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SEED Coagulation

Sysmex Educational Enhancement and Development
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The role of the D-dimer test in clinical diagnostics

What are D-dimers?

When the clotting cascade is triggered, thrombin is generated which culminates in conversion of fibrinogen into fibrin monomers. These fibrin monomers, comprised of a so-called 'E' domain and two 'D' domains, are stabilised through the process of cross linkage mediated via activated factor XIII to form an insoluble fibrin clot. This cross linkage takes the form of a covalent bond which irreversibly binds the D domains of two adjacent fibrin molecules to each other. In health, the process of clot formation is finely balanced with the simultaneous initiation of clot breakdown, so-called fibrinolysis, to ensure that the blood vessel lumen is not occluded. The process of fibrinolysis is mediated via the enzyme plasmin which has the ability to cleave both fibrin and fibrinogen into a heterogeneous mixture of so-called fibrin(ogen) degradation products (FDPs). The covalent bond formed between two D domains is however resistant to plasmin degradation. FDPs containing 2 such D domains are referred to as D-dimers and are exclusively produced from cross-linked fibrin (Fig. 1 and Fig. 2).

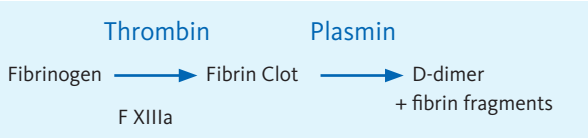

Fig. 1 *Sequence of reactions giving rise to D-dimers*

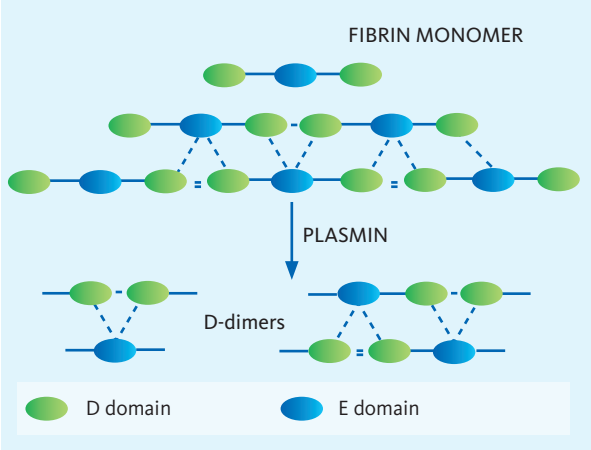

Fig. 2 *Schematic representation of D-dimer formation*

Why do we test for D-dimers?

a) To screen for an underlying thromboembolic event

The D-dimer is generated only from cross linked fibrin. Its presence is an indication that clot formation (thrombosis) and subsequent fibrinolysis has occurred. Increased levels of D-dimers occur in a variety of conditions where the coagulation system has been activated. D-dimers are therefore used as a screening test for underlying thrombosis and embolisation. Embolisation refers to the process whereby a blood clot that formed at a local site (thrombus) becomes free and is swept further downstream and subsequently gets trapped in a smaller vessel causing obstruction. This 'relocated' thrombus is referred to as an embolus. The commonest clinical presentation of this phenomenon is a pulmonary embolus, where a blood clot which was originally formed in the veins of the lower limb, pelvis or abdomen becomes lodged in the lungs.

b) To assist in the laboratory diagnosis and response to treatment of an underlying disseminated intravascular coagulopathy (DIC)

The D-dimer test is used to assist in the diagnosis of an underlying DIC which is a clinical syndrome comprised of both thrombosis and bleeding due to unregulated release of thrombin into the general circulation. This gives rise to widespread microvascular micro-clot formation which in turn causes tissue ischaemia resulting in organ damage. The body responds to this by switching on the fibrinolytic system. Plasmin is generated which breaks down fibrin in an attempt to maintain vascular patency. Fibrinogen is however also degraded in this process and results in bleeding which is exacerbated by concomitantly decreasing levels of clotting factors due to consumption during unregulated clot formation. D-dimers are also used as a monitoring test to assess if patients with DIC are getting better or not.

c) To assess the response to therapeutic fibrinolysis

Under conditions where a thrombosis is life threatening, drugs may be used to try and speed up the fibrinolytic process. The D-dimer test is used to monitor the effectiveness of therapeutic fibrinolysis in such patients.

What causes D-dimers to be elevated?

