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LABORATORY TESTING FOR HEPARIN INDUCED THROMBOCYTOPENIA

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Abstract

Heparin-induced thrombocytopenia (HIT) is a common clinical condition. There are two types of Heparin-induced thrombocytopenia: type I and type II. Type I is a self-limiting condition, where platelet counts will return to normal within a few days. Type II is a very critical clinical condition associated with thrombotic events in arterial and venous systems. Though its clinical status raises a high degree of suspicion, definitive diagnosis includes laboratory testing for platelet activation (SRA) and antigen assays (PF4 ELISA). The purpose of this review is to provide an overview of the biochemical markers for Heparin-induced thrombocytopenia, especially for type II.

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INTRODUCTION

Heparin was discovered by Jay McLean, a second-year medical student in 1916[1]. Heparin has a rapid onset of function, predictable action, predictable laboratory testing, is widely available, inexpensive and easily reversible action. Because of the above characteristics, Heparin is used in cardiopulmonary bypass, extracorporeal membrane circuits, deep vein thrombosis etc[2]. Heparin is one of the most commonly used medication. More than 1 trillion units of heparin are used in the United States annually[3] and about 12 million patients receive heparin as treatment annually[4].

Heparin-induced thrombocytopenia is a well-known complication following heparin therapy. This occurs in 5% of patients who receive heparin treatment.

There are two types of Heparin-induced thrombocytopenia, type I and type II. Heparin-induced thrombocytopenia Type I is a transient non-immune mediated form, causing moderate thrombocytopenia (<100,000) without thromboembolism. Incidence is around 10-20% of patients who are exposed to Heparin and occurs within 48-72 hours. Platelet count returns to normal within 4 days. This is not associated with the risk of thrombosis[5].

Heparin-induced thrombocytopenia type II is immune-mediated and has a more critical presentation. The principal antigen is a multimolecular platelet factor 4 (PF4). The incidence of Heparin-induced thrombocytopenia type II varies from 0.4% to 4.9 %. Thrombotic events occur in 10-20 % of patients with type II thrombocytopenia [6].

Type II Heparin-induced thrombocytopenia is characterized by a drastic fall of platelet-count of more than 50% in 5 to 10 days after commencement of Heparin. Associated with thrombocytopenia, this leads to hypercoagulability and thrombotic complications. The incidence of thrombotic events in type II occurs in 30-80% of patients. These thrombotic events can occur either in arterial or venous systems. Other complications include deep vein thrombosis, pulmonary embolism, myocardial infarction, stroke and end-organ failure [7].

DISCUSSION

Heparin-induced thrombocytopenia Type I is caused by a non immunological response to Heparin, mainly by the interaction between Heparin and platelets, which leads to platelet clumping and sequestration thereby causing thrombocytopenia. Heparin therapy can be continued and platelet count will return to normal within few days [8].

Heparin-induced thrombocytopenia Type II is due to the production of antibodies, which activates platelets after Heparin administration. The principal antigen is a complex between Heparin and Platelet factor 4 (PF4). This Heparin and PF4 complex then generates antibodies, which leads to the release of prothrombotic platelet-derived microparticles, platelet consumption, and thrombocytopenia. These microparticles, in turn, promote excessive thrombin generation, frequently resulting in thrombosis. These reactions lead to interactions between the antigen-antibody complex and macrophages which then lead to the creation of tissue factor production. The above factors lead to activation of the coagulation cascade and thrombin generation[9].

Though the diagnosis of Heparin-induced thrombocytopenia Type II is a clinical one, a number of biochemical markers can assist in confirming the diagnosis. There are two types of serologic tests to diagnose Heparin-induced thrombocytopenia in the laboratory. Activations tests detects platelet activation in the presence of patient antibodies and heparin. An example of this type of test is the Serotonin Release Assay (SRA). Antigen tests detect the presence of Heparin-dependent platelet-reactive antibodies. In this test, antibody binding to complexes of PF4 bound to heparin or a heparin-like molecule is detected. An example of this type of test is the PF4 ELISA technique[10].

Among the available laboratory tests, Serotonin Release Assay (SRA) is the gold standard test in the diagnosis of Heparin-induced thrombocytopenia Type II. In this test, normal donor platelets are radiolabelled with serotonin and washed. These platelets become very sensitive to serum from patients with HIT II. To these radiolabeled platelets,

Heparin (both therapeutic and high concentrations) and the patient's serum are added. With a positive result, the release of serotonin occurs with significant activation at therapeutic levels of Heparin and an absence of a significant effect at high levels of Heparin. SRA has high sensitivity (90% to 98%) and specificity (above 95% in early phases and 80% to 97% for late-phase platelet declines)[11].

Another sensitive test is the Platelet factor 4 (PF4) enzyme-linked immunosorbent assay technique (PF4 ELISA), which measures antibodies to PF4 complex with Heparin or other polyanions. The PF4 ELISA has a sensitivity of >90% [12]. There are two types of ELISA techniques available for Heparin-induced thrombocytopenia: IgG specific ELISA technique and Polyspecific Assay. Diagnostic accuracy is superior in IgG specific immunoassay[13] which has a high sensitivity (> 99%). This technique detects nonpathogenic and weak antibodies resulting in too many false positive tests, hence it has a poor specificity (30%-70%) [14].

Heparin-induced platelet aggregation (HIPA) is another available test; this test assesses the aggregation of platelets induced by the serum of patients in the presence of Heparin. A positive aggregation study was defined as a difference of 20% or more in aggregation response between patient and normal PPP (platelet-poor plasma) in the presence of heparin. The test is interpreted by visual assessment of aggregation[15].

Rapid screening for HIT antibodies can be performed using PF4-dependent particle-based immunoassays. One such assay, the particle gel immunoassay, has the advantage of higher diagnostic specificity than the ELISA techniques (although lower than the platelet activation assays), but the main disadvantage is false-negative results in approximately 5% of HIT cases [16].

There is no single test which has 100% sensitivity and specificity, but SRA, PF4 ELISA, HIPA, particle gel immunoassay are definitely close to this goal.

CONCLUSION

Heparin-induced thrombocytopenia is a common clinical condition. Though HIT type I is a self-limiting condition, HIT type II is a

very critical clinical condition. If undetected and untreated, it can lead to potentially life-threatening complications including death. Laboratory tests are aimed at detecting the presence of immunogenic Heparin-PF4 complexes, which are a characteristic feature of Heparin-induced thrombocytopenia. Though the high degree of clinical suspicion is necessary, laboratory tests such as Serotonin Release assay (SRA) and IgG-specific PF4 ELISA are very helpful in confirming the diagnosis of heparin-induced thrombocytopenia.

Conflict of Interest Statement-

There is no conflict of interest.

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