Indian J Hematol Blood Transfus (July-Sept 2019) 35(3):478–484
https://doi.org/10.1007/s12288-018-01072-8

ORIGINAL ARTICLE

Utility of Lupus Anticoagulant Assays (APTT-LA, KCT, DPT and DRVVT) in Detection of Antiphospholipid Syndrome (APS) in High Risk Pregnancy Cases

Ankur Ahuja[1] · Seema Tyagi[2] · Hara Prasad Pati[2] · Renu Saxena[2] · Venkatesan Somasundaram[3] · Prabhu Manivannan[2] · Preeti Tripathi[2] · Dinesh Chandra[4]

Received: 15 February 2018 / Accepted: 29 December 2018 / Published online: 23 January 2019

Abstract Routine investigation for recurrent pregnancy loss includes measurement of antiphospholipid antibodies. The lupus anticoagulant has long been associated with increased risks for thrombosis and adverse obstetric outcomes. But there are some disadvantages with lupus anticoagulant (LAC) tests which includes varied sensitivity of different clot based assays. ISTH recommends only 2 assays (preferably DRVVT and APTT-LA) for the identification of lupus anticoagulant but there are some studies which don't support this contention. Our study analyzed 526 samples from high risk pregnancy cases for APLA by all four LAC tests from tertiary centre of northern India. Among all the cases studies 65 cases were positive for lupus anticoagulant 25 of this became negative after 12 weeks. Among the 40 repeated positive assays, dRVVT could able to diagnose 36 cases followed by APTT-LA which could able to diagnose 28 cases, while KCT could able to diagnose 23 cases and dPT could able to diagnose only 14 cases. There were 12 cases in whom all lupus assays were positive. Our study thus concluded that DRVVT was the most sensitive followed by APPT-LA, KCT, dPT. The combination of dRVVT with APTT-LA or KCT appeared to be superior to other combinations. No individual test per se is 100% sensitive for the diagnosis of APLA in high risk pregnancy cases. Further results confirmed that repeated LAC result is required even in a high-risk setting. Positive LAC assay in majority were not associated with exclusively recurrent pregnancy loss but were associated with sporadic stillbirth and thrombosis.

Keywords Antiphospholipid syndrome · Lupus anticoagulant · DRVVT · dPT · KCT · APTT-LA · High risk pregnancy · Recurrent abortions

✉ Seema Tyagi
drseematyagi@hotmail.com

Ankur Ahuja
ankurahuja74@gmail.com

Hara Prasad Pati
harappati@yahoo.co.in

Renu Saxena
renusaxena@outlook.com

Venkatesan Somasundaram
ltcolsvenkatesan@gmail.com

Prabhu Manivannan
drprabhumanivannan@gmail.com

Preeti Tripathi
contactdoctorpreeti@gmail.com

Dinesh Chandra
dinesh3224@gmail.com

1 Department of Lab Sciences and Molecular Medicine, Army Hospital (Research and Referral), New Delhi, India

2 Department of Haematology, All India Institute of Medical Sciences, New Delhi 110010, India

3 Department of Pathology, Armed Forces Medical College, Pune, India

4 Department of Haematology, SGPGI, Lucknow, India

Introduction

Antiphospholipid syndrome (APS) is characterized by multisystem manifestations like arterial and venous thrombosis, recurrent pregnancy loss, intrauterine death and other obstetric morbidities like premature birth and preeclampsia accompanied by persistence of antiphospholipid antibodies (aPL) [1, 2]. aPL are detected on the basis of solid phase assays [comprised of anticardiolipin (acl) antibody and anti β2 glycoprotein I (aβ2GPI)] and liquid

