurement and use.

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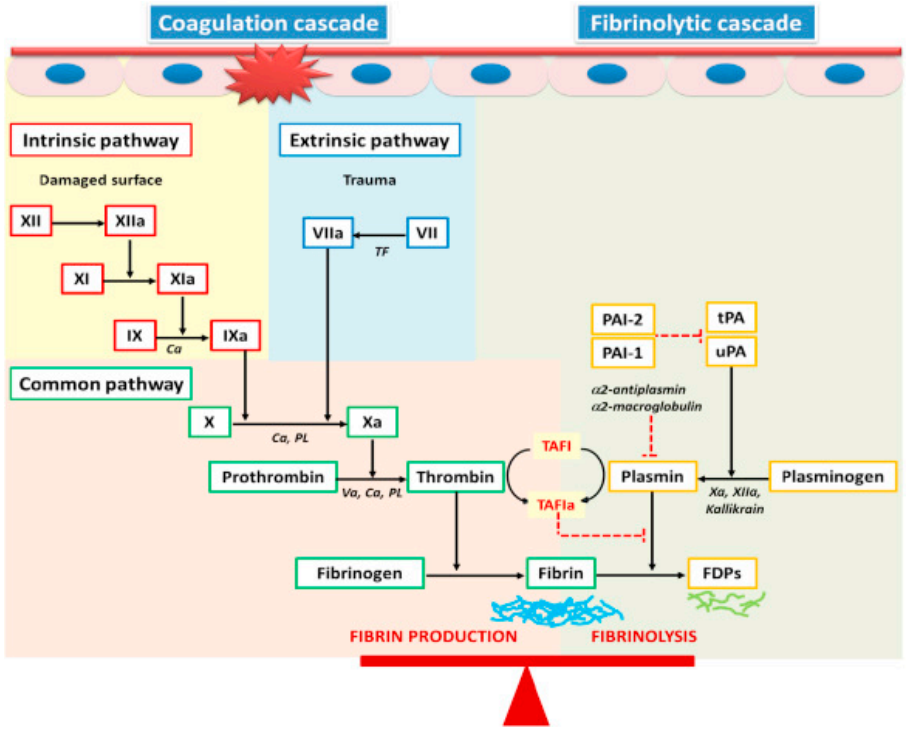
The haemostatic pathways



The fine balance

* Together coagulation, anticoagulation and fibrinolysis maintain a delicate physiological balance. If this balance is lost then thrombosis or bleeding will occur

