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Original Research Article

Evaluation of Rosners index vs Brandt correction and Chang's %, in the interpretation of mixing studies at varying dilutions

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ABSTRACT

Background: For evaluation of unexplained prolongation of PT and PTT, mixing tests forms a great diagnostic tool. On mixing equal volume of patient plasma with normal pooled plasma, if there is correction it indicates factor deficiency and non-correction indicates inhibitors.
Methods: Sysmex CS-5100 Coagulometer with Pathrombin SL APTT reagent, LA1 and LA2 reagents supplied by siemens were used. All data were expressed as Mean±SD. Statistical analysis was done using unpaired students t test. A p value of <0.05 was used to indicate statistical significance in all analyse.
Results: APTT with (1:1) and (4:1) mixing study for detection of factor deficiency showed a sensitivity of 91% and 92% for RI, 88% and 90% for Changs %, and 75% for Brandt correction PNP aPTT + 5 secs respectively. For Inhibitors, RI shows a sensitivity of 79% and 89%, Changs 71 and 80% and Brandt test 50% for APTT (1:1) and (4:1) mix, respectively.
Conclusions: Mixing tests forms an important diagnostic tool in differentiating factor deficiency from inhibitors especially in LAC patients. This study recommends mandatory use of mixing tests in LAC cases as also advocated by BSH, ISTH and CLSI. Rosners Index is more sensitive than changes % and Brandt correction in the interpretation of mixing studies. It can be safely concluded that RI can be used as a reference method for evaluation of mixing studies and its sensitivity is greatly increased by using PP4:1 PNP. It's a matter of debate that whether these indices can be effective with other Analysers and reagents?

Keywords: Activated partial thromboplastin time, Lupus anticoagulant, Pooled normal plasma, Mixing study, Rosner's Index, Changs % correction

INTRODUCTION

For evaluation of unexplained prolongation of PT and PTT, mixing tests forms a great diagnostic tool. On Mixing PP 1:1 NPP, if there is correction it indicates Factor deficiency and if mixing results shows non correction then it indicates inhibitors.[1]

The principle of the mixing study seems simple but results often are difficult to interpret in practice. There is no uniform agreement as to what criteria should be used to judge correction. The normal range usually can be used as the guide for correction, but the drawback is weak LAC can show correction with a 1:1 mix and in contrast, factor deficiencies with a markedly prolonged PT or aPTT may not be corrected to normal in a 1:1 mix of PP with PNP.[2,3]

A 1:1 mix of PP with CNP frequently seemed to "overcorrect" (false high percent correction); missing a weak LAC and can be misclassified as a factor deficiency. Few studies suggest a 4:1 mix of PP with

CNP is more sensitive for the detection of a lupus anticoagulant. This study incorporates a 1:1 and 4:1 mix of PP with CNP.

Mixing study test principle

If PT and/or aPTTis prolonged, then mixing test is indicated. A patient would generally need a level >40% of each factor that is being detected by the test procedure to achieve a normal aPTT or PT test result. Therefore, a patient with an inadequate level, meaning less than 40%, of one or more coagulation factor will have a prolonged PT or aPTT test. In the mixing study, an aliquot of abnormal patient plasma is mixed with an equal amount of Pooled Normal Plasma (PNP), which contains approx. 100% of all coagulation factors. The new mixed plasma sample contains at least a 40% level of each factor after the mix, including the factors that may have been present in very low levels in the original sample.[2,3]

