Diagnostic test combinations associated with thrombosis in lupus anticoagulant positive patients

Katrien Devreese1; Kathelijne Peerlinck2; Marc F. Hoylaerts2
1Coagulation Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Gent, Belgium; 2Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

Dear Sir,

The laboratory diagnosis of the antiphospholipid syndrome (APS) via antiphospholipid antibody (aPL) tests, including lupus anticoagulant (LAC), anticardiolipin (aCL), or anti-beta2 glycoprotein I (aβ2GPI) antibodies remains a challenge (1). All are associated with clinical APS-criteria (thrombotic and/or pregnancy complications) with limited specificity, and multiple positivity seems clinically more relevant (2–6).

A large number of studies computing the risk for clinical complications in patients with aPL concluded that LAC were associated best with the occurrence of thrombosis (7). Subclassification of patients according to number and type of positive tests, and their antibody isotype (IgG), as well as more specific characteristics (such as antibodies against domain I of β2GPI) proved useful in the risk assessment of thrombosis and obstetric complications (3–5, 8, 9). However, prognostic serological markers for the prediction of thrombosis or pregnancy complications are not yet available (10).

While the role of the three current laboratory criteria is still debated, at present, all assays have a place in the diagnostic work-up for APS (1, 11–13). Via a retrospective study in a small cohort of LAC-positive patients, similar to that recently described (14), we have now assessed the diagnostic performance of combinations of these tests, including quantitative measurements of LAC and the procoagulant marker P-selectin.

Our cohort consisted of confirmed LAC-positive patients (n=54) diagnosed following established criteria (2, 14, 15). Thrombosis patients (n=36) mostly presented with deep venous thromboembolism or pulmonary embolism (n=30), three had arterial thrombosis, three manifested pregnancy complications and thrombosis; 18 patients had no thromboembolic complications (TEC). Patient plasma was analysed for LAC via thrombin generation assays, expressing LAC-activity quantitatively (in arbitrary units AU) (14, 16).

aCL and β2GPI antibodies (expressed in μg/ml) were measured by the ELISA Bindazyme™ aCL and aβ2GPI S EIA kits (The Binding Site, Birmingham, UK), selected based on the clinical performance and low inter-assay imprecision characteristics (< 7%) (17). In house cut-off values were determined by the 99th percentile of a normal population (aCL IgG 0.91 μg/ml, aβ2GPI IgG 0.59 μg/ml) (2, 18, 19). We restricted our analysis to IgG measurements, because available evidence so far indicates that these are more relevant than IgMs (3, 6, 11, 12, 20–22). Human soluble P-selectin (sP-selectin) (ng/ml) was also measured by ELISA (Bender Med Systems, Vienna, Austria). For each of these parameters, receiver-operating characteristics (ROC) analysis was done, to find the best discriminator for disease. These values are given in Table 1 for each parameter: samples below and above this cut-off value are identified as low (L) and high (H), respectively. The corresponding sensitivity, specificity, positive and negative predictive value (PPV, NPV) for all assays are shown in the table, together with the p-value (Fisher’s exact test)

Correspondence to:
Katrien Devreese
Coagulation Laboratory, Laboratory for Clinical Biology
Ghent University Hospital
De Pintelaan, 185 (2P8)
B-9000 Gent, Belgium
Tel.: +32 9 332 65 67, Fax: +32 9 332 45 89
E-mail: katrien.devreese@uzgent.be

Received: September 23, 2010
Accepted after minor revision: December 24, 2010
Prepublished online: February 8, 2011

doI:10.1160/TH10-09-0606

Thrombosis and Haemostasis 2011; 105: 736–738

© Schattauer 2011
for the corresponding 2x2 tables for the distribution in TEC and non-TEC patients.

In the control population, very weak aCL-titres did not correlate with aβ2GPI antibody titres (r=0.156, p=0.255) but in the patient population (n=54), aCL and aβ2GPI antibody titres were much higher and correlated linearly (r=0.825, p<0.0001) (see Supplemental file available online at www.thrombosis-online.com).

Similar analyses with other commercial kits (17) led to a similar result (not shown), i.e. antibodies measured in aCL assays are largely anti-β2GPI-dependent in APS patients. Via 2x2 frequency tables for aCL and aβ2GPI antibodies, both parameters were shown to discriminate between patients with and without clinical criteria (Table 1). Sensitivity, specificity, PPV and NPV were highly comparable between both assays in our cohort (Table 1).