phase assays (which identifies lupus anticoagulants [LACs]). LACs are being detected by clot based tests. Four clot based assays are available namely diluted Russell viper venom test (dRVVT), activated partial prothrombin time—lupus anticoagulant (APTT-LA), kaolin clotting time (KCT) and dilute prothrombin time (dPT). The International Society on Thrombosis and Hemostasis (ISTH) has proposed use of two assays to measure lupus anticoagulant: the dilute Russell viper venom time (dRVVT) and a sensitive partial thromboplastin time (PTT-LA) [3, 4]. Earlier recommendations have included kaolin clotting time (KCT) and dilute prothrombin time (dPT) as sensitive measures of the lupus anticoagulant [4, 5]. Though it is still debatable [6, 7], however updated guidelines no longer support the use of KCT and dPT because of difficulties in performing them as there is a risk of increased chance of false-positivity with the use of more than 2 assays [3]. However there are several studies published where one or other tests have been considered as superior to others [6, 8–10]. Till now no superior combinations of the LAC assays have been observed in the literature [4, 6]. It is very important to diagnose APS correctly as it is a treatable condition and thus requires a test with good sensitivity. The association of LAC tests with various obstetric morbidities is also debatable [2, 11–17]. Another debatable issue in the setting of APLA diagnosis is the number of LAC tests required to perform in the clinically suspected cases of APS. ISTH guidelines have forewarned for false-positivity if more than two lupus anticoagulant assays are performed [3] but there are studies available which don't agree with these guidelines. Also there is lack of association between any one particular assays and clinical manifestations thus indicating a need of more than one assay.

In view of the above varied literature and a fewer available data in Indian population, this study was aimed to find out the most suitable test, to know the fact that whether there is increase in the sensitivity of LAC tests by including more assays or not, and finally to find out the association of LAC tests in various obstetric morbidities in an Indian population.

Materials and Methods

Descriptive cross sectional study was done. This prospective study was conducted in the tertiary centre of northern India from the period 01 July 2014 to 30 September 2015. Five hundred and twenty-six patients of high risk pregnancy conditions (Recurrent Pregnancy loss early spontaneous abortions, Stillbirths, IUDs, Ectopic pregnancies, IUGR, HELLP syndrome, Eclampsia and preeclampsia) were consecutively studied in whom the tests were being advised as a part of routine work up for LAC tests. The study was approved by the Ethics Committee and was performed in accordance with ethics standards laid down in the 1975 Declaration of Helsinki and its later amendments. A written informed consent was obtained from the patients prior to the procedures.

Patients who were on drugs (procainamide and chlorpromazine, anticoagulants) and who were suffering from infections (HIV, hepatitis, malaria, CMV and others) were excluded. Patients with only 1 pregnancy loss were excluded.

LAC test was done with a minimum gap of 3 months and maximum gap of 5 years of the last abortion as per the guidelines [18].

Fifty sex and age matched healthy controls with no history of pregnancy loss, thrombotic episode, and uneventful previous pregnancy was also incorporated.

All tests except KCT was done on STAGO STA Compact. In dPT, the recombinant thromboplastin [Tissue Factor + Phospholipid] was diluted 1:50 and 1:500. The ratio of the clotting time with the dilute recombinant thromboplastin [1:500] and the normal reagent [1:50] was compared to a normal control. Ratio of 50 normal controls were calculated and 99th percentile was taken (1.3). The ratio above the 99th percentile (> 1.3) was considered positive.

In the confirmatory protocol, a uniquely formulated phospholipid reagent was used to demonstrate the phospholipid-dependent nature of the LA detected in samples that tested positive in the screening protocol. Kaolin was performed manually.

Kaolin clotting time was calculated by Rosner Index (RI):

$$RI = \frac{KCT\ (50{:}50mix) - KCT\ (Normal)}{KCT\ (patient)}$$

RI > 0.145 was considered positive (99th percentile of matched 50 controls)

DRVVT was performed by STA®-Staclot® screen and STA®-Staclot® confirm whose reference range was established from 30 individual normal plasma samples. The final result was expressed as a screen ratio = screen clotting time (CT) of the plasma to be tested/screen CT of reference pool. Screen clotting time of reference pool was 40.3 s. 99th Screen clotting time of the 30 controls was 48.41 s. Therefore the cut-off of the screen ratio was 1.2. It was considered positive when it was ≥ 1.2 after which then mixing study was performed.

When not corrected by mixing study then DRVVT (confirm) test was performed in the similar manner by choosing DRVVT (confirm) mode and confirm ratio was calculated as confirm ratio = confirm clotting time (CT) of the plasma to be tested/confirm CT of reference pool.

Further normalized ratio was calculated as normalised ratio = screen ratio/confirm ratio. It was considered as positive when it was > 1.2.

APTT of the test plasma studied was compared with pooled plasma. The test was done in the Stago STA compact automated coagulation analyzer. 99th percentile of the 30 control was 45.2 s. Thus the clotting time of > 45.2 s was considered positive.

Open Epi version 3 was used to calculate the sensitivity and the specificity. Univariate odds ratio and 95% CI were estimated for the various parameters by Med Calc software. p value of < 0.05 was considered significant.