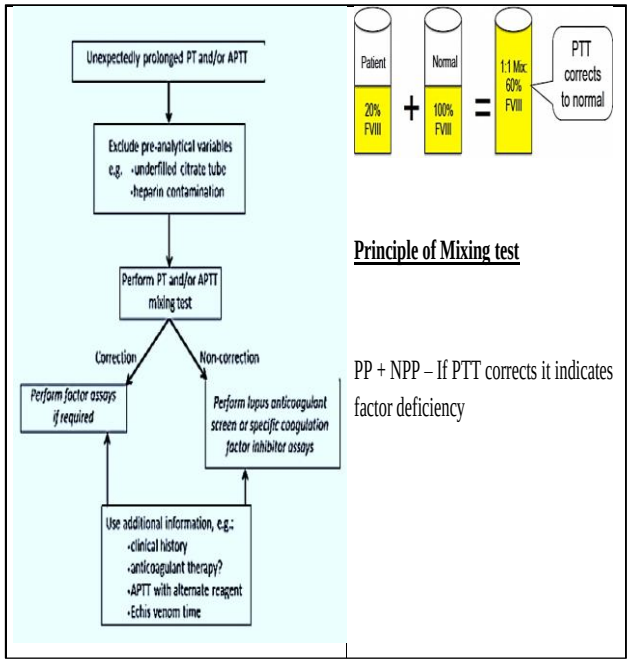

Figure 1: Mixing study-flow chart.

Objectives of the study was to compare the efficacy of RI as reference method against Changs % and Brandt correction, in mixing studies at (1:1) and (4:1) dilutions, to evaluate the sensitivity and specificity of these indices in interpretation of mixing studies.

METHODS

Preanalytical variables[4]

- 3.2% Citrated Plasma: Blood (1:9)
- HCT > 55% (adjust citrate)
- Adequate sample (filled up to mark), check for clots and hemolysis
- Sample should be processed within 4 hours
- Storage: At ≤200 up to 2 weeks and for prolonged storage at -700c
- Centrifugation at for 15 min 3700 rpm for PPP (PLT count <10x109/L

Check points

- Ensure Coagulation factor level is 100% in PNCP
- Assess the sensitivity of aPTT by running dilutions of PNCP with specific factor deficient plasma. This ensures that it will detect a normal result, even if the factor level is as low as 40%.
- In Mixing study, if PT/APTT is prolonged in control tubes, it indicates detoriation of heat-labile factors
- Check for reagents activity.[2]

This is a prospective study of 1-year duration (from March 2018 to March 2019) carried out in a tertiary care hospital, medical college and research centre.

Statistical analysis of data

All data were expressed as Mean±SD. Statistical analysis was done using unpaired students t test. A level of p value <0.05 was used to indicate statistical significance in all analyses.

The blood samples were run in Sysmex CS-5100 Coagulometer and the APTT reagent used is Pathrombin SL, LA1 (DRVVT) and LA2 (confirmatory) which were supplied by siemens.

Inclusion criteria

- Coagulation factor deficiency and Factor specific inhibitors were included

Exclusion criteria

- LAC cases
- Liver disease, DOACs, Warfarin

Mixing study in which patient plasma is mixed with pooled normal plasma in the ratio of (1:1) and (4:1) PP: PNP = (1 :1) and (4:1) mix.

Diagnostic criteria

- Factor deficiency (<40%) - Stage 1 APTT based assay.
- Positive LAC =LA1/LA2 ratio >1.15

The definitions of correction suggested by Brandt et al4 were as follows

- aPTT 1:1 mix result less than or equal to the upper limit of normal.
- aPTT 1:1 mix result less than or equal to the CNP aPTT plus 5 seconds.

Table 1: Interpretation of mixing test.

Interpretation	Tube 1 (PCNP)	Tube 2 (PP)	Tube 3 (1:1 PNCP: PP)	Tube 4 (1:1 PNCP: PP)
	37^0C for 2 hrs.	37^0C for 2 hrs.	37^0C for 2 hrs.	No incubation
Incubate	perform APTT	Perform APTT	perform APTT	Perform APTT immediately
Normal Study	Normal	Normal	Normal	Normal
CF deficiency	Normal	APTT-Prolonged	Normal	Normal
Factor VIII Inhibitor (time dependent)	Normal	APTT-Prolonged	APTT-Prolonged	Normal
Factor IX inhibitor (immediate acting)	Normal	APTT-Prolonged	Normal	APTT-Prolonged

Table 2: Rosners index and Chang's % cut off values.

