Stago

Diagnostics is in our blood.

MAYO formación

Laboratory diagnosis of antiphospholipid syndrome. Where do we stand?

Armando Tripodi

Milan, Italia.

06/11/2022

Antiphospholipid Syndrome

Condition defined by clinical and lab criteria

- *Laboratory criteria*

- Lupus anticoagulant and/or solid-phase antiphospholipid antibody positive tests (confirmed on 2 occasions 12 weeks apart)

- *Clinical criteria*

- Pregnancy complications, venous and/or arterial thrombosis

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Solid-phase Antiphospholipid Antibodies

- *Which Test(s)*
  - Anti-cardiolipin
  - Anti-β_2GPI
  - Anti-PS/PT (not yet endorsed by guidelines)
- *Which Isotype(s)*
  - IgG
  - IgM

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Assays for aCL and a-ß$_2$GPI

- Many commercial ELISA-based assays
  - Poorly standardized
  - Gross degree of variation across labs
- Application of international guidelines helps standardization

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Laboratory Detection of LA

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RECOMMENDATIONS AND GUIDELINES

jth

Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis

Update of the guidelines for lupus anticoagulant detection and interpretation

Katrien M. J. Devreese[1,2] | Philip G. de Groot[3] | Bas de Laat[3] | Doruk Erkan[4] | Emmanuel J. Favaloro[5] | Ian Mackie[6] | Marta Martinuzzo[7] | Thomas L. Ortel[8,9] | Vittorio Pengo[10] | Jacob H. Rand[11] | Armando Tripodi[12,13] | Denis Wahl[14,15] | Hannah Cohen[16,17]

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Issues on LA Detection

- ***Who should be tested***
- Which test(s)
- Diagnostic criteria
- When testing
- Results reporting
- Interpretation

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Indications to search for APS

- Occurrence of (accidentally-found) prolongation of the aPTT without known etiology
- Patients with venous and/or arterial thrombosis at young age (<50 years)
- Patients with thrombosis at unusual sites, or associated with autoimmune diseases
- Women with pregnancy complications

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Which Test

- ***Two tests based on different principles***
- dRVVT
- Sensitive aPTT-based test (low phospholipids and silica as activator)

LA should be considered as positive if at least one of the two tests is positive

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Diagnostic Criteria for LA Detection

- ***Screening***
- Prolongation of phospholipid-dependent clotting test
- ***Mixing***
- Evidence that the prolongation is due to the presence of an inhibitor
- ***Confirmation***
- Evidence that the inhibitor is directed against phospholipids

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Cut-off values to interpret results

- Cut-off values should be determined in each lab
- Testing plasma from healthy donors
- Take as cut-off the 99th centile of the distribution

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ORIGINAL ARTICLE

Variability of cut-off values for the detection of lupus anticoagulants: results of an international multicenter multiplatform study

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Essentials

- Cut-off values for LA detection were calculated in 11 labs each testing plasma from 120 donors with 3 commercial platforms
- Major variations were observed even within the same platform
- Cut-off values determined in any given lab are not necessarily interchangeable

Issues on LA Detection

- Who should be tested
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When Testing

- ***Problem***
- Results interpretation is difficult during acute thrombosis and/or during antithrombotic drugs
- ***Recommendation***
- Blood should be collected before starting anticoagulation or after a sufficient period from its discontinuation

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Effect of Anticoagulation on LA Testing

- Unfractionated Heparin (UFH) mimics LA
  - *Many LA tests do contain UFH neutralizers*
- LMWH may mimic LA
  - *Depending on the brand of LMWH used*
  - *Especially at peak*
- VKA give rise to false-positive (or false-negative) LA
- DOAC give rise to false-positive LA

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Approaches to Overcome Anticoagulation

- Dilution (1:1) of patient plasma into pooled normal plasma (PNP)
- Integrated assays (screen and confirm)
- Tests (reportedly) less affected by anticoagulants
- Antidotes or neutralizers to quench *in vitro* the activity of anticoagulants
- Discontinuation of anticoagulation

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Dilution (1:1) of patient plasma into PNP

- *Rationale*
  - Deficiency of coagulation will be corrected by the PNP
- Limitations
  - Applicable only to VKA & with good quality PNP
  - Dilution reduces (by 50%) the LA potency
  - Correction by PNP is dependent on the aPTT or dRVVT
  - No conclusive evidence on the value of the procedure
  - False-negative or false-positive LA should be expected

