Lupus Anticoagulant testing: analyzing the influence of prolonged INR.

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INTRODUCTION: Lupus Anticoagulant (LA) is a risk factor for arterial and venous thromboembolisms and obstetrics complications. At the laboratory is a test difficult to standardize. Guidelines recommend using two techniques (one of them a dVVRT test) for diagnose it. We want to describe the results obtained in our laboratory in a large series of patients in a year comparing the results of two tests, diluted Russell Viper Venom Time (dRVVT) and Silica Clotting Time (SCT), and the relationship with routine tests, specifically the influence of prolonged INR.

METHODS: In our laboratory we receive samples from hospitals and general practice centers. Some of the patients whom we analyze are receiving oral anticoagulants (Vitamin K inhibitors) with INR >1.5.
We use the ACL-Top 700 analyzers (Werfen®) with HemosIL ® SynthASil (for aPTT), dVVRTScreen/dVVRT Confirm, and Silica Clotting Time reagents.
Heparin was ruled out by thrombin time analysis.
For dVVRT and SCT techniques:
- Defining reference time for screening and confirmatory test: by analysis of 40 normal samples and obtaining the mean time for each technique
- Screening ratio: patient screening time/reference screening time. Positive if higher than 1.2.
- Confirmatory ratio : patient confirmatory time/reference confirmatory time.
- Normalized Ratio was defined as screening ratio/confirmatory ratio. Positive if >1.2

If in one of the LA test the Normalized Ratio (NR) is >1.2 the result is considered positive.

Mixing test: the same tests (SCT and dVVRT) using a mixture 1:1 between patient plasma and normal pooled plasma to try to overcome a possible deficit of clotting factors caused by ant vitamin-K treatment.
The conditions to analyze Lupus anticoagulant mixing test were: negative results (NR<1.2) but both Screening Ratio and Confirmatory Ratio >1.2, specially if the INR of the patient was >1.5. Also, we performed mixing test if there were positive or discrepant LA results and INR greater than 1.5.
Antiphospholypid antibodies –Anticardiolipin (ACAIgG/IgM), and Anti-B2Glycoprotein-I (AB2GPIIgG/IgM)- are analyzed by Fluoroimmunoanalysis (Phadia,ThermoFisher).

RESULTS: From January to November 2015 were performed 1435 LA tests. The distribution of results of each test and their correlation with prolonged aPTT and INR are shown in Table 1. A significant number of dVVRT positive SCT negative tests was found to have INR > 1.5 and normal aPTT. Prolonged aPTT results with normal INR and Thrombin time were considered related to Lupus Anticoagulant.

	N	aPTT >1.25	INR>1.5
SCT-dRVVT-	1150	2	103
SCT+dRVVT-	58	0	0
SCT-dRVVT+	146	71	59
SCT+dRVVT+	81	73	6
TOTAL	1435	146	168

Table 1, LA tests results according to routine test

Of the 1435 orders, only 1038 included antiphospholipid antibodies (AAF) testing.
80% of this AAF test were negative, but if some AL test was positive we found a 40% with at least one AAF positive.
In Table 2 is shown the different AAF positive for each subset of LA test results.

	N	INR>1,5	TTPR >1,25	IgG ACA +	IgM ACA +	IgG AB2GPI +	IgM AB2GPI+	AAF all +	AAF all-
SCT-dVVRT-	840	42	87	41	106	32	32	0	683
SCT+dVVRT-	43	0	21	6	2	18	13	0	33
SCT-dVVRT+	90	32	34	6	17	1	16	1	62
SCT+dVVRT+	65	6	57	23	14	4	4	5	29
total	1038	80	199	76	139	55	65	6	807

Table 2, LA subsets results, routine test prolonged values and AAF positive results

MIXING TESTS:
39 samples (not included in the previous series of patients) were analyzed by mixing test as described above (results in Table 3)
-17 samples were positive for dVVRT and only one for SCT.
- Of 16 samples with a SCT-dRVVT+ results and INR>1.5, 15 gave negative dRVVT results after mixing test, and then considered negative.
- Only one sample considered negative with SCT and dVVRT became positive after dVVRT-M

APTT-R	INR	SCT NR	SCT-M NR	dVVRT NR	dVVRT-M NR
2,53	**6,38**	0,66	0,54	0,80	0,85
1,46	**4,39**	0,97	0,83	1,17	**1,41**
1,7	**3,95**	0,96	0,80	**1,28**	0,93
1,75	**3,55**	0,99	0,82	**1,48**	1,01
1,85	**3,43**	0,85	0,78	**1,39**	1,07
1,49	**3,31**	1,09	0,86	**1,29**	0,89
1,38	**3,20**	0,96	0,82	1,11	1,01
1,75	**3,18**	1,05	0,84	**1,60**	**1,36**
1,48	**3,02**	1,11	0,85	1,16	1,00
1,5	**2,96**	1,03	0,96	0,99	0,91
2,25	**2,95**	**2,84**	**1,99**	**2,83**	**2,34**
1,71	**2,93**	0,88	0,77	**1,52**	1,15
1,4	**2,86**	0,95	0,82	**1,29**	0,89
1,8	**2,80**	0,83	0,78	1,11	0,87
1,6	**2,75**	0,86	0,79	**1,36**	1,09
1,12	**2,74**	0,82	0,76	1,03	0,96
1,52	**2,66**	0,76	0,75	**1,23**	1,06
1,63	**2,63**	0,90	0,80	**1,63**	1,00
1,54	**2,59**	0,90	0,78	1,06	0,93
1,34	**2,51**	0,91	0,79	**1,22**	0,98
1,82	**2,49**	1,14	0,96	1,01	0,89
1,75	**2,46**	0,97	0,82	**1,47**	1,13
1,28	**2,43**	0,97	0,84	**1,46**	1,06
1,53	**2,42**	1,05	0,84	**1,20**	1,19
1,27	**2,36**	0,89	0,78	0,99	0,91
1,4	**2,21**	0,93	0,89	**1,38**	1,02
1,29	**2,21**	1,19	0,99	0,97	0,89
1,14	**2,09**	1,01	0,84	**1,29**	1,03
1,41	**2,04**	1,12	0,98	1,17	0,90
1,37	**1,89**	1,14	1,03	0,92	0,87
1,16	**1,82**	1,03	0,96	0,99	0,86
1,36	**1,55**	0,88	0,83	1,12	1,03
1,16	1,38	0,94	0,86	1,09	1,09
1,03	1,26	0,94	0,87	1,09	1,05
1,18	1,14	0,84	0,80	1,02	0,96
1,61	1,12	0,97	0,92	0,99	0,90
1,36	1,11	0,84	0,77	1,01	0,89
1,42	1	1,07	1,09	0,96	1,18
1,07	0,99	0,75	0,75	0,98	1,03

Table 3, Mixing test LA results

CONCLUSION:
– Anticoagulated patients can give false positive results for dRVVT so we recommend to withdraw it before testing.
– Mixing tests can be helpful in doubtful or discrepant cases.