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Pre-analytical Variables in Coagulation Testing: Avoiding Diagnostic Errors in Hemostasis

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Abstract

Emergency physician tends to assume that the sample analysed by the lab and the results authenticated and released reflects what is happening in the patient. It is important to note that the clinical decision making process based on correlation with lab reports will be flawed and may have disastrous consequences if a system exists where the pre-analytical variables of Lab testing, especially coagulation tests are not considered and monitored. This review essentially highlights most important pre-analytical variables with the underlying mechanisms that if ignored can lead to correct analysis but wrong results if correlated to patients condition.

Keywords: Coagulation; Hemostasis; Phlebotomy; Fibrinolytic System.

Introduction

In Accident & Emergency Medicine "Doing the right thing" alone will not suffice, "Doing the thing right also holds equal importance". Large multi-speciality tertiary care hospitals usually have busy Emergency Medicine department with a constant inflow of patients suffering from varying acute disorders together with a good number of road traffic accident cases. Emergency Physicians are always under pressure to deliver best possible care within a short time frame and save as many lives as possible. Although clinical decision making relies heavily on symptomatology and physical sign elicitation, backup results authenticated by Lab of basic parameters play a vital role in confirmation of primary diagnosis or narrowing down the differential diagnosis, choice of medications and short term prognostication.

Pre-analytical, Analytical & Post-analytical phase of Diagnostic Services

Test for Hemostasis is an important component of all primary tests ordered by the Emergency Physician in most of the patients streaming to the Emergency Department. These tests are used to assess whether a particular patient is at risk to bleed or clot. Choice of tests ordered, time to draw blood, positive patient identification, phlebotomy process, choice of anticoagulant, sample transport to Lab, sample accessioning by the Lab and sample storage before analysis form the pre-analytical aspect of Lab testing. Actual testing procedure constitutes the analytical phase of Lab testing. Laboratory Information System (LIS) with its integration to the Hospital Management System ensures that the test reports authenticated by the Lab Physician are accessed by the Emergency Physician in a regulated and timely fashion. This forms the post-analytical phase of Lab testing.

Brief overview of Hemostasis

Most of the time, we equate hemostasis with coagulation cascade and assessment of its surrogate markers. However hemostasis is far more complicated. It is a complex dynamic interaction between several key players in the body namely the vascular endothelium, platelets, coagulation factors, the fibrinolytic system and inhibitors of the fibrinolytic system [1, 2]. Checks and balances are present in every step. Bleeding or thrombosis occurs when the interactions are disrupted due to deficiency or dysfunction of the important components [3].

Hemostasis unit of the Hematopathology department usually performs the routine coagulation tests like the Prothrombin time (PT), Activated plasma thromboplastin time (APTT), Thrombin time (TT), Fibrinogen assay and D-Dimer / FDP tests. A major issue in coagulation testing is overlooked and which has in the recent times begun to be the topic of interest and research: Pre-Analytical Variables.

Concept of Pre-Analytical Variables

Are essentially the problems and deviations that may arises prior to sample testing including but not limited to sample collection, handling, transport, processing in accession and storage prior to testing. When a sample is inadequate, it is rejected by the lab. Accredited Labs have well defined sample rejection guidelines, its standard operating procedure (SOP) and records of its implementation. However the system is flawed because a major portion of the pre-analytical events occur outside the purview of the lab. Lab is unaware most of the time that an adverse event has occurred with a particular sample. Hence it is not always clear when an unsuitable sample has arrived in the lab, tested in good faith and results released [4, 5].

Pre-Analytical Variables

Patient Identification

Patient misidentification is associated wrong reports resulting in worst clinical outcome due to misdiagnosis and inappropriate treatment. In both outdoor and indoor settings double identifiers is preferred. Identification from the current prescription and talking with the patient / relatives in OPD and from the bar coded wrist band together with verbal communication with the patient / attendant / nursing staff will help reduce the incidents of blood being drawn from the wrong patient [6].