Results

A total of 526 female patients were studied. The median age of the patient was 28 years with the range of 20–42 years. Median age of the control was 27 years with the range of 20–39 years. None of the control cases were found to be positive by all four LAC tests.

Table 1 shows 65 lupus anticoagulant (LAC) positive cases out of the 500 and 26 high risk pregnancy cases. After 12 weeks of repeated tests, 25 patients who showed initial positivity were turned out to negative. Among the 40 repeated positive assays, overall dRVVT could able to diagnose 36 cases followed by APTT-LA which could able to diagnose 28 cases, while KCT could able to diagnose 23 cases and dPT could able to diagnose only 14 cases. There were 12 cases in whom all lupus assays were positive (Table 1).

Among the 526 women studied, there were 291 women who had the previous history of late abortions alone, 176 women had the history of early abortions alone, while 59 women had the history of both early and late abortions. Among the early abortions 10 out of 176 patients showed confirmed LAC positivity while among the late abortions group 23 out of the 291 patents showed the LAC positivity (Table 2).

In addition to the pregnancy losses, 19 women also had at least one documented and proven episode of thrombosis. Of these 19 cases 10 women had the history of stroke, 07 women had DVT while 01 woman had history of hepatic and 01 woman had history of portal vein thrombosis. Out of the 19 cases 04 had LAC positivity. 123 women in addition had the history of still birth of which 15 cases showed the LAC positivity. 27 had the history of eclampsia/pre-eclampsia out of which only one woman was positive for LAC (Table 3).

There was significant increase in the history of thrombosis with positive LAC assays in comparison to LAC negative assay(s) [10% vs. 3%; with incidence rate difference 0.07 (95% CI 0.004–0.13), $p = 0.04$; with Odds ratio of 3.26; (95% CI 1.03–10.27), $p = 0.04$].

Similarly, there was significant increase in the history of still birth with the positive assay(s) in comparison to LAC negative assay(s) (37.5% vs. 22.25%; incidence rate difference 0.18 (95% CI 0.02–0.33), $p = 0.026$, OR = 2.1 (95% CI 1.07–4.1), $p = 0.03$).

There was no apparent significant difference among LAC positive and LAC negative assays in women with the early abortion group [25% vs. 34% with incidence rate difference − 0.1121 (95% CI − 0.30 to 0.079) $p = 0.25$ with OR = 0.70 (95% CI 0.33–1.47), $p = 0.3497$] as well as late abortion group [57.5% vs. 54.5% with incidence rate difference 0.001235 (95% CI − 0.24 to 0.25), $p = 0.99$; OR of 1.23 (95% CI 0.63–2.37), $p = 0.53$] and in the women with preeclampsia [2.5% vs. 5.3% with incidence rate difference incidence rate difference − 0.03 (95% CI − 0.10 to 0.04), $p = 0.41$ OR of 0.45 (95% CI 0.06–3.4), $p = 0.44$ observed between the LAC positive and the LAC negative (Table 4).

Table 1 Positive LAC High risk pregnancy cases

LAC assays	Repeated after 12 weeks positive (n = 40)	Repeated after 12 weeks negative (n = 25)
All	12	1
KCT + APTT LA + dRVVT	7	11
KCT + APTT LA	3	1
KCT + dRVVT	1	0
APTT LA + dRVVT	5	3
KCT	0	1
APTT LA	1	1
dRVVT	9	7
dRVVT + dPT	02	0
Total	40	25

Table 2 Distribution of recurrent abortions

Recurrent abortions	LAC positive n = 40	LAC negative n = 486
Early abortion (< 12 weeks) (n = 176)	10 (5.7%)	166 (94.3%)
Late abortion (12–20 weeks) (n = 291)	23 (7.9%)	268 (92.1%)
Both early and late abortion (n = 59)	7 (11.9%)	52 (88.1%)

Table 3 Distribution of additional clinical events in women with recurrent abortions

Additional clinical events in women with recurrent abortions	LAC positive n = 40	LAC negative n = 486
Still birth (> 20 weeks) (n = 123)	15 (12.2%)	108 (87.8%)
Thrombosis (n = 19)	4 (21%)	15 (79%)
Eclampsia/preeclampsia	1 (3.7%)	26 (96.3%)

