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High frequency of inadequate test requests for antiphospholipid antibodies in daily clinical practice

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Abstract

Background: We have empirically noted that many physicians routinely request anti-phospholipid antibodies (aPL) without a correct clinical indication. The aim of this study was to evaluate retrospectively whether aPL testing at our Thrombosis Centre was justified.

Methods: Medical records from 520 subjects for aPL screening tests for various clinical conditions were reviewed. The aPL screening tests were: lupus anticoagulant (LA), anti-cardiolipin antibodies (aCL) and anti-β_2 glycoptotein I (aβ_2 GPI). Requests for aPL screening were divided into justified, potentially justified or not adequately justified.

Results: aPL testing requests were considered justified in 358 (69%) patients, potentially justified in 66 (12.6%) and not adequately justified in 96 (18.4%). LA was positive in 65 (18%) of justified requests and in only one (1%) of the 96 potentially justified requests. None of the 66 not adequately justified for aPL testing was positive for LA. aβ_2 GPI was positive in 63 (17.6%) of the 358 justified, in four (6%) of the 66 potentially justified and in five (5.2%) of the 96 not adequately justified requests; aCL IgG were positive in 59 (16.4%) of the 358 justified and in five (7.5%) and six (6.2%) of the potentially justified and not adequately justified requests, respectively. The presence of the triple aPL positivity was found exclusively in the justified requests.

Conclusions: This study suggests that requests for aPL tests should be addressed more adequately. This work could be an example of how to focus attention on requests for laboratory tests especially on the basis of valid clinical criteria before the analyte is measured.

Keywords: anti-cardiolipin antibodies; anti-β_2 glycoprotein I antibodies; Dilute Russell's viper venom time (dRVVT); inadequate test request; silica clotting time (SCT).

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Introduction

Laboratory investigation in the diagnosis of antiphospholipid syndrome (APS) is based on the presence of lupus anticoagulant (LA) in plasma and/or in combination with anti-cardiolipin (aCL) and anti-β_2 glycoprotein antibodies ($\alpha\beta_2$ GPI) (1, 2). Taken together, they represent the laboratory pattern of anti-phospholipid antibodies (aPL). Suggested indications for aPL testing are: prolongation of activated partial thromboplastin time (APTT) mixing test with normal plasma, venous or arterial thrombosis, especially if occurring before the age of 50 years, thrombosis at unusual sites or associated with autoimmune diseases, or complications of pregnancy (2). However, a ''grey zone'' of potentially clinical utility may exist in the case of autoimmune diseases. In particular, there is good evidence that patients with systemic lupus erythematosus (SLE) may acquire features of APS and vice-versa (3). Requests for aPL testing in other conditions are, in general, discouraged to avoid false-positive results (4–6). The aims of this study were to evaluate retrospectively whether aPL testing at our Thrombosis Centre (TC) between July 2008 and August 2009 was justified and followed the above criteria, and to analyse the physicians approach to aPL testing which may be just one example of how laboratory tests are excessively requested in daily clinical practise. In general, the phenomenon of ordering superfluous tests is well recognized, but it is difficult to contrast. Physicians often do not know the meaning of the tests they order. This may be the case with aPL (7).

Materials and methods

Patients

The records of 720 consecutive unselected subjects, referred to our TC from July 2008 to August 2009 for aPL screening tests, were considered. Two hundred records were excluded as the reasons for the request were unknown. A total of 520 requests were considered. The reasons for performing the tests were retrospectively identified by consulting the medical records. Requests were divided into three groups: justified (clinical evidence of thrombotic events and miscarriage), potentially justified (autoimmune disease without clinical evidence of thrombotic events and miscarriage) or not adequately justified (all other conditions).

Coagulation tests

Silica clotting time (SCT) The test consists of paired APTTs performed on test plasmas with micronized silica (Haemosil™ Silica Clotting Time, Instrumentation Laboratory, Milan, Italy) as activator and two phospholipid concentrations (low SCT1 and high SCT2) as platelet substitutes. The test was performed using an automated

coagulometer (ACL Advance, International Laboratory, Milan, Italy) and is based on the ratio between the SCT screen and SCT confirm. A SCT screen ratio was first calculated by dividing the clotting time obtained in the patients using low SCT1 by the mean clotting time obtained in a group of normal subjects. A SCT confirm ratio was calculated by dividing the clotting time obtained in the patients using high SCT2 by the mean clotting time obtained in a group of normal subjects. A final ratio was obtained by dividing the results of SCT screen by that of SCT confirm. If the cut-off value of 1.21 was exceeded, the test was repeated after mixing 1:1 the plasma sample with normal plasma. It was considered positive when a 1:1 mixture of test plasma and normal plasma yielded a ratio more than 1.21. This cut-off value was calculated using the 99th percentile of coagulation times obtained in frozen plasma samples from 50 healthy subjects.