Sample Identification

Post phlebotomy collection vials usually are identified by bar coded tube labels pasted on it[7].These bar coded tube labels should be generated bedside after collection is completed for that particular patient. The practice of scribbling by pen few patient data on the filled vials and moving to the next patient for phlebotomy keeping the generation of bar coded tube labels and pasting on the vials based on the scribbled data for a later time at the nursing station has to be discouraged .

Phlebotomy Process

Tourniquet time of more than one minute results in hemoconcentration. Further it stimulates the endothelium and activates the coagulation cascade at multilevel in the vessel. In both the cases results are altered as ratio of plasma to anticoagulant is changed in the former event and consumption of factors occurs at the later event. Use of tourniquet of < 1 minute and release of the same as soon as blood flow into the vial, is recommended.

Slow venepuncture and difficult venepuncture irritates the vessel wall and causes in vivo activation of cascade resulting from local release of tissue factor. If the vein is located by multiple passes by the needle / manipulation of the needle or blood comes out in a slow stream another venipuncture site have to be selected.

Drawing blood from IV infusion line or phlebotomy from a site downstream to the infusion site is discouraged. It leads to dilution of coagulation factors leading to erroneous results, when drawing blood from peripheral or central venous lines predraw flushing and discard of the initial sample is necessary. This avoids sample dilution and sample contamination. Too large needle (<16G) and too small needle (>25G) needs to be avoided. Former causes more tissue damage in the wall of the vein causing premature start of the clotting process resulting in false low results. Later cases cause hemolysis of the sample. Heparinised needles used in blood gas analysis needs to be avoided at all costs [11].

According to CLSI guidelines on order of blood draw [8], coagulation test sample to be preferably drawn first (second to blood culture set). These avoid sample contamination from subsequent anticoagulants and clot activators. Also effect of local release of tissue factor is minimal. If winged collection set is used, it is necessary to discard the first sample to minimise the effect of contaminants and air in the tube [9].

Sample vial should be filled up to the mentioned mark or 90% of the total prescribed volume. Underfilling leads to low sample volume and excess calcium binding citrate causing falsely prolonged coagulation results [10]. Blood drawn into the citrate vial if not adequate in volume should never be topped up from another vial having same or different anticoagulant. In the former case it results in doubling up of the anticoagulant and dilution of plasma sample. In the later case introduction of calcium chelating EDTA or clot activators results early and spurious start of the coagulation process and results in false low test results.

Thorough mixing of sample after its collection in the vial with the anticoagulant present in the vial is to be done by end over end inversion 3-6 times gently. This prevents clot formation. Clot if formed however small it may be leads to erroneous results. The practice of manually removing a clot if formed in the vial and the sending the vial to the lab for testing instead of rejecting the sample is to be strongly discouraged. Conversely vigorous shaking of the filled vial by the phlebotomist / others can lead to hemolysis of the sample or spurious factor activation resulting in wrong results with disastrous consequences to medication and safety.

Choice of the Anticoagulant

CLSI guidelines recommend use of 3.2% citrate instead of 3.8% citrate except for few specific applications. Samples drawn into 3.8% citrate overestimate PT & APTT results and lower fibrinogen values. Biological reference ranges derived from literature review is mostly based on coagulation study on sample drawn in 3.2% citrate vials [12].

Sample transport to the Lab

Current recommendation states, sample to be transported to the Lab as soon as possible in non refrigerated state at ambient temperature (15-22®C) [13]. Emergency Physician needs to understand that correct results for all coagulation tests are possible if analysis is done within 4 hrs. Hence sample should be send to lab stat and not collectively at predetermined time. Putting the sample in the refrigerator before despatch to Lab is to be avoided at all costs. It is important to note that APTT test of patients getting unfractionated heparin needs to be done within 1hr [14]. Delay causes heparin neutralization by platelets resulting in wrong results.

Conclusion

Preanalytical process variation remains an important cause of diagnostic errors. A large number of wrong results can be intercepted before release of reports if the concepts and knowledge of Pre-Analytical Variables is available to the Emergency Physician.

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