$Rosner\ Index^2 = \frac{x1{:}1\ mix\ PTT\ -\ PNP\ PTT\ x\ 100}{patient\ PTT}$	$Chang's\ \%\ correction^{5,6} = \frac{APTT\ patient\ plasma\ -\ 1{:}1\ Mix\ aPTT\ X\ 100}{APTT\ patient\ plasma\ -\ PNCP}$
Cut off values ≤ 10 = Correction ≥ 15 = Inhibitor 11- 15 = indeterminate	>70% indicates correction (Factor deficiency) < 58 indicates Inhibitor 58-70 = indeterminate

RESULTS

A total of 200 plasma samples with elevated APTT were studied of which 100 were factor deficient cases and 100 were inhibitors (LAC= 60, DOAC= 30 and F VIII inhibitors= 10).

For factor deficiency RI shows a sensitivity of 91% and 92% for APTT (1:1) and (4:1) mix respectively. For Inhibitors, RI shows a sensitivity of 79% and 89% for APTT (1:1) and (4:1) mix, respectively. These results show that for LAC, RI sensitivity increased from 78% for APTT (1:1) mix to 92% with (4:1) mix. These results clearly indicate that for weak LAC, RI was negative with 1:1 mix and showed more sensitivity in detection of LAC with (4:1) mixing study (Table 3).

Table 3: RI sensitivity for factor deficiency and inhibitor at APTT 1:1 and 4:1 dilution.

APTT Mix	Group	APTT (1:1) Mix Sensitivity%	APTT (4:1) Mix Sensitivity%
Rosners index (RI)	Factor def. (100)	91	92
	Inhibitors (100)	79	89
≤ 10= Correction	LA -60	47(78.3%)	55 (91.6%)
≥ 15 = Inhibitor	DOAC -30	22 (73.3%)	26 (86.6%)
	F8 Inhibitor-10	10	08
RI: 11-15 = indeterminate	Factor def.	09	08
	Inhibitors	21	11

Table 4: Chang % sensitivity for Factor def. and inhibitor at aPTT 1:1 and 4:1 dilution.

APTT Mix	Group	APTT (1:1) Mix Sensitivity%	APTT (4:1) Mix Sensitivity%
Changs % Correction	Factor def. (100)	88	90
	Inhibitors (100)	71	80
>70% = correction	LA -60	41(68%)	48(80%)
(factor def.)	DOAC -30	20 (66%)	24 (80%)
< 58 = Inhibitor	F8 Inhibitor-10	10	08
58-70 = indeterminate	Factor def.	12	10
	Inhibitors	29	20

For factor deficiency Changs % shows a sensitivity of 88% and 90% for APTT (1:1) and (4:1) mix respectively. For Inhibitors Changs % shows a sensitivity of 71% and 80% for APTT (1:1) and (4:1) mix, respectively. These results show that for LAC determined by Changs %, the sensitivity increased from 68% for APTT (1:1) mix to 80% with (4:1) mix. These results clearly indicate that for weak LAC, Changes % was negative with 1:1 mix and showed more sensitivity in detection of LAC with (4:1) mixing study (Table 4).

Table 5: Brandts correction sensitivity for factor def. And inhibitor at APTT 1:1.

APTT (1:1) Mix	Category	Sensitivity %
Upper limit of normal (local lab based)	Factor def (100) Inhibitor (100)	70 45
PNP APTT + 5 seconds	Factor def Inhibitor	75 50

Table 6: Interpretation of APTT 4:1 mixing study.

Immediate % correction	Incubated % correction	Results
>50%	>10%	Factor deficiency
<50%	>10%	Mild factor deficiency
>50%	<10%	Inhibitor
<50%	<10%	LAC

Brandt correction using upper limit of normal reference range criteria shows 70% and 45% sensitivity for factor deficiency and Inhibitor respectively. Brandt correction PNP aPTT + 5 secs criteria shows 75% and 50% sensitivity for factor deficiency and Inhibitor respectively (Table 5).

Based on this result as the APTT 4:1 mix is more sensitive for inhibitor identification, immediate % correction and incubated % correction was studied by using the above criteria. % correction Sensitivity for detection of Factor deficiency = 92% and for Inhibitor = 86% (Table 6).

DISCUSSION

Routine coagulation screening and specific tests are used in investigation of Factor deficiencies, monitoring of DOACs and warfarin, detection of factor 8 and 9 specific inhibitors and LAC.

For evaluation of unexplained prolongation of PT and PTT, