

D Dimer...Pre Analytical Phase.....In Covid Era

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D-dimer is a plasmin derived soluble degradation product of cross-linked fibrin. Formation of D dimer requires the sequential activity of three substances as thrombin, activated factor XIII and plasmin. The first step in the formation D-dimer involves the action of thrombin on fibrinogen and convert it into fibrin monomers. Fibrinogen is a soluble plasma glycoprotein composed of three different pairs of polypeptide chains that are α , β , and γ connecting two-outer D-domains to the central E-domain. Thrombin cleaves two cryptic polymerization sites located on the E-domain, causes generation of both highly self-adhesive fibrin monomers and fibrinopeptides A and B. Fibrin monomers then bind to one another to form a soluble meshwork. Simultaneously, the complex between soluble fibrin polymers, thrombin, and plasma factor XIII promotes the formation of factor XIIIa that is activated factor thirteen, which catalyzes covalent cross-linking of fibrin polymer via intermolecular bonds and leads to the formation of stable and insoluble clots. Now the role comes of fibrinolytic pathway which causes degradation of stabilized clots through plasmin activation. Plasmin is activated from plasminogen by tissue plasminogen activator (t-PA) at the fibrin surface and cleaves fibrin at specific sites. The products of this reaction is called fibrin degradation products and this create a vast array of molecular weights. In the first steps of fibrinolysis, FDP are large. Continual breakdown generates the fragment D-dimer/fragment E complex (DD/E), which has two covalently-bound D-domain.

Thus, the process begins by action of thrombin during the coagulation pathway which converts soluble fibrinogen to fibrin monomers. Fibrin is strengthen by factor XIIIa which is a transglutaminase activated by thrombin, which cross-links the D domains of adjacent fibrin monomers, as well as the α -chains of opposing monomers to form D-dimer and α -polymers, respectively. Therefore, formation of cross-linked fibrin, the substrate for D-dimer formation, requires activation of coagulation with the resultant generation of thrombin, conversion of fibrinogen to fibrin monomers, polymerization of the fibrin monomers to form fibrin polymers, and cross-linking of the fibrin polymers by factor XIIIa. Therefore, the presence of D-dimer reflects simultaneous activation of both coagulation and fibrinolysis.

Thereby this test interpretation must be done by considering all parameters most importantly preanalytical variables and interfering substances, patient drug therapy and any underlying disease which affect test result. Here we discuss preanalytical phase which is a “processes that start, from the clinician’s request for the test, preparation and identification of the patient, collection of the primary sample and the transport to and within the laboratory and end when the analytical examination begins.

Firstly, the collection of sample is done with the use of straight needles with a diameter ranging from 19 to 22 gauge, which is recommended if not considered it cause lysis of red blood cells and due to this hemolysis erroneous result occur. The use of butterfly device is usually discouraged. This can only be considered in geriatrics, oncology, pediatrics patients. Excessive manipulation of veins should not be done as it can cause clotting of blood so results come to be wrong.

Secondly the material of glass tubes which must be silicone coated or polypropylene plastic tubes are preferred for hemostasis testing because these are of inert material thus it will prevent initiation of clotting in the tube.

For D dimer assay use of 3.2% buffered sodium citrate anticoagulant in ratio of 9/1 is to be maintained. Thus, sample must be correctly filled, not hemolysed, not lipaemic. Blood should always flow freely into the tube and be promptly mixed within 30 seconds after

phlebotomy also there must be 3 to 6 complete inversions so that complete distribution of anticoagulant with the sample. The inversions must be gentle so that there will be no trauma to red cell membrane.

Routinely samples are collected by using Tourniquets as it temporarily obstruct the vein flow and helps the phlebotomist to identify vein. The tourniquet should be removed as soon as the needle is in the vein. The most common drawback of prolonged tourniquet is hemoconcentration and clot formation, which may lead to erroneous result. Many studies suggested as prolong tourniquet application of three minutes are significantly increased result of D dimer by 13.4%.

Samples should be delivered to the laboratory at temperature of 15 - 22°C in the shortest possible time generally < 1h after collection. Before being processed samples should be carefully checked for the presence of inappropriate additive, identification errors, insufficient volume or 9/1 ratio not maintained and even for the presence of clots.

Thus, various interferences occurring in the preanalytical phase of D dimer testing are categorized into paraproteinemia, icterus, lipemia, and hemolysis. In vitro hemolysis still represents one of the most frequent cause of preanalytical error in clinical laboratories, with a prevalence ranging between 50 - 70%. Causes of hemolysis of samples are either from underlying disease of patients as hemolytic anemia, metabolic disorders, infectious agents. Patient preparation error as during phlebotomy includes needle gauge, tourniquet time, traumatic draw, no mixing or vigorous mixing of sample, transport, processing and storage of samples.

Thus in nut shell we as lab physician or as clinician or as surgeons must be aware of sample processing steps of all the test which are to be interpreted in concern to patients. So just to conclude the report is not sufficient, specially in this covid era because D dimer is a very sensitive test which must be interpreted by clinicopathology correlation otherwise it may lead to erroneous diagnosis, wrong treatment and ultimately the economic burden and mental trauma to the patient and during this Covid era we have to work up on patient extensively as sometimes a little ignorance costs very high. Thus, clinicians and intensivists must know all the preanalytical variables which affect D dimer test.

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