Amongst the single positive LAC assay, dRVVT was the most sensitive with sensitivity rate of 90% followed by APTT-LA with the sensitivity of 70% and KCT with the sensitivity of 57.5% and the sensitivity of dPT time was 35%. In the group of dual assays the sensitivity of APTT LA and/or dRVVT and KCT and/or dRVVT were 97.5% each, while the sensitivity of KCT and/or APTT-LA was 72.5% APTT-LA and/or dPT approached 75% and KCT and or dPT had a sensitivity of 62.5%. In the triple combination of dRVVT and/or KCT and/or APTT LA the sensitivity and NPV was 100% as also in the combination of dRVVT and/or APTT-LA and/or dPT. In APTT-LA and/or KCT and/or dPT the sensitivity was 77.5% and in dRVVT and/or KCT and/or dPT the sensitivity was 97.5% (Table 5).

Table 4 Odds ratio and comparison of LAC positive and LAC negative of various clinical events

	Incidence rate difference between LAC positive vs LAC negative group	Odds ratio (OR) between LAC positive vs LAC negative group
Still birth (> 20 weeks) (n = 123)	0.18	OR = 2.1
	(95% CI 0.02–0.33)	(95% CI 1.07–4.1)
	$p = 0.026$	$p = 0.03$
Thrombosis (n = 19)	0.07	OR = 3.26
	(95% CI 0.004–0.13)	(95% CI 1.03–10.27)
	$p = 0.04$	$p = 0.04$
Early abortion (< 12 weeks) (n = 176)	− 0.1121	OR = 0.70
	(95% CI − 0.30 to 0.079)	(95% CI 0.33–1.47)
	$p = 0.25$	$p = 0.3497$
Late abortion (12–20 weeks) (n = 288)	0.001235	1.23
	(95% CI − 0.24 to 0.25)	(95% CI 0.63–2.37)
	$p = 0.99$	$p = 0.53$
Both early and late abortion (n = 59)	0.0536	2.25
	(95% CI − 0.06 to 0.16)	(95% CI 0.92–5.48)
	$p = 0.35$	$p = 0.07$
Eclampsia	− 0.03	0.4536
	95% CI − 0.10 to 0.04	(95% CI 0.06–3.4)
	$p = 0.41$	$p = 0.44$

Table 5 Sensitivity and negative predictive value of various LAC assays and their combinations

LAC assays	Sensitivity (%)	NPV (%)
KCT and/or dPT and/or APTT-LA	77.5	98.2
KCT and/or APTT LA and/or dRVVT	100	100
KCT and/or dPT and/or dRVVT	97.5	99.8
APTT-LA and/or dPT and/or dRVVT	100	100
KCT and/or APTT LA	72.5	97.8
KCT and/or dRVVT	97.5	99.8
KCT and/or dPT	62.5	97.0
APTT LA and/or dRVVT	97.5	99.8
APTT LA and/or dPT	75	97.9
KCT	57.5	96.6
APTT LA	70	97.6
dPT	35	94.9
dRVVT	90	99.2

NPV negative predictive value

Discussion

The study which was aimed to focus on the utility of all four LAC tests in high risk pregnancies firstly has confirmed the fact that repeated LAC assay after 12 weeks as suggested in the guidelines is required in order to avoid false positivity as was also seen in our 25 cases that turned out to be negative after 12 weeks as evident in Table 1. Secondly, our study has confirmed an association between the LAC positivity and maternal thrombosis (Table 4) which has also been reported previously by other studies [19–21]. In our study the incidence rate of thrombosis was 10% (Table 3) which was same as documented in the previous studies [19–21]. This suggests an importance of counselling in patients suspected to have APLA to avoid any other concomitant thrombophilic conditions, e.g. oestrogen-progesterone combination oral contraceptives, high altitudes, cigarette smoking.

Similarly, stillbirths have been significantly associated with LAC positivity (Tables 3, 4).

Our study (Tables 2, 4) didn't observe any association of LAC positivity with early abortions which was in concurrence with various studies [13, 18, 22–24]. However one study [14] observed the association of positive LAC assays with early abortions. It is hypothesized that this study [14] was conducted in the era before Sydney guidelines were introduced when LAC assays were repeated after 06 weeks period instead of 12 weeks period, hence it might be possible that it may be false positive. However since it's an old study it is not appropriate to prove and analyze their results.

Eclampsia/Pr-eclampsia was also not significantly associated with LAC positivity in our study thus debating the indication of these costly tests in these conditions.