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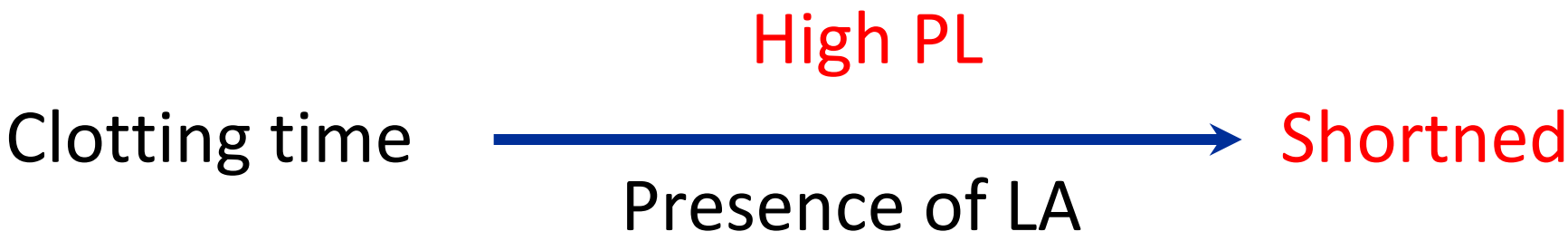
Schematic representation of Integrated LA Test



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Integrated LA Tests

- Earlier reports suggested that screen and confirm integrated tests in patients on VKA or UFH are proportionally prolonged
- Hence, they are reliable even in patients on UFH or VKA
- Later reports showed that screen and confirm in patients on DOAC are not proportionally prolonged
- Screen tends to be more prolonged than confirm
- Consequently, the ratio screen/confirm tends to be higher than expected and may lead to false-positive LA

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Tests (reportedly) less affected by anticoagulants

- Snake venoms (Taipan & Ecarin) might be useful to detect LA during anticoagulation, as they are able to activate FII
- Taipan is a PL- and calcium-dependent activator, whilst Ecarin is not
- If used in combination, they may help detecting LA during anticoagulation
- There is information from literature on their diagnostic efficacy on patients on VKA, but not conclusive evidence

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Antidotes/Neutralizers to Quench Anticoagulants

- Idarucizumab, Andexanet alfa
- Added in vitro to neutralize dabigatran or anti-Fxa drugs
- DOAC-Stop®, or DOAC-Remove®
- Activated charcoal added in vitro to adsorb DOAC
- DOAC-Filter
- Cartridge filtering DOAC

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Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Neutralising rivaroxaban induced interference in laboratory testing for lupus anticoagulant (LA): A comparative study using DOAC Stop and andexanet alfa

Emmanuel J. Favaloro[a,b,*], Grace Gilmore[c], Sandya Arunachalam[d], Soma Mohammed[a], Ross Baker[c]

Rivaroxaban caused falsely LA positivity with dRVV

Rivaroxaban plasma treated with DOAC-Stop showed correction of the screen/confirm ratio for most LA tests

Participants in the study correctly identified the rivaroxaban plasma treated With DOAC-Stop as LA-negative

Andexanet alfa left some residual interference

Thrombosis Research 204 (2021) 32–39

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Impact of a commercially available DOAC absorbent on two integrated procedures for lupus anticoagulant detection

Armando Tripodi [a,b,*], Erica Scalambrino [a], Veena Chantarangkul [a,b], Oriana Paoletti [c], Marigrazia Clerici [a], Cristina Novembrino [a], Massimo Boscolo-Anzoletti [a], Flora Peyvandi [a,d], Sophie Testa [c]

Essentials

- DOAC-stop proved effective in reducing DOAC concentration
- Results mimicking LA were observed in patients on DOAC before absorption, especially for rivaroxaban when testing was performed with dRVVT
- The rate of results mimicking LA was considerably reduced after exposure of the patient plasma to DOAC-stop

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ISLH International Journal of Laboratory Hematology WILEY

ORIGINAL ARTICLE

Evaluation of DOAC Filter, a new device to remove direct oral anticoagulants from plasma samples

Pierre-Olivier Sevenet[1] | Virginie Cucini[2] | Tristan Hervé[1] | François Depasse[1] | Audrey Carlo[3] | Geneviève Contant[2] | Olivier Mathieu[2]

TABLE 1 Postfiltration results of pools of plasmas spiked with dabigatran, rivaroxaban, apixaban, and edoxaban

	Prefiltration	Postfiltration	
	DOAC concentration (ng/mL)	Number of result below LoD of dedicated assay	LoD (ng/mL)
Dabigatran	306	27/27 (100%)	15
Rivaroxaban	321	35/35 (100%)	25
Apixaban	320	33/33 (100%)	23
Edoxaban	264	27/27 (100%)	20

Abbreviations: DOAC, direct oral anticoagulant; LoD, limit of detection.

