Lupus Anticoagulants and Their Relationship with the Inhibitors against Coagulation Factor VIII: Considerations on the Differentiation between the Two Circulating Anticoagulants, Armando Tripodi,1 Maria Elisa Mannuccio,1 Veena Chantarangkul,1 Marigrazia Clerici,1 Rossella Bader,1 Pier Luigi Meroni,2 Elena Santagostino,1 and Pier Mannuccio Mannucci1 (1 Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine and Dermatology, University and Foundation IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; 2 IRCCS Istituto Auxologico Italiano, Milan, Italy; * address correspondence to this author at: Via Pace 9, 20122 Milan, Italy; fax 39-02-50320723, e-mail armando.tripodi@unimi.it)

Lupus anticoagulants (LAs) are a heterogeneous group of immunoglobulins directed against negatively charged phospholipids in complex with proteins [β₂-glycoprotein I (β₂-GPI), prothrombin and others] (1). LAs prolong phospholipid-dependent coagulation tests and are associated with increased risk of thrombosis and fetal loss (1). There are other types of anti-phospholipid (aPL) antibodies with or without LA activity that are detected by ELISAs that use as capture antigen cardiolipin, β₂-GPI, or prothrombin (1). Although the exact distribution of the two types of antibodies is unknown, it is widely accepted that LAs and aPL antibodies coexist in a large number of patients classified as having the antiphospholipid syndrome.

The relationship of these factors to inhibitors of individual coagulation factors seen in other conditions is controversial. Among the inhibitors of individual coagulation factors, those that inhibit factor VIII (anti-FVIII) are the most frequent, with an estimated incidence of 30% in patients with severe hemophilia A (2). They may also occur in nonhemophiliacs, producing a clinical condition known as acquired hemophilia (2). Anti-FVIII inhibitors are time-dependent and are associated with the risk of bleeding (3). Although LA and anti-FVIII inhibitors are dissimilar in terms of target and clinical presentation, they are somewhat related, as both of them prolong the phospholipid-dependent coagulation tests. Over the years there has been much debate on the possibility that some hemophiliacs may bear both types of anticoagulants (4–6), but this is still an unresolved question because tests to detect LA without interference from the anti-FVIII inhibitors are lacking (7).

In an attempt to clarify these issues and to explore the suitability of current diagnostic strategies to differentiate LAs from specific inhibitors to coagulation factors, we used a variety of phospholipid-dependent tests to detect LA in two populations of hemophiliacs with and without anti-FVIII inhibitors. We also searched for the three main types of aPL antibodies (i.e., anti-cardiolipin, anti-prothrombin, and anti-β₂-GP) in these patients.

We studied 49 hemophiliacs (median age, 31 years; range 3–65 years) who were regularly followed up at our center. Hemophilia was classified as severe (FVIII, <0.01 kU/L) in 39, as moderate (FVIII, 0.02–0.03 kU/L) in 6, and mild (FVIII, 0.05–0.16 kU/L) in 4. Anti-FVIII inhibitors were present (range, 0.5–5000 Bethesda units/mL) in 27 of the 49 patients. Overall, 36 of 49 patients had blood-borne infections caused by hepatitis B (HBV), hepatitis C (HCV), or HIV viruses.

After receipt of informed consent, we collected blood in evacuated tubes (Becton Dickinson) containing 105 mmol/L trisodium citrate (ratio of blood to anticoagulant, 9/1). Blood was centrifuged at 2500 g for 15 min. The plasma was then divided into two portions, and one was filtered through 0.22 μm pore-size cellulose filters (Millipore). Filtered and unfiltered plasmas were aliquoted and stored at −70 °C until tested. Testing was performed, in batches, no later than 6 months from blood collection.

The titer of the anti-FVIII inhibitors was measured on unfiltered plasmas according to the Nijmegen modification of the Bethesda method (8). LA detection was performed on filtered plasmas according to the criteria of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Hemostasis (ISTH) (9) with screening and confirmatory procedures carried out with 3 tests: home-made silica clotting time (SCT), home-made and commercial dilute Russell viper venom tests (dRVVT), and an activated partial thromboplastin time (APTT) with hexagonal phospholipids. SCT and home-made dRVVT were carried out as described elsewhere (10, 11). The procedures consisted of recording paired coagulation times (SCT or dRVVT) at low and high phospholipid concentrations. Results were expressed as the percentage correction between the two clotting times (10, 11). By definition, the higher the percentage correction the greater the likelihood of LA positivity. The commercial dRVVT assay (LAC-Screen and Confirm; Instrumentation Laboratory) was carried out according to the manufacturer’s instructions. Paired APTT tests (Staclot-LA; Stago) were performed with (APTT1) and without (APTT2) hexagonal phospholipids on a mixture of test and normal plasma. Results were expressed as the difference between APTT1 and APTT2. Anti-cardiolipin, anti-β₂-GP, and anti-prothrombin antibodies were detected by ELISA (12–14).