ISTH suspected the risk of high false-positivity if ≥ 2 LAC assays are being performed. This issue is quite debatable as discussed below also and required to be studied.

While the reported global prevalence of LAC positivity in high risk pregnancy was 2–8% [17], the prevalence of LAC positivity in high risk pregnancy in this study was 7.6% thus ruling out any spurious increase in prevalence even after using four assays.

The disadvantage of recommending all four assays is that it may lead to increased costs, but the advantage is that it may increase the sensitivity of LAC tests. While it is recommended to use any of the two assays by ISTH [18], there has been unsolved debate over the choice of assays [3, 4, 6, 8, 10, 25–27]. We have seen in our study that there is an increase in the sensitivity to 100% if we increase the number of assays to three by incorporating APTT-LA, dRVVT and KCT (Table 5). While among the single assay the sensitivity of dRVVT was 90% which was the best in a single LAC assay group and thus our study also stresses upon inclusion of dRVVT at least in LAC tests. Since no individual test per se was 100% sensitive for the diagnosis of APLA in high risk pregnancy cases, there is a need of using more than one assay which is also supported by the ISTH guidelines [3]. The best combination on using two assays group was the combination of dRVVT and APTT-LA or KCT assay as it approached to 97.5% in the each group but unfortunately it never reached to 100% when considered only in pairs. While ISTH strongly recommends dRVVT and APTT-LA in the work up of APS [3], Pengo et al. reported that a combination of both dRVVT and KCT was more useful [18]. Our study has supported both the above recommendations [3, 8]. However in contrast to our study, some of the studies [6, 15] have observed that there is no superior combination among LAC assays.

Though dPT and KCT, are specifically not recommended in the ISTH guidelines [3], some of the studies [6] have concluded that the result of KCT was "most abnormal" among all LAC positive cases. However in our study we did not find KCT to be the most abnormal and its sensitivity was near that of APTT-LA (Table 5). The drawback of the KCT mentioned in the literature [28] was its technique, but since more than two decades we have been performing LAC assays in our laboratory and no methodological issues were raised with respect to performance of the KCT assay.

Thus our study has highlighted all important issue which often comes across while investigating APLA.

Conclusions

Our study has concluded that dRVVT should be incorporated with either APTT-LA or KCT in the setting of high risk pregnancy cases especially with the history of stillbirths and thrombosis, however for the recurrent abortions more studies with large number of patients are required to confirm their association with positive LAC assay(s).

Acknowledgements We acknowledge the support of our patients and technical staff, as with their support the study had been feasible.

Author contributions RS, HPT, AA and ST were involved in conceptualization, designing, writing and critical review of the manuscript and were responsible for overall supervision. PM, AA, DC, PT and VS were involved in literature search, writing and manuscript editing. ST is the overall guarantor of the article.