Bleeding

Thrombosis

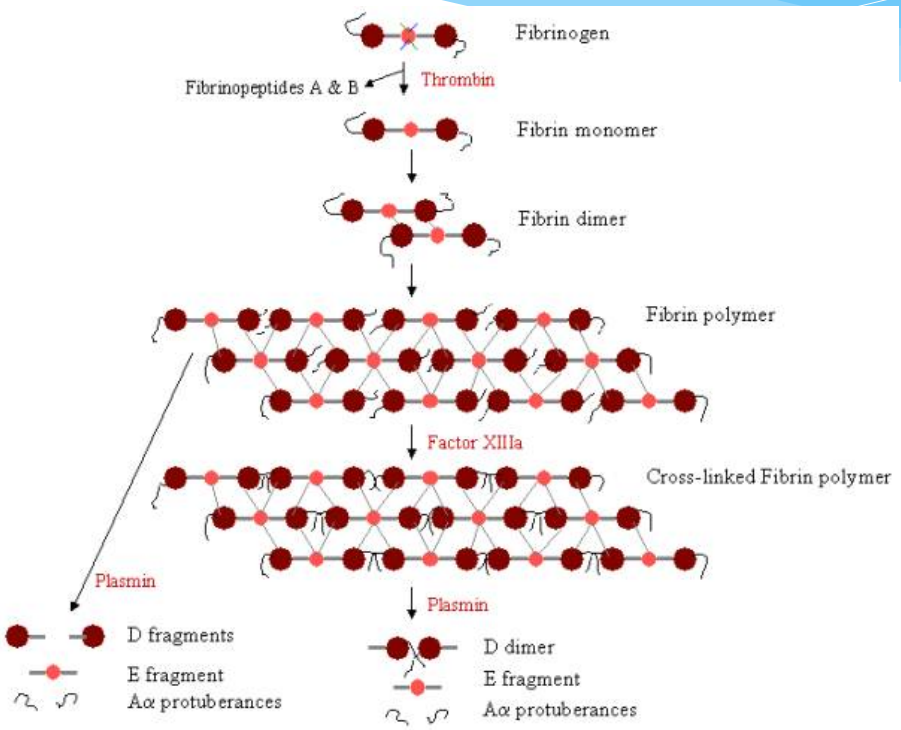
Tests of fibrinolysis

* Platelet count – useful in DIC diagnosis
* Fibrinogen - useful in DIC diagnosis
* Fibrin degradation products
    - Fibrinopeptide A
    - Fibrinopeptide B
    - Fragment D
    - Fragment E
    - D-dimer ("cross-linked" fibrin degradation product)

The D-dimer is useful for DVT and PE as it shows the fibrinolytic pathway is active.

What is a DDIMER

* Coagulation activation results in the cleavage of fibrinogen to fibrin monomer. The fibrin monomers spontaneously aggregate to fibrin and are cross-linked by factor XIII.
* This produces a fibrin clot. In response to the coagulation process the fibrinolytic system is activated resulting in the conversion of plasminogen into plasmin.
* Plasmin cleaves fibrin into the fragments D and E.
* Due to cross-linkage between D-domains in the fibrin clot, the action of plasmin releases fibrin degradation products with cross-linked D-domains.
* The smallest unit and end product of fibrinolysis is D-dimer.


Fibrinogen
Fibrinopeptides A & B
Thrombin
Fibrin monomer
Fibrin dimer
Fibrin polymer
Factor XIIIa
Cross-linked Fibrin polymer
Plasmin
Plasmin
D fragments
E fragment
Aα protuberances
D dimer
E fragment
Aα protuberances

How we used to measure FDPs (not just Ddimer)

Taken from a paper from July 1966

* **Precipitin Test:** A micro-capillary tube was filled with fibrinogen antiserum and an equal volume of test material added. The tube sealed with heat and the mixture incubated for 18 hours at 37 C. The amount of precipitate was observed and after 18 hours measured in mm.

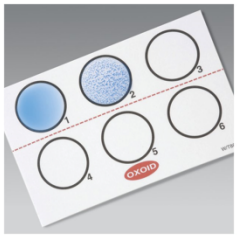
* **Fi Test:** The sample was diluted 0.1 M glycine buffer containing 1.0 per cent NaCI. One drop of dilute sample was mixed with two drops of Fi test reagent onto a glass slide. The mixture was observed for agglutination. Macroscopic or microscopic evidence of agglutination which was considered a positive reaction.

The one I remember.

* **Tanned Red Cell Hemagglutination Inhibition immunoassay (TRCHII):** Doubling dilutions of plasma were made in citrate buffer and an equal volume of antiserum added. One volume of tanned red cells was added and left at 4C for 12-18hrs and then checked for agglutination. The first negative tube was regarded as the end point .

Latex particles to measure FDP (1972)

* A rapid slide test for the detection of degradation products of fibrinogen/fibrin (FDP) using a new antibody-coated latex particle. The latex particle had been specifically coated with antibody to D fragments and agglutination was observed
* This became the routine test for FDPs until Ddimer specific tests were introduced in the 1990s.



Current laboratory methodologies.

* ELISA – considered the gold standard but time consuming and not suitable for rapid testing.
* VIDAS test combines ELISA with final detection by fluorescence.
* Nycocard – a semi-quantitative method with rapid results.
* Immuno-turbimetric – the agglutination of antibody coated latex or polystyrene similar particles on an analyser platform.

Common technology.

* Most hospitals measure DDIMER on a coagulation analyser using an immunoturbimetric assay.
* Polystyrene or latex particles covalently coated with a monoclonal antibody are aggregated when mixed with samples containing D-dimer. The D-dimer cross-linkage region has a stereo symmetrical structure.
* Consequently, one antibody suffices in order to trigger an aggregation reaction, which is then detected turbidimetrically via an increase in turbidity which is measured by light transmission.
* This has high sensitivity and intermediate specificity.