Among the whole population of hemophiliacs for whom results were available 0 of 49 (0%), 8 of 47 (17%), 1 of 49 (2%), and 9 of 47 (19%) patients were positive according to the home-made dRVVT, commercial dRVVT, SCT, and StaClot-LA, respectively. The prevalence of LA positivity as detected by all methods was higher in patients with anti-FVIII inhibitors than in those without: 22% vs 10% for the commercial dRVVT, 3.7% vs 0% for SCT, and 30% vs 5% for StaClot-LA. The relative rate of LA detection in patients with anti-FVIII inhibitors was the highest for StaClot-LA (30%), intermediate for the commercial dRVVT (22%), and the lowest for SCT (3.7%). None of the patients tested LA positive according to the home-made dRVVT. aPL antibodies were in all cases negative, except for one hemophiliac without anti-FVIII inhibitors, who tested positive for anti-prothrombin IgM.

The details on aPL and LA status according to different assays for those patients (n = 14) with at least one positive
test are given in Table 1. All but 2 patients were positive for HCV, HIV, or HBV. Three (patients 19, 26, and 47) of the 14 patients were positive for LA with 2 tests (commercial dRVVT and StaClot-LA). One (patient 19) of these 3 patients was negative and the other 2 (patients 26 and 47) were positive (82 and 5000 Bethesda units/mL) for anti-FVIII inhibitors. One (patient 27) of the 14 patients was positive for LAs with 3 tests (commercial dRVVT, SCT, and StaClot-LA). This patient was positive (24.0 Bethesda units/mL) for anti-FVIII inhibitors. One patient (patient 40) was slightly positive only for anti-prothrombin IgM. This patient was positive for HCV and negative for anti-FVIII inhibitors.

The detection of LAs is still an unresolved issue because no specific tests were developed for this purpose, and clinical laboratories still rely on a set of diagnostic criteria issued by the SSC of the ISTH for those patients with chronic infection (9). Because of the different nature of LAs, the diagnostic criteria call for more than one test to be performed with both screening and confirmatory procedures (9). Because of the heterogeneous nature of LA, it is also reasonable to assume that the more different is the assay design of the tests used, the greater is the chance of detecting all the antibodies.

We attempted to reinvestigate the LA/aPL pattern in hemophiliacs by use of 4 phospholipid-dependent coagulation tests and solid-phase assays using 3 of the known antigenic targets to which the aPL are directed. The prevalence of positive patients as detected by LA tests varied both between and within tests (see the different prevalence of positive patients as detected by LA tests). The observed prevalence (27%) compares favorably with that in another series (5). However, for the vast majority of hemophiliacs in our series, the positivity was weak (see Table 1). Furthermore, the prevalence of positive patients decreases considerably when one considers as positive only patients who had 2 (8%) or 3 (2%) concomitantly positive tests. Finally, the entire panel of phospholipid-dependent coagulation tests used in this and other investigations was previously reported to be affected by the presence of inhibitors to FVIII (7, 10, 15–19). The vast majority of patients in our cohort who tested positive for LAs also tested positive for anti-FVIII inhibitors. All of these findings together seem to indicate that the prevalence of true LA-positive patients among hemophiliacs is lower than generally believed. These conclusions are further supported by the findings of a very low prevalence of patients who tested positive in three tests for aPL antibodies. The latter finding contrasts with other reports in which the prevalence of positive anti-cardiolipin in hemophiliacs was high and associated with the occurrence of such markers of infections as HIV (4), HCV (20), or both (20). The low prevalence of HIV (8 of 49 patients) in our cohort may in part explain the contrasting findings. However, the prevalence of HCV was quite high (37 of 49 patients), thus pointing to alternative explanations such as differences in the anti-cardiolipin assay design and cutoff values used in different studies. Relevant to this is the persistent, between-laboratory variability of aPL assay results (12, 21).

Although the question of whether LA/aPL antibodies coexist with anti-FVIII inhibitors in hemophiliacs may seem of academic interest only, it may have practical implications. Differentiation of LA from anti-FVIII inhibitors is crucial for the clinical laboratory (22) because of the different therapeutic interventions that may be required for patients bearing one or the other condition (i.e., antithrombotic agents in the former or hemostatic agents in the latter). Although it is not possible to draw definite

<table>
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<tr>
<th>Patient</th>
<th>HCV</th>
<th>HIV</th>
<th>HBV</th>
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<td>aCL, aβ2GPI, aFII</td>
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* Values in parentheses represent the results obtained with the relevant test. Cutoff values were as follows: home-made dRVVT, >17%; commercial dRVVT, >1.1; SCT, >22%; StaClot-LA, ≥3 s; aCL, <10 units; anti-β2GPI, <0.16 and <0.25 absorbance for IgG and IgM, respectively; anti-FII, <17.8 and <46.7 arbitrary units for IgG and IgM, respectively.

aFVIII, inhibitors to FVIII (Bethesda units/mL); aCL, anti-cardiolipin; aβ2GPI, anti-β2GPI; aFII, anti-prothrombin.

Commercial dRVVT.

Home-made dRVVT.
conclusions, it is reasonable to assume that using only one test to rule in or out LA when the clinical history of the patient being investigated is unknown may be risky, particularly if only one test is used. Two or more positive test results, particularly if they are from assays with different designs, probably are more informative and more likely to differentiate LA from anti-FVIII inhibitors.

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References


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