Funding This study was funded by Haematology Research Society (Grant Number 01) and we are very grateful to them for helping us in research process.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical Approval The present study is in compliance with ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Lubbe WF, Liggins GC (1985) Lupus anticoagulant and pregnancy. Am J Obstet Gynecol 153:322–327
2. Branch DW, Scott JR, Kochenour NK, Hershgold E (1985) Obstetric complications associated with the lupus anticoagulant. N Engl J Med 313:1322–1326
3. Pengo V, Tripodi A, Reber G et al (2009) Update of the guidelines for lupus anticoagulant detection. Subcommittee on lupus anticoagulant/antiphospholipid antibody of the scientific and standardisation committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost 7:1737–1740
4. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC et al (1999) International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arthr Rheum 42:1309–1311
5. Galli M, Reber G, de Moerloose P, de Groot PG (2008) Invitation to a debate on the serological criteria that define the antiphospholipid syndrome. J Thromb Haemost 6:399–401
6. Galli M, Finazzi G, Bevers EM, Barbui T (1995) Kaolin clotting time and dilute Russell's viper venom time distinguish between prothrombin-dependent and beta 2-glycoprotein I-dependent antiphospholipid antibodies. Blood 86:617–623
7. Keeling D, Mackie I, Moore GW, Greer IA, Greaves M (2012) British committee for standards in haematology. Br J Haematol 157:47–58
8. Pengo V, Biasiolo A, Gresele P et al (2007) Participating centres of Italian Federation of Thrombosis Centres (FCSA). Survey of lupus anticoagulant diagnosis by central evaluation of positive plasma samples. J Thromb Haemost 5:925–930
9. Jennings I, Mackie I, Arnout J, Preston FE (2004) UK national external quality assessment scheme for blood coagulation. Lupus anticoagulant testing using plasma spiked with monoclonal antibodies: performance in the UK NEQAS proficiency testing programme. J Thromb Haemost 2:2178–2184
10. Arnout J, Vanrusselt M, Huybrechts E, Vermylen J (1994) Optimization of the dilute prothrombin time for the detection of the lupus anticoagulant by use of a recombinant tissue thromboplastin. Br J Haematol 87:94–99
11. Bowie EJ, Thompson JH Jr, Pascuzzi CA, Owen CA Jr (1963) Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. J Lab Clin Med 62:416–430
12. Mueh JR, Herbst KD, Rapaport SI (1980) Thrombosis in patients with the lupus anticoagulant. Ann Intern Med 92:156–159
13. Clarke CA, Davidovits J, Spitzer KA, Laskin CA (2013) Lupus anticoagulant: results from 2257 patient attending a high risk pregnancy clinic. Blood 18(122):341–347
14. Vora S, Shetty S, Salvi V, Satoskar P, Ghosh K (2008) Thrombophilia and unexplained pregnancy loss in Indian patients. Natl Med J India 21:116–119
15. Swadzba J, Iwaniec T, Pulka M, De Laat B, De Groot PG, Musial J (2011) Lupus anticoagulant: performance of the tests as recommended by the latest ISTH guidelines. J Thromb Haemost 9:1776–1783
16. Helgadóttir LB, Turowski G, Skjeldestad FE, Jacobsen AF, Sandset PM, Roald B et al (2013) Classification of stillbirths and risk factors by cause of death—a case–control study. Acta Obstet Gynecol Scand 92:325–333
17. Duley L (2009) The global impact of pre-eclampsia and eclampsia. Semin Perinatol 33:130–137
18. Miyakis S, Lockshin MD, Atsumi D, Branch DW, Brey RL, Cervera R et al (2006) International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 4:295–306
19. Alijotas-Reig J, Ferrer-Oliveras R, Ruffatti A, Tincani A, Lefkou E, Bertero MT et al (2015) The European Registry on Obstetric Antiphospholipid Syndrome (EUROAPS): a survey of 247 consecutive cases. Autoimmun Rev 14:387–395
20. Bouvier S, Cochery-Nouvellon E, Lavigne-Lissalde G, Mercier E, Marchetti T, Balducchi JP et al (2014) Comparative incidence of pregnancy outcomes in treated obstetric antiphospholipid syndrome: the NOH-APS observational study. Blood 16(123):404–413
21. Fischer-Betz R, Specker C, Brinks R, Schneider M (2012) Pregnancy outcome in patients with antiphospholipid syndrome after cerebral ischaemic events: an observational study. Lupus 21:1183–1189
22. Barbour LA (2001) ACOG committee on practice bulletins-obstetrics. Int J Gynaecol Obstet 75(2):203–212
23. Branch DW, Silver RM, Porter TF (2010) Obstetric in antiphospholipid syndrome: current uncertainties should guide our way. Lupus 19:446–452
24. Skrzypczak J, Rajewski M, Wirstlein P, Goździewicz T, Zimmer M, Wołczyński S et al (2011) Frequency of antiphospholipid syndrome in women with pregnancy loss in multicenter study in Poland. Ginekol Pol 82:749–754
25. Martinuzzo ME, Cerrato GS, Varela ML, Adamczuk YP, Forastiero RR (2012) New guidelines for lupus anticoagulant: sensitivity and specificity of cut-off values calculated with plasmas from healthy controls in mixing and confirmatory tests. Int J Lab Hematol 34:208–213

26. Exner T, Rickard KA, Kronenberg H (1978) A sensitive test demonstrating lupus anticoagulant and its behavioural patterns. Br J Haematol 40:143–151
27. Pengo V, Biasiolo A, Rampazzo P, Brocco T (1999) dRVVT is more sensitive than KCT or TTI for detecting lupus anticoagulant activity of anti-beta2-glycoprotein I autoantibodies. Thromb Haemost 81:256–258
28. Urbanus RT, de Groot PG (2011) Antiphospholipid antibodies—we are not quite there yet. Blood Rev 25:97–106