Units of measurement

* Some laboratories use mg/L (or µg/mL) others use µg/L (or ng/mL). Eg 0.5mg/L= 500ng/mL
* Technically, most D-Dimers are measured as "FEUs" or Fibrinogen Equivalent Units
* An alternative is to use D-Dimer units. 1 D-Dimer unit is 2 FEU.
* Potential confusion! eg 0.5 FEU µg/mL = 500 FEU ng/mL = 250 DDU µg/L (!!)

VTE exclusion or reference range

* Each laboratory will have their own Ddimer cut off for the exclusion of VTE based on analyser and reagent variations.
* This is usually around 500ng/ml FEU or 250ng/ml DDU (non FEU).
* You need to be aware of your own laboratories ranges and VTE exclusion cut off.
* We add a comment to all Ddimer results :- “NB cut off in assessment of VTE is 500” to avoid misinterpretation.

NEQAS participation

Table 12. Quantitative Results D-Dimer Kit	FEU	Total n	µg/ml n*	ng/ml n*	Median ng/ml	CV (%)	Range ng/ml	*std error of the median*
AQT90 Flex DDimer	No	3	1	2	579	-	540.0-581.0	-
DG Latex D-Dimer	No	2	0	2	235.5	-	191.0-280.0	-
Helena	No	6	0	6	152.5	-	134.0-190.0	-
Hemosil D-Dimer	No	28	1	27	433	142.7	10.0-5000.0	145.963
HemosIL D-Dimer HS	No	257	3	254	267	10.0 (25.3)	166.0-920.0	2.082
Not Stated	No	4	1	3	602	-	295.0-782.0	-
Sclavo AutoD-Dimer	No	4	0	4	160.5	-	144.0-174.0	-
Siemens D Dimer plus	No	4	0	4	669.5	-	564.0-760.0	-
Siemens Dimertest	No	2	0	2	629	-	628.0-630.0	-
Siemens Innovance (non-FEU)	No	20	0	20	316.5	27.5	251.0-562.0	24.328
Siemens Innovance CA600 series	No	3	2	1	720	-	710.0-920.0	-
Stago Liatest (non-FEU)	No	4	0	4	343.5	-	332.0-352.0	-
DG Latex D-Dimer	Yes	1	0	1	648	-	-	-
HemosIL D-Dimer HS	Yes	4	0	4	649.5	-	551.0-846.0	-
HemosIL D-Dimer HS500	Yes	83	20	63	740	8.4	588.0-905.0	8.529
Siemens Innovance	Yes	218	97	121	630	10.2	309.0-880.0	5.44
Siemens Innovance CA600 series	Yes	1	1	0	1790	-	-	-
Stago Di Test	Yes	1	1	0	300	-	-	-
Stago Liatest	Yes	41	29	12	680	14.0	313.0-1030.0	18.585
Stago Liatest D-Di Plus	Yes	66	41	25	700	12.2	335.0-820.0	13.14
Vidas D Dimer Exclusion	Yes	16	4	12	998.7	5.8	913.0-1145.6	-
Overall (ng/ml) non-FEU		**337**			**270**	**41.7 (88.9)**	**10.0-5000.0**	**7.666**
Overall (ng/ml) FEU		**431**			**670**	**16.0 (17.8)**	**300.0-1790.0**	**6.455**

Wells score background

* **Deep-vein thrombosis** The original 1997 DVT Wells score used a three-level risk stratification system. The 2003 version (which is referred to in the literature as 'updated', 'modified', 'revised' or 'two-level') uses two levels of risk stratification:
* ***Wells score (1997) (original)*** In 1997, Wells et al.[1] developed a nine-component clinical prediction rule for DVT. Two points are deducted if an alternative diagnosis to DVT is at least as likely. This gives a possible score range of –2 to 8. There were three risk categories: high (3 points or more), intermediate (1–2 points) and low (less than 1 point). This is also sometimes referred to as the Hamilton score, with a slight change of wording.
* ***Wells score (2003) (two level)*** In 2003 a further component, previously documented DVT, was added to the original Wells score. Additionally, the duration of risk after surgery was increased from 4 weeks to 12 weeks[2]. This gives a possible score range of –2 to 9. This version reduced the number of risk categories from three to two: likely (2 points or more) and unlikely (less than 2 points).

