Challenges in laboratory detection of haemostasis inhibitors

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Introduction

Acquired functional inhibitors of coagulation comprise inhibitors that interfere with individual coagulation factors leading to bleeding complications. These inhibitors are immunoglobulins that arise in certain circumstances after autologous (i.e., leading to acquired haemophilia) or allogeneic (i.e., in congenital haemophilia) exposure to coagulation factors. The immunoglobulins bind to functional epitopes on individual coagulation proteins, thereby inhibiting their function and which results in prolongation of clotting tests and clinically in bleeding complications. This type of inhibitors are clearly distinct from lupus anticoagulants (LA), immunoglobulins which also prolong clotting tests. LA are directed against a complex of phospholipids and coagulation factors rather than against individual coagulation factors, and are, in contrast with these inhibitors, potential thrombogenic. Unfortunately, LA and inhibitors against individual coagulation factors are not always easy to distinguish from each other, either by clinical means or by laboratory assays. Therefore, the recognition and classification of coagulation inhibitors remains a challenge, even for more experienced diagnostic laboratories.

Prolonged coagulation screening assays of native plasma may indicate the presence of inhibitors. However, the interference of coagulation factor deficiencies, which also prolong the clotting time, has to be excluded. Therefore, mixing tests with patient plasma and normal plasma are indicated. A prolonged clotting time that is corrected in the mixing test is suggestive for a factor (or factors) deficiency and absence of an inhibitor, although this may be contingent on the way in which mixing studies are performed and a correct interpretation of ‘normalised’ test results. For example, an immediately performed mixing test that results in a correction will not exclude an inhibitor to factor (F) VIII, since such inhibitors are often time and temperature dependent.

A prolonged clotting time that is not corrected in the mixing test is suggestive for the presence of an inhibitor. However, LA, heparin and/or other potential therapeutically administered direct anti-IIa and anti-Xa inhibitors (e.g., argatroban, dabigatran, rivaroxaban) still have to be excluded. Quantification of inhibitors can be performed by the Nijmegen or Bethesda assays. Both methods have been extensively tested and described for the quantification of FVIII inhibitors but may also be applied for other coagulation inhibitors.

Inter-laboratory surveys on inhibitor detection and quantification include or exclude the presence of LA, and decreased factor activities should be confirmed or invalidated, when possible, by assay with chromogenic substrate methods.

The most remarkable observation from a workshop, organized by the ECAT in 2008, was the lack of a clear step-by-step analysis of the nature of an inhibitor once a positive mixing test has been detected. Based on general experiences as well as on the results of the workshop, we suggest a diagnostic route for the confirmation or exclusion of haemostasis-inhibitors as described below.

Interpretation of laboratory inhibitor testing

The results of laboratory testing may have direct implications for the clinical management of patients. Unfortunately, especially the differentiation between LA and mild inhibitors to individual coagulation factors remains a problem, mainly in non-specialized laboratories. No data are available on the problem in “real life”, but results of external quality survey programs show significant evidence of misclassification. A correct differential diagnosis between LA and factor inhibitor is extremely important for adequate management of patients. LA positive patients generally have to be protected against the risk of undesirably thrombotic events, whereas patients with inhibitors to individual coagulations factors generally suffer from a bleeding diagnosis of variable severity, up to life-threatening hemorrhages. False diagnosis of LA and/or inhibitors to individual factors may therefore result in mistreatment, e.g., antithrombotic treatment in patients with factor inhibitors or supply of coagulation factor products in LA patients. Therefore, in order to obtain a high level of quality, a positive mixing test should always be followed by a LA confirmation test to include or exclude the presence of LA, and decreased factor activities should be confirmed or invalidated, when possible, by assay with chromogenic substrate methods.

Diagnostic algorithm for inhibitor testing

1. When a screen test appears to be prolonged, repeat the assay in 1:1 mixture with pH-stabilized normal plasma (pH = 7.4), immediately after mixing and, if required because of suspicion of FVIII inhibitor, 1-2 hours after incubation of the mixture at 37 °C. A parallel assay is performed on a reference sample with identical clotting time (e.g., haemophilic plasma without inhibitors).

Each laboratory has to develop its own experience on the evaluation of mixing tests and include appropriate control samples in the assay. When the results of the mixing 3 tests are within the reference ranges, the presence of a weak inhibitor may still be possible. Therefore, after excluding heparin, the assay of coagulation factors in the native sample and, when one or more factors are decreased, in a mix of patient plasma and nor-
normal plasma after 1-2 hours at 37 °C is indicated. A decreased recovery in the mix sample is suggestive for an inhibitor. An inhibitor assay has to be performed for the clinically significant factor(s).

2. If the mixing test is prolonged, perform a TT and rule out an incorrect type of sample, e.g., EDTA-anticoagulated plasma and serum (17). If the TT is prolonged, perform a TT in a patient/normal plasma mix and a heparin neutralization test (and eventually anti-Xa assay) to exclude or confirm the presence of heparin and/or another anticoagulant inhibitor.
   - If heparin is present (i.e., normalization of TT after neutralization and/or positive anti-Xa activity), the sample is ideally discarded and a new sample obtained for further testing.
   - If no heparin is present (i.e., persisting prolonged TT in mixture after neutralization with protamin sulphate) differential diagnosis may result in thrombin inhibitor or abnormal fibrinogen (18,19).

3. If TT of undiluted plasma is normal, perform LA test with LA sensitive reagents, and, dependent on the clinical anamnesis, clotting factor assays with LA insensitive reagents to determine whether there is an isolated factor deficiency (congenital or acquired).

4. If the LA confirmation test is negative but one or more clotting factor activities are abnormal with a lack of parallelism in factor assays, most probably this is caused by the presence of LA (20). Perform chromogenic assays of coagulation factors.

A discrepancy between the one-stage assay and the chromogenic assay indicates the presence of LA, despite the negative confirmation test. If there is no discrepancy between the one-stage assay and the chromogenic assay, the presence of an inhibitor against an individual coagulation factor is likely. Perform an inhibitor assay against the concerning factor.

A flowchart of the diagnostic algorithm for acquired coagulation inhibitors is shown in Figure 1.

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**References**