Dilute Russell's viper venom time (dRVVT) The test consists of paired clotting times (Hemosil™ dRVVT LAC screen and confirm, Instrumentation Laboratory, Milan, Italy) performed using an automated coagulometer (ACL Advance, Instrumentation Laboratory, Milan, Italy). It is based on the ratio between the dRVVT screen (low phospholipids concentration) and dRVVT confirm (high phospholipids concentration). For the generation of the final ratio we followed the same procedure described for SCT. It was considered positive when a 1:1 mixture of test plasma and normal plasma yielded a ratio more than 1.34. This cut-off value was calculated using the 99th percentile of coagulation times obtained in frozen plasma samples from 50 healthy subjects.

$a\beta_2$ GPI

IgG and IgM anti-β_2 GPI antibodies were measured using ELISA kits (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Concentrations of $a\beta_2$ GPI antibodies were expressed in units/mL and values of more than 8 and 12.5 were considered positive for IgM and IgG isotypes, respectively. The cut-off values have been derived locally from 50 healthy subjects using the 99th percentile.

aCL

The presence of IgG and IgM aCL were detected using quantitative ELISA kits (Anticariolipine Bouty, Italy) according to the manufacturer's instructions. Measured values of aCL were expressed in units/mL, and values of more than 12 U/mL for either IgG or IgM were considered positive. The cut-off values have been locally derived from 95 healthy subjects using the 99th percentile.

Only abnormal tests (LA, aCL and $a\beta_2$ GPI), confirmed after 3 months, were considered positive.

Statistical analysis

Statistical analysis was performed using MEDCALC software (version 10.0.1.0). Data were expressed as median and range or mean $\pm$ SD where appropriate. Frequency data were compared using Fisher's exact test. p-Values <0.05 were considered statistically significant.

Results

A total of 720 tests for an aPL screen performed in our TC from July 2008 to August 2009 were examined. Requests for 520 of these requests were correctly detected by examining the medical record. A total of 358 out of 520 requests for aPL screening were considered as justified. Justified, potentially justified and non-adequately justified requests are listed in Table 1.

A total of 168 patients (32%) tested positive on two occasions for at least one of the considered tests (dRVVT, SCT, $a\beta_2$ GPI, aCL). To determine whether the presence of aPL was more prevalent in individuals with justified requests, the distribution of test positivity in all groups (Table 2) was evaluated. Sixty-five (18%) of justified requests were positive for SCT and/or dRVVT, only one (1%) of the 96 potentially justified requests was positive for SCT, none of the 66 not adequately justified were positive for SCT and/or dRVVT; $a\beta_2$ GPI was positive in 63 (17.6%) of the 358 justified, in four (6%) of the 66 potentially justified and in five (5.2%) of the 96 not adequately justified requests; aCL IgG was positive in 59 (16.4%) of the 358 justified and in five (7.5%) and six (6.2%) of the potentially justified and non-adequately justified requests, respectively. When the combination of aPL test was considered, the presence of triple aPL positivity, i.e., LA, aCL and a $a\beta_2$ GPI was found exclusively in the adequate request patients only. The other possible combinations showed a higher frequency of aPL positivity in the group of patients with an adequate request, but the differences were not significant because the subgroups were too small.

Discussion

We have empirically noticed that many physicians routinely request aPL screening for patients in whom these requests do not seem to be adequate. We therefore questioned the adequacy of aPL screening at our TC.

There are several reasons why physicians request aPL screening for their patients in daily practice: the occurrence (accidentally identified) of prolongation of the APTT without known aetiology, to provide an explanation for arterial or venous thrombosis, to assess the risk of miscarriage (pregnancy loss) recurrence, thrombosis in unusual sites or associated with autoimmune diseases (2). These requests have been classified in this study as justified. Even in autoimmune diseases, requests for aPL may be ordered since a relationship between some of these analytes and antiphospholipid syndrome does exist (3). In this case, we classified the requests as potentially justified.

Generalised searches on asymptomatic individuals other than the above should be discouraged to avoid the risk of false-positive results, of wasting time and resources, and of obtaining a positive result which could be misleading in the diagnosis and management of the single patient leading to the use of inappropriate anti-thrombotic drugs. These requests were classified as not justified.

Results of this retrospective study show that an important percentage of potentially justified and not justified requests were processed in our laboratory. In particular, only one patient showed LA positivity in the group of patients with potentially justified request, while none showed LA positi-

Table 1 Justified, potentially justified and not adequately justified requests for aPL screening tests.