Two-level DVT Wells score

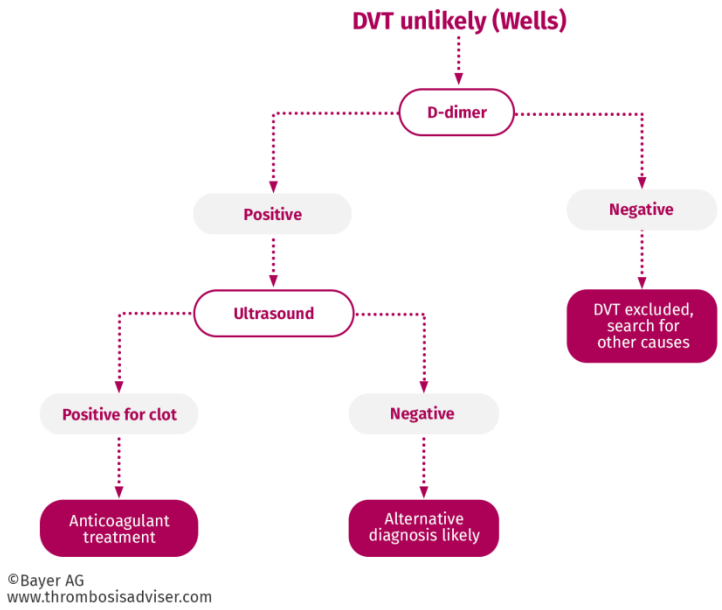
Clinical feature	Points
Active cancer (treatment ongoing, within 6 months, or palliative)	1
Paralysis, paresis or recent plaster immobilisation of the lower extremities	1
Recently bedridden for 3 days or more or major surgery within 12 weeks requiring general or regional anaesthesia	1
Localised tenderness along the distribution of the deep venous system	1
Entire leg swollen	1
Calf swelling at least 3 cm larger than asymptomatic side	1
Pitting oedema confined to the symptomatic leg	1
Collateral superficial veins (non-varicose)	1
Previously documented DVT	1
An alternative diagnosis is at least as likely as DVT	−2
Clinical probability simplified score	
DVT likely	2 points or more
DVT unlikely	1 point or less

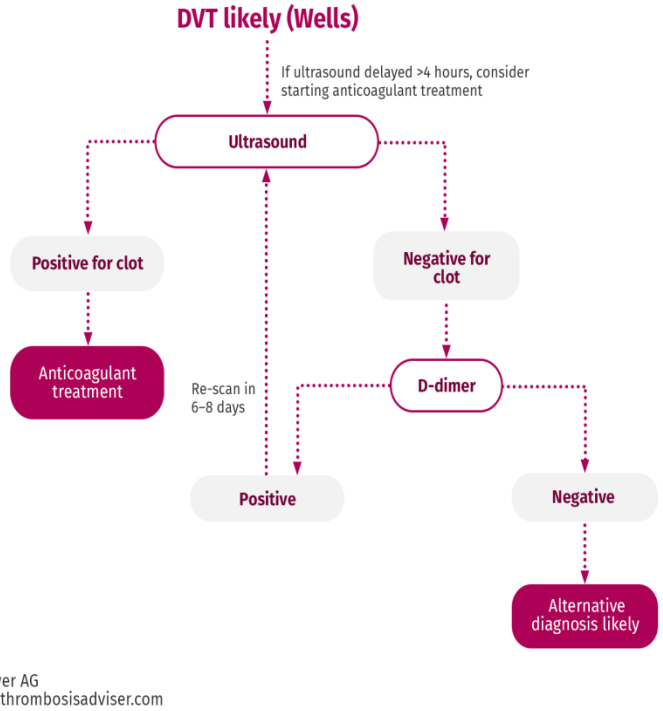
Other Causes of D-Dimer Elevation

* DIC
* Recent Surgery
* Trauma
* Malignancy
* Pregnancy
* Liver Disease
* Renal Disease

Therefore the Ddimer cannot be used reliably to exclude a VTE in patients with any of these conditions. VTE exclusion is usually only appropriate in out patient presentations. Eg ED or GP surgeries

DVT flowchart.





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Point of care

* There are now a number of point of care Ddimer kits
* These are usually qualitative and are either positive or negative.
* They can be used on whole blood from a finger prick.
* They allow rapid testing in GP surgeries or ED departments.
* Any POC testing needs to be careful managed with appropriate training and EQA considered.