Table 1 also shows that LAC titres, aCL IgG titres and aβ2GPI IgG titres were all informative of TEC in our patient population, with odds ratios (ORs) varying from 4.90 to 7.00. Since all three current aPL assays showed low sensitivity and NPV (Table 1), we have investigated whether combining these parameters would increase their diagnostic power. Figure 1 shows for TEC and non-TEC patients how patient numbers distribute over the different combined test groups. As also suggested by other studies, the highest patient number coincide with triple high LAC/aCL/aβ2GPI titres in the TEC group (n=15). However, the combined double LAC/aCL and LAC/aβ2GPI antibody titre analysis in the TEC subpopulation revealed 16/36 and 15/36 samples with LAC/aCL and high LAC/aβ2GPI titres, respectively (see Supplemental file available online at www.thrombosis-online.com), i.e. triple high LAC/aCL/aβ2GPI titre positivity was not more informative of thrombosis than either double high LAC/aCL or double high LAC/aβ2GPI titre positivity.

In contrast, single high titres in selected test combinations (LAC/aCL or LAC/aβ2GPI) resulted in comparable sensitivity and specificity with ORs even worse than for separate assays (Table 1), further indicating that double measurement of aCL and aβ2GPI is redundant.

On the other hand, the ORs for sP-selectin and LAC titre were comparable, with similar Fisher exact’s test (Table 1), confirming the relationship between sP-selectin and thrombosis (14). Upon combining aPL tests and sP-selectin in a single high titre approach (i.e. high in one of two assays), sensitivity increased to 94.4% for the combination of sP-selectin with either of the established APS criteria (Table 1), with acceptable PPV and NPV values in all cases and highly significant Fisher’s exact test (Table 1). Compared to individual APS assays, the specificity dropped to 38.9%, low specificity being a hallmark of sP-selectin. Adding sP-selectin to either LAC, aCL or aβ2GPI increased the OR to the highest value found in our present analysis (Table 1).
1), i.e. combining sP-selectin with one established APS parameter enhances the diagnostic power of the clinical factor. However, combining sP-selectin with two established laboratory criteria did not further ameliorate the diagnostic power (Table 1).

Via the availability of LAC-units and application of ROC-analysis, we could presently enhance the power of the LAC-assay and distinguish between single and simultaneously high values, taking all relevant aPL assays into consideration. This analysis revealed identical specificities for aCL and β2GPI antibodies, in agreement with the strict correlation between aCL and β2GPI antibody titres, which we found in LAC-positive patients. In spite of current debate (11, 12), we therefore believe that methodologically correct aCL assays (based on microtitre plates coated with cardiolipin and β2GPI [18]), do have diagnostic value.

We also found very similar sensitivity for aCL and β2GPI tests. Recent studies already showed show that the risk of thrombotic events increases with the number of positive tests in APS patients (3, 6, 8, 23, 24). We found that simultaneously high LAC/aCL-titres (n=16) or LAC/β2GPI-titres (n=15) were only found in patients with thrombosis, and further found redundancy in aCL and β2GPI IgG titre determinations. Also, in our study, the OR for aCL was higher than for β2GPI antibodie.

The conclusion that double high LAC/aCL titres are as informative of thrombosis as triple high LAC/aCL/β2GPI titres, is not in contradiction with the earlier conclusion by Prego et al., considering the assays and methodology selected then and now (4). Also in other reports, β2GPI antibody titres were closely related to other aPL (25, 26). The aCL and β2GPI antibody detection in a larger APS population also showed comparable specificity for both IgG tests and higher ORs for aCL IgG than for β2GPI IgG for three out of four assays (27). However, the conclusion that aCL and β2GPI IgG titre determinations are redundant requires confirmation in larger prospective cohorts.

Combining aPL tests did not improve the diagnostic power of thrombosis associations in single high titre positivity, unless sP-selectin was included, raising sensitivity and NPV. Or, a non-APS-specific assay can help to confirm or exclude thrombosis, in the APS sub-population with low of double low aPL tests, where combined LAC/aCL and LAC/β2GPI screening is poorly informative.

Rather than providing conclusive data on the occurrence of thrombosis in APS, in this small cohort of selected LAC-positive patients, the present study has validated how combining various APS tests and sP-selectin associate with thrombosis, compared to separate tests.

The diagnostic associative conclusions reached in our present study may guide a simplified testing scheme in larger prospective studies.

Acknowledgements

The authors thank M. Luypaert for his technical assistance in the performance of the measurements.

References