Diagnosis	n	%
Justified (n = 358)		
Deep venous thrombosis	98	18.9
Recurrent pregnancy losses	96	18.5
Antiphospholipid syndrome	47	9
Ischaemic stroke	33	6.3
Transient ischemic attack	31	5.9
Superficial venous thrombosis	16	3
Pulmonary embolism	16	3
Myocardial infarction	16	3
Avascular bone necrosis	3	0.5
Ischaemic neuritis	1	0.1
Prolonged APTT	1	0.1
Potentially justified (n = 66)		
Autoimmune diseases[a]	66	12.6
Not adequately justified (n = 96)		
Artrhalgias or arthritis	27	5.1
Positivity for autoantibodies	13	2.5
Raynaud phenomenon	8	1.5
Familial history of thrombosis	7	1.3
Other conditions[b]	18	3.4

[a]Autoimmune diseases: systemic lupus erythematosus (n = 24, 36.3%), systemic sclerosis (n = 27, 41%), rheumatoid arthritis (n = 10, 15%), Sjögren's syndrome (n = 5, 7.5%), without clinical evidence of thrombotic events or pregnancy losses. [b]Other conditions: headache, infective diseases, hepatitis, urticaria, venous insufficiency, dermatitis, ecchimosis, fever, neoplasm, erythema nodosum, pericarditis, aftosis.

vity in the group of patients with unjustified requests. Moreover, it is worth noting that triple positivity (LA, aCL and aβ_2 GPI) was absent in both the potentially justified and not justified group, but present in the justified request group. Aβ_2 GPI and aCL were present in the potentially justified and not justified groups, but we observed that in different combinations, LA was never present. Even though the group of patients with a potentially justified request was small, our results show that the percentage of positive aCL and aβ_2 GPI tests was as low as that of the patients with unjustified requests. It should be remembered that only LA was significantly associated with thromboembolism. Both aCL and aβ_2 GPI were found to not be so closely related with thromboembolism (8, 9).

Requests for aCL and aβ_2 GPI appear less useful in the management of the single patient without clinical and environmental evidence of either a thrombotic event or foetal loss. An educational program could be planned in order to limit inappropriate aPL requests (10, 11). This approach has been successfully adopted to improve, for instance, the appropriateness of antiepileptic drug monitoring (12). A computer-based intervention could be built, forcing physicians to order aPL tests only if the clinical criteria of appropriateness are satisfied. A proposal for an algorithm is presented in Figure 1. Requests for aPL testing should be considered if there is clinical suspicion of APS. In particular, LA testing should be the first test performed. If the request is not justified, physicians should be discouraged from ordering aPL tests. In fact, asymptomatic cases meeting only the laboratory criteria for APS are not to be treated with oral anticoagulants. A particular condition may be that related to patients with potentially justified requests, that is, those with autoimmune disease. In these patients, ordering aPL testing may be useful only if anti-thrombotic prophylaxis is required.

Table 2 Distribution of aPL positivity in adequate and non-adequate requests.

	Justified n = 358	Potentially justified n = 66	Not adequately justified n = 96
LA positivity[a]	65 (18.1%)	1 (1.5%)	0
LA + aβ_2 GPI + aCL	46	0	0
LA + aβ_2 GPI	3	0	1 (1%)
LA + aCL	3	0	0
aβ_2 GPI-IgG[b]	63 (17.6%)	4 (6%)	5 (5.2%)
aβ_2 GPI-IgM	59 (16.5%)	4 (6%)	7 (7.2%)
aCL IgG	59 (16.5%)	5 (7.5%)	6 (6.2%)
aCL IgM	47 (13.1%)	6 (9%)	10 (10.4%)

Values are given in numbers (percentage). LA, lupus anticoagulant; aCL, anticardiolipin antibody; aβ_2 GPI, anti-β_2 glycoprotein antibody. [a]$p < 0.0001$ vs. potentially and not justified requests. [b]$p < 0.004$ vs. potentially and not justified requests.

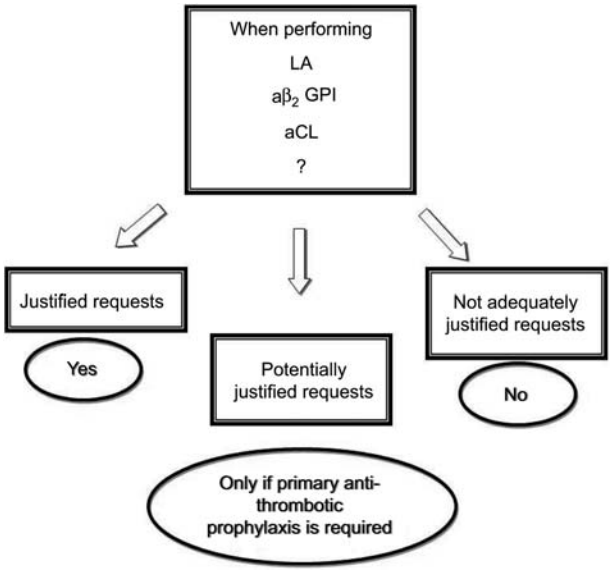

Figure 1 Proposed algorithm for aPL screening.

This paper has some limitations. It is a retrospective study, but we wanted to investigate the daily clinical and laboratory practice of aPL testing at our TC with the aim of limiting inappropriate requests in the future. We hope that this work is an example of how to focus attention on the requests for laboratory tests, especially on the basis of valid clinical criteria to determine test appropriateness before it is measured.

Conflict of interest statement

Authors' conflict of interest disclosure: None declared.
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