Lupus Anticoagulants and Their Relationship with the Inhibitors against Coagulation Factor VIII: Considerations on the Differentiation between the 2 Circulating Anticoagulants, Armando Tripodi,1 Maria Elisa Mancuso,1 Veena Chantarangkul,1 Marigrazia Clerici,1 Rossella Bader,1 Pier Luigi Meroni,2 Elena Santagostino,1 and Pier 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 (β2GPI), 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, β2GPI, or prothrombin (1). Although the exact distribution of the 2 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 bind to FVIII over a period of time 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 2 populations of hemophiliacs with and without anti-FVIII inhibitors. We also searched for the 3 main types of aPL antibodies (i.e., anti-cardiolipin, anti-prothrombin, and anti-β2GPI) 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 U/mL) in 39, as moderate (FVIII, 0.02–0.03 U/mL) in 6, and mild (FVIII, 0.05–0.16 U/mL) in 4. Anti-FVIII inhibitors were present (range, 0.5–500 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 nonfiltered 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 nonfiltered 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 APTT2 and APTT1. Anti-cardiolipin, anti-β2GPI, 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 1 hemophilic 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 1 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 have been developed for this purpose, and clinical laboratories still rely on a set of diagnostic criteria issued by the SSC of the ISTH (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 detected by LA tests varied both between and within tests (see the different behaviors of the 2 dRVVT 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 taken 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 3 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

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<th>HBV</th>
<th>aFVIII</th>
<th>aPL antibodies</th>
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*a 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.

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

*c Commercial dRVVT.

*d Home-made dRVVT.
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 conclusions, it is reasonable to assume that using only 1 test to rule in or out LA when the clinical history of the patient being investigated is unknown may be risky, particularly if only 1 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


Use of Dual TaqMan Probes to Increase the Sensitivity of 1-Step Quantitative Reverse Transcription-PCR: Application to the Detection of SARS Coronavirus, Seung Yiin,1 Shing Shun T. To,1 Polly H.M. Leung,1 Tsz Shan Cheung,1 Peter K.C. Cheng,2 and Wilina W.L. Lim2 (1 Department of Health Technology & Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China; 2 Public Health Laboratory Centre, Centre for Health Protection, Department of Health, Hong Kong SAR, China; * address correspondence to this author at: Department of Health Technology & Informatics, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China; fax 852-2364-9663, e-mail shea.ping.yip@polyu.edu.hk)

Severe acute respiratory syndrome (SARS), caused by a novel coronavirus (SARS-CoV) (1–5), has affected 8096 people and produced 774 deaths in 29 countries/regions (6). The vital step in preventing and controlling future epidemics is to block transmission of infection through an effective quarantine policy, which in turn hinges on early diagnosis and confirmation of the disease, particularly by laboratory tests (7). The need for rapid, ultrasensitive assays that can detect infection very early in the course of the disease is obvious.

The antibody response to SARS-CoV infection is detectable only after ~10 days of illness (8); hence early laboratory diagnosis rests on early detection of the virus itself. Detection relies on reverse transcription followed by PCR (RT-PCR) (7). We designed a 1-step real-time quantitative RT-PCR assay for SARS-CoV with the use of 2 TaqMan probes, instead of 1 probe, hybridizing to the same PCR product to further improve the sensitivity. This simple modification using dual TaqMan probes for quantification has wide applications in areas in which ultra-sensitivity is critically required.

Our 1-step assay was designed to amplify the ORF1b regions of the SARS-CoV by TaqMan EZ RT-PCR Kit in a 7500 Real Time PCR System (Applied Biosystems). We compared assays using 1 and 2 TaqMan probes (Fig. 1, A and B). The 25-μl reaction mixture contained 1 × TaqMan