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REGULAR ARTICLE

Evaluation of a new silica clotting time in the diagnosis of lupus anticoagulants

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Abstract

Introduction: A new commercial silica clotting time (SCT), the HemosIL™ SCT assay (Instrumentation Laboratory, Milan, Italy) was evaluated in the laboratory diagnosis of lupus anticoagulants (LAC). This integrated test system for screening and confirmation was compared with the frequently used aPTT-based PTT-LA and Staclot-LA (Diagnostica Stago, Asnières, France) in a patient population investigated for LAC and in a subpopulation who met the clinical criteria for antiphospholipid syndrome (APS).
Materials and methods: 201 samples were analysed with the HemosIL™ SCT assay. Own reference values were calculated. Results are expressed as measured clotting times in seconds or as normalised ratios.
Results: SCT screen and PTT-LA had a sensitivity of, 61.1% and 63.8%, respectively. Normalising the results gained sensitivity up to 72.2% and 90%, respectively. The confirmation SCT and the Staclot-LA had a sensitivity of 30.6% and 63.9% with a specificity of 86.7% and 100%, respectively. Sensitivity of SCT for detecting LAC in clinical criteria positive patients was lower compared to aPTT and dRVVT (45.8% versus 66.7% and 65%). Combination of SCT/dRVVT and aPTT/dRVVT gave a sensitivity of 51.2% and 63.6%, with a specificity of 50.0% and 52.3%, respectively.
Conclusions: In comparison with PTT-LA as screening test, the SCT screen shows an acceptable sensitivity. However, the HemosIL™ SCT assay including the confirmation step, has a much lower sensitivity in the diagnosis of LAC in comparison with the Staclot-LA test. Combining the HemosIL™ SCT assay with dRVVT results in a better sensitivity, although lower than the combined aPTT/dRVVT based method as usually performed in our lab.

Abbreviations: LAC, lupus anticoagulants; APS, antiphospholipid syndrome; ISTH, International Society of Thrombosis and Haemostasis; aPTT, activated partial thromboplastin time; dRVVT, diluted Russell's viper venom time; SCT, silica clotting time; aPL, antiphospholipid; β2 GPI, β2 -glycoprotein I; aCL, anticardiolipin; ROC, receiver operator curve; CV, coefficient of variation; NP, normal pooled plasma; sec, seconds; CSCT, colloidal silica clotting time; KCT, kaolin clotting time.

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Introduction

The antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid (aPL) antibodies such as β_2-glycoprotein I (β_2GPI) dependent anticardiolipin (aCL) antibodies and/or lupus anticoagulants (LAC). These antibodies are thought to be involved in the development of venous and/or arterial thrombosis and pregnancy morbidity [1].

Laboratory diagnosis of APS is based on phospholipid-dependent coagulation tests to demonstrate the presence of a coagulation inhibitor (the LAC) and the demonstration of the presence of aCL antibodies by an immunological method [2,3].

An overview of clinical studies indicated that the presence of LAC correlates better with thromboembolic complications than the presence of aCL antibodies [4]. Besides, several reports show that β_2GPI antibodies identify LAC positive patients at risk for thrombosis [5]. When these antibodies are responsible for LAC activity, there is a high association with thrombotic events [5–10].

The clinical and serological criteria necessary for the diagnosis of APS are summarized in the Sapporo criteria [1], which were amended recently [11]. Since the publication of the Sapporo criteria in 1999, new clinical, laboratory and experimental insights have led to an updated international consensus statement on the classification criteria for APS [11].

APS requires the combination of one clinical and one laboratory criterion.

Laboratory criteria consist of the presence of a LAC, aCL antibodies or β_2GPI antibodies [11].

Laboratory diagnosis of LAC performed according to the revised criteria proposed by the Subcommittee for Standardisation of Lupus Anticoagulant of the International Society of Thrombosis and Haemostasis (ISTH) includes mixing studies, screening and confirmation tests [12]. Prolongation of phospholipid dependent clotting assays are used as positive criterion for the screening assays, evidence of inhibition is demonstrated by mixing studies and lack of a specific inhibitor of any specific coagulation factor has to be ruled out [12].

Besides screening tests and mixing studies, confirmation tests are essential in the diagnosis of LAC. This step is extremely important to distinguish LAC from specific factor inhibitors because the latter are associated with a bleeding risk. In these tests the phospholipid dependence of an anticoagulant is demonstrated by relative correction of the abnormal clotting time following addition of phospholipids or platelets [3,12].

No definite recommendations are given on the assays of choice for LAC testing but both activated partial thromboplastin time (aPTT)-based assays and dilute Russell's viper venom time (dRVVT) are considered as suitable [3,13,14].

One positive test suffices for LAC positivity. As no single test is 100% sensitive for LAC, it is advised to use two or more tests with different assay principles before the presence of LAC can be excluded [11].

Since the laboratory demonstration of LAC is considered to have a stronger link to thromboembolic events than aCL, the search for sensitive and specific test systems for detecting LAC goes on. Moreover, the dRVVT test was found to be predictive for the risk of thrombosis [11,15,16].

I evaluated a new commercial Silica Clotting Time (SCT), the HemosIL™ SCT assay (Instrumentation Laboratory, Milan, Italy), on a LAC positive and a LAC negative patient population. The kit contains a low concentration phospholipid screening reagent and a high concentration phospholipid confirmation reagent.

Aim of the study was to evaluate the performance of the SCT as screening and confirmation test in the intrinsic coagulation pathway, in comparison with the frequently used aPTT in the determination of LAC. In this study the sensitivity and specificity of this SCT was also evaluated in the detection of LAC in patients who meet the clinical criteria for APS. These results were compared with those obtained by aPTT and dRVVT assays.

Materials and methods

Patients

Plasmas from patients with request for screening for LAC, who were registered at our department from October 2005 until March 2006, were included in the study.

373 samples were recruited from consecutive patients investigated for a work-up for hypercoagulability, thrombocytopenia, unexplained miscarriage, autoimmune disease or neurological manifestations.

72 samples were considered as LAC positive and 301 samples as LAC negative, based on the combination of results of the screening, mixing and confirmation tests, routinely used in our laboratory.

Thrombotic episodes or pregnancy morbidity were retrospectively identified by consulting the medical records. Of hundred and thirty-one patients clinical manifestations were documented. Sixty-six patients met the clinical criteria for APS according the international consensus statement on the update of the classification criteria for definite APS [11]. Sixty-five patients did not fulfil the clinical criteria for APS.