TABLE 2 Pre- and postfiltration concentrations (in ng/mL) in 18 individual patient's plasma samples treated with dabigatran, rivaroxaban, and apixaban

Dabigatran		Rivaroxaban		Apixaban	
Prefiltration	Postfiltration	Prefiltration	Postfiltration	Prefiltration	Postfiltration
29.6	<15	34.1	<25	147.2	<23
67.0	<15	57.7	<25	165.8	<23
100.9	<15	106.6	<25	194.2	<23
160.0	<15	170.4	<25	205.6	<23
288.4	<15	238.0	<25	213.3	<23
438.7	<15	294.6	<25	298.0	25.1

TABLE 4 Pre- and postfilter LA testing in plasma samples from patients referred for LA testing

	DRVV Screen			DRVV Confirm			PTT-LA		
Pool	Prefilter (s)	Postfilter (s)	Difference (%)	Prefilter (s)	Postfilter (s)	Difference (%)	Prefilter (s)	Postfilter (s)	Difference (%)
1	46.7	45.4	−3	43.3	40.6	−6	44.7	44.4	−1
2	36.4	35.8	−2	37.5	35.9	−4	40.0	37.9	−5
3	36.9	36.8	0	37.2	35.6	−4	38.7	38.0	−2
4	91.5	90.8	−1	39.6	38.8	−2	100.3	108.2	8
5	74.2	78.5	6	42.4	40.7	−4	54.6	51.9	−5

Abbreviation: LA: lupus anticoagulant.

Approaches to Overcome Anticoagulation

- Dilution (1:1) of patient plasma into pooled normal plasma (PNP)
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- Discontinuation of anticoagulation

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Discontinuation of Anticoagulation

- Oral anticoagulation may be temporarily stopped and switched to LMWH
- LMWH would protect from thrombosis, making LA detection possible
- This strategy may be considered in individual patients after full consideration of pros and cons

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Issues on LA Detection

- Who should be tested
- Which test(s)
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- When testing
- ***Results reporting***
- Interpretation

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Results Reporting

LA detection should be reported with analytical results and an interpretative comment

(i.e., ***LA yes, or no***)

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Issues on LA Detection

- Who should be tested
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- Diagnostic Criteria
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- Results reporting
- ***Interpretation***

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Clinical interpretation of results

- *Interpretation should consider the results of all the 3 tests*
- The syndrome is defined if at least one of the tests (LA, aCL or aß$_2$GPI) is positive
- Positivity for all the 3 tests *(triple positivity)* identifies patients at very high risk

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LA Detection
Main unresolved issues

- Standardization of existing procedures
- *Application of SSC guidelines*
- Urgent need for LA specific tests
- *Understanding of pathogenic mechanisms may help*
- Tests able to identify LA patients who develop clinical events
- *dRVVT better than APTT-based tests ?*
- *aβ2-GPI domain I*
- Quantification of LA potency
- *Establishment of "international standards" ?*

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Journal of Thrombosis and Haemostasis, **7**: 1767–1773 DOI: 10.1111/j.1538-7836.2009.03588.x

ORIGINAL ARTICLE

The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study

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Table 2 Association between aPL and thrombosis

	Odds ratio (95% confidence interval)
Anti-domain I IgG	**3.5 (2.3–5.4)***
Non-domain I Anti-beta2GPI IgG	0.4 (0.3–0.6)
Anti-beta2GPI IgM	0.9 (0.6–1.3)
LAC	**1.8 (1.1–3.1)***
aCL	1.1 (0.6–2.1)

To estimate whether there is a significant increase in association of anti-domain I IgG antibodies with thrombosis an odds ratio was calculated within the total population of 511 patients. *One is not included in 95% confidence interval. Bold: Significant association of assay with clinical symptom.