Because clot formation and clot breakdown are part of the normal haemostatic process maintaining the integrity of the vascular system, low levels of D-dimers are a normal finding. As part of the normal wear and tear of living, D-dimers tend to rise with age. Direct injury to a blood vessel, such as in cases of trauma or a surgical incision, will result in elevated D-dimer levels. D-dimers also become progressively elevated in pregnancy as a result of the haemostatic balance swinging in favour of clotting in preparation for anticipated blood loss during childbirth. As is to be expected, D-dimers become markedly elevated during childbirth in response to bleeding from the uterine wall after expulsion of the placenta. The D-dimers remain elevated for about a week post-partum and then return to baseline levels. D-dimers will also rise in response to less obvious damage, namely as a result of any inflammatory process that damages the endothelial lining of a blood vessel. D-dimers are thus the end result of the normal physiological response of the body to keep blood vessels intact and blood flowing. As previously alluded to, the D-dimer test is conventionally used as a screening test for the detection of pathological clotting, either a localised clot e. g. deep vein thrombosis or as in a systemic process e.g. DIC, for which there are several causes. The challenge is to identify which patients have elevated D-dimers that reflect what would be expected as a normal response, e.g. due to recent surgery, and those patients in whom a pathological thrombosis has occurred because the normal balance of haemostasis has been disturbed. In this regard it is important that a D-dimer test result is always interpreted in conjunction with the clinical history of the patient. The causes of elevated D-dimers are summarised in Tab. 1.

a) Pathological disturbance of the haemostatic balance	b) Endothelial damage	c) Phyisological response to natural process
■ Deep vein thrombosis and pulmonary embolism	■ Disseminated intravascular coagulopathy	■ Surgery
■ Arterial thrombosis such as stroke and myocardial infarction	■ Other microangiopathic conditions	■ Pregnancy and post-partum period
■ Cancer	■ HIV	■ Increasing age
		■ Significant bleed

Tab. 1 *Commonest causes of raised D-dimers*

HIV and D-dimers

Whilst healthcare provision in the past decade has largely focused on the prevention and management of HIV, the link between HIV infection and thrombosis has gone largely unrecognised. The availability of antiretroviral treatment has converted HIV from a 'death sentence' to a chronic disease with dramatic improvement in lifespan with individuals surviving into middle and older age. The downside of this is that they are now exposed to cardiovascular disease risk factors and live long enough to develop long term complications. An unanticipated side effect with many of the drug regimens is an altered lipid profile which has resulted in an alarming incidence of vascular complications, such as heart attacks and strokes, in HAART (Highly Active Anti-Retroviral Therapy) treated HIV positive individuals. Furthermore viral replication is associated with inflammation, endothelial cell activation and an increased tendency to thrombosis. Studies have shown that increased D-dimer levels are strongly associated with increased mortality in HIV infected individuals as well as being predictors of the immune reconstitution inflammatory syndrome (IRIS) after commencing antiretroviral treatment. IRIS is a condition that is seen in some AIDS patients shortly after starting antiretroviral therapy. Recovery of the immune system is accompanied by an overwhelming response to a previously acquired opportunistic infection that paradoxically makes the symptoms of infection worse. Because elevated D-dimers have been shown to be very good predictors of patients at high risk, there is a strong possibility that testing for D-dimers will become part of the HIV treatment programmes in the not too distant future. This would allow high risk patients to be identified to possibly receive an alternate drug regimen and almost certainly to be assigned to a much closer follow-up.

What specimens are needed for D-dimer testing?

Blood samples must be collected into 0.9% sodium citrate (light blue top tube) as for all other routine coagulation tests. The whole blood samples must be spun down and the platelet poor plasma removed. The test is performed on the plasma.

How are D-dimers tested?

D-dimer tests can be divided into manual semi-quantitative tests and automated quantitative tests. Both are based on the same test principle. Polystyrene or latex beads are coated with a monoclonal antibody that is directed against the D-dimer fragment. The antigen:antibody reaction is restricted to the cross-linked fibrin only making it highly specific for D-dimers and not other FDPs. Specificity is defined as the proportion of patients without the disease who have a negative test result. A test with high specificity will have minimal false positive results, hence a low probability that disease (in this case thromboembolism) will be incorrectly diagnosed.

The D-dimer cross linkage region has what is called a stereo-symmetrical structure i.e. the binding site for the monoclonal antibody appears twice. What this means is that one D-dimer molecule has the ability to bind to two separate beads. Consequently, when plasma containing D-dimer molecules is mixed with these antibody coated beads, the beads aggregate. The extent of aggregation is proportional to the concentration of D-dimer present. The level of D-dimer detected depends on several related factors *in vivo*, such as the severity of the thrombotic episode, the rate of cross-linked fibrin formation, and the time elapsed after the thrombotic event until blood is drawn from the patient. Irrespective of which method is adopted, positive and negative controls must be included in each batch of samples.

