Laboratory Guidelines for Antithrombin, Proteins C and S, and APC-Resistance Testing

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Venous thromboembolism (VTE) is one of the most common causes of mortality and morbidity in the Western World. Both acquired and genetic causes of VTE have been identified. Some genetic causes of thrombosis have been identified in patients with Thrombophilia. Laboratory evaluation of patients with thrombophilia can be undertaken to help diagnose the cause of VTE. As with any laboratory test, numerous (patient, pre-analytical and analytical) variables can cause erroneous interpretation of results. Three major genes (Protein C [PC], Protein S [PS] and Antithrombin [AT], and one polymorphism, Activated Protein C Resistance [APC-R]) have been identified as having significant relevance in Thrombophilia expression. These are routinely screened by plasma assays during the investigation of the cause(s) of VTE.

This presentation will describe common problems associated with thrombophilia testing. This presentation will discuss the most common causes of erroneous diagnosis. A brief review of pre-analytical and post-analytical variables affecting results and interpretations will be presented. The majority of the lecture will focus on discussions of analytical issues. The major recommendations for testing of plasma levels of PC, PS, AT and APC-R are presented below.

General Recommendations:

1. Patient variability, pre-analytical variables and post-analytical issues may cause an erroneous result and an incorrect interpretation. These problematic sources must be kept to a minimum to ensure accurate diagnosis.
2. Laboratory diagnosis of abnormal levels does not always reflect a genetic deficiency or clinical phenotype expression. Repeating abnormal values in 4-8 weeks to confirm a deficiency must be performed.
3. Not all kits or Laboratory Developed Tests measure plasma PC, PS, AT or APC-R in the same manner. Normal or abnormal values may be generated on the same sample or patient when using different kits or assay methods.
4. Testing at the correct time is paramount to an accurate diagnoses. Testing at incorrect times may result in inaccurate results and incorrect interpretation.
5. Testing in the presence of anticoagulants may result in an incorrect interpretation.
6. Testing in the presence of acquired abnormalities or conditions (such as Lupus Anticoagulant, DIC or surgery) may result in an incorrect interpretation.
7. Comparison of patient's result to the incorrect Reference Interval can lead to an incorrect interpretation and incorrect diagnosis.

Protein C Testing:

1. To determine the presence of PC deficiency, use either a chromogenic or clot based assay. The chromogenic PC assay is recommended as a cost savings measure but has more limitations.
2. If the PC activity is abnormal, then a PC antigen assay is performed to determine if the PC deficiency is a Type I or Type II.
3. Patient's age must be taken into account for final diagnosis. Pediatric and newborn Reference Intervals are lower than adult ranges.

Protein S Testing:

1. The initial or screening assay should be a Free PS antigen assay.
2. The initial assay should NOT be a PS activity assay. A significant percentage of spuriously low values of PS activity have been reported in normal individuals.
3. If the Free PS antigen assay is abnormal, then PS activity and Total PS antigen assays should be performed to determine the PS deficiency type.
4. For determination of PS deficiency, compare the patient's value to age and possibly gender specific Reference Intervals.
5. PS assays should NOT be performed during pregnancy or hormone therapy.

Antithrombin Testing:

1. When screening for AT deficiency, a chromogenic assay using factor Xa or thrombin and heparin is recommended.
2. If the activity assay is abnormal, then an AT antigen assay is performed. The ratio confirms Type I or Type II AT deficiency.
3. If Type II deficiency is detected, further specialized testing can be performed to determine the Type II AT deficiency subclass (Reactive Site, Heparin Binding Site or Pleiotropic).

APC-R Testing:

1. When screening for APC-R, a second generation clotting assay (sample diluted in factor V deficient plasma) should be used is recommended.
2. Normalized ratio of the APC-R result will eliminate potential artifacts of acquired conditions.
2. If the result is abnormal, then a genetic confirmation of the abnormality (factor V_{Leiden}) should be performed.

References:

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