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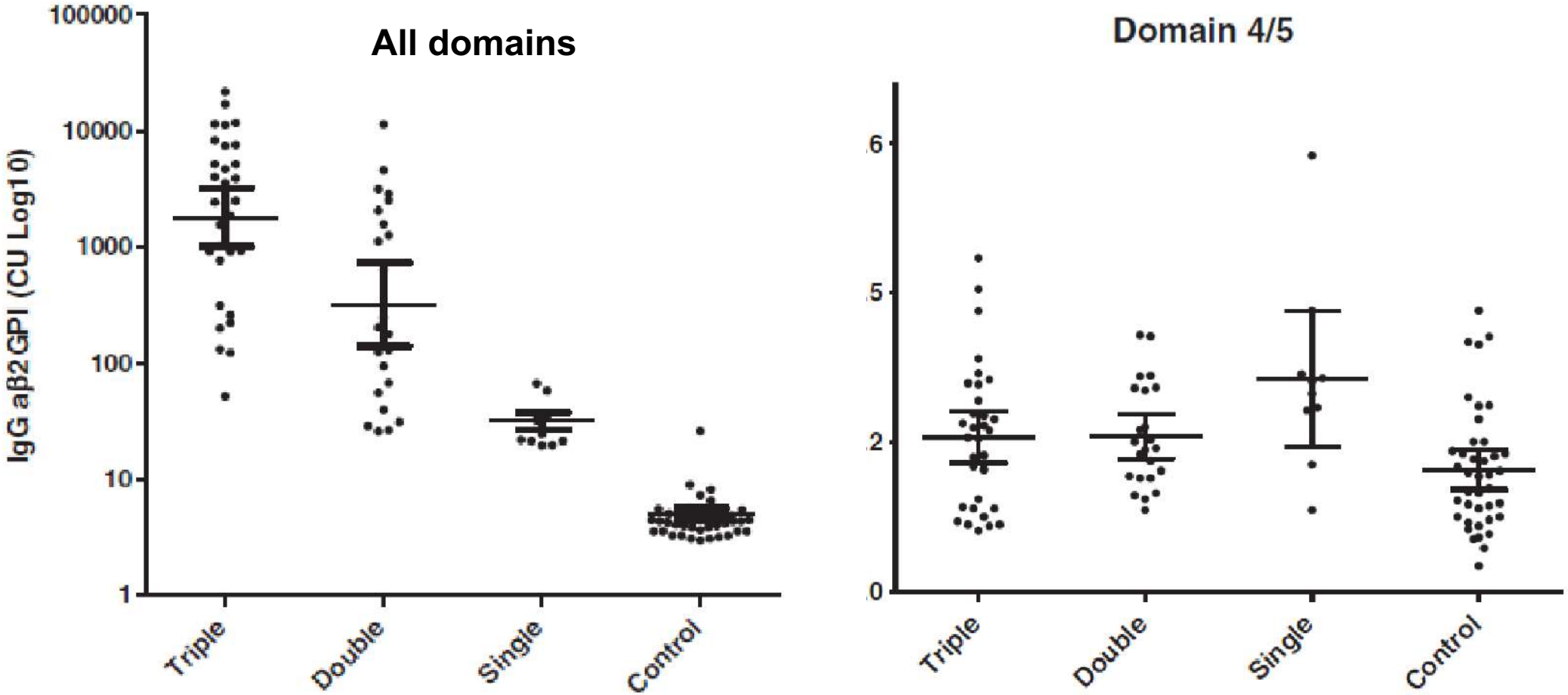
Thromb Res 2915; 136: 161-63

Antibodies to Domain 4/5 (Dm4/5) of β2-Glycoprotein 1 (β2GP1) in different antiphospholipid (aPL) antibody profiles

V. Pengo [a,b,*], A. Ruffatti [a,b], M. Tonello [a,b], A. Hoxha [a], E. Bison [a], G. Denas [a], S. Padayattil Jose [a], G. Zoppellaro [a], A. Bracco [a], A. Banzato [a]



Summary & Conclusions

- Accuracy of lab diagnosis is essential as APS patients are candidates for long term anticoagulation
- Diagnosis should be established far from acute events and off therapy
- APS requires one the following
  - *Positive aPTT-based or dRVV*
  - *aCL IgG or IgM above normal limits*
  - *aß2GPI, IgG or IgM above normal limits*
- Triple positivity identifies patients at high risk

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