a) Manual D-dimer test

The plasma and the reagent containing the beads are mixed on a glass slide. If bead agglutination is visually observed the test is positive. The level at which the test is positive in undiluted plasma is the level above which D-dimers are considered to be abnormally high. The actual concentration of D-dimers present can be determined in a semi-quantitative fashion by repeating the test on serial dilutions of patient plasma. The highest dilution which gives a positive result is then used to calculate the value.

b) Automated D-dimer test

D-dimer assays on automated analysers are performed using the immunoturbidometric detection principle (Fig. 3). Here the agglutinated particles block the passage of light. The degree of light transmission is indirectly proportional to the amount of D-dimers present, i.e. low light transmission equates to high levels of D-dimers and vice versa. This is converted into an absolute quantity by means of a calibration curve. D-dimer assays can be performed on the CA-560, CA-660, CA-1500, CA-7000 and CS-series of Sysmex coagulation analysers.

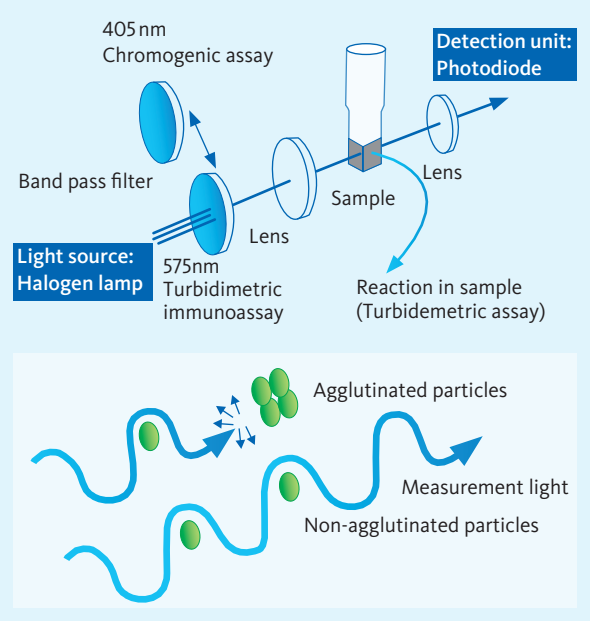

Fig. 3 *Illustration of immunoturbidometric assay detection principle used for the automated D-dimer test on the Sysmex CA-560 coagulation analyser*

How are D-dimer results reported?

It is important to be aware of the fact that the unit of measure of reporting quantitative D-dimer assays is not uniform. Depending on the specific set of reagents, the results may be expressed as either D-dimer units or fibrinogen equivalent units (FEU). In broad terms, 1 D-dimer unit is equal to 2 FEU. This is because one D-dimer molecule is made up of components from 2 fibrinogen molecules. This is very important to consider as the clinically important cut-off level to assign a positive or negative result will be double the value for an assay reported in FEU. Furthermore it should be noted that the concentration of D-dimers is reported in a non-consistent way, although they are all metrically equal (Tab. 2). Irrespective of whether values are measured in D-dimer units or FEU, the units used to report the concentration levels are the same, e. g. mg/L. Reference should always be made to whether the result obtained reflects FEU mg/L or D-dimer units mg/L but this is seldom done. The potential for confusion and incorrect result interpretation is high. The reference values used by a laboratory must be specific for the test that is in use, i. e. FEU or D-dimer units. They are not interchangeable.

D-dimer units	Fibrinogen Equivalent units
0.25 mg/L	0.5 mg/L
250 µg/L	500 µg/L
250 ng/mL	500 ng/mL

(Values shown are all equivalent and reflect the usual cut-off level used to assign a positive or negative determination)

Tab. 2 *Examples of units used to report D-dimer concentrations in plasma*

How are D-dimer results interpreted?

In the context of thromboembolism exclusion, it is strongly recommended that D-dimer testing is conducted in conjunction with clinical pre-test probability (PTP) assessment. PTP assessment categorises patients into different groups based on how likely it is, based on clinical assessment alone, that they have in fact had a thrombosis. This greatly enhances predictive power of D-dimer screening test. As the PTP rises, a negative test is increasingly unlikely to be false.

If D-dimers are being used solely as a screening test, a positive or negative finding is sufficient. The cut-off value which defines this must however be carefully established to ensure that there are not too many false negative results. A negative test rules out a thromboembolic event and those patients are spared any further radiological investigations. However, a positive test result in a patient with a clinical suspicion of thrombosis will have to undergo some kind of imaging process e. g. doppler ultrasound, CT scan etc. to objectively confirm the presence of clot. In other words, a positive result is not diagnostic of a thrombosis as there are other causes of elevated D-dimers as shown in Tab. 1.

A quantitative result is especially important for the diagnosis of DIC and serial monitoring thereafter to assess response to any treatment intervention taken. Likewise, quantitative D-dimers are required to monitor patients undergoing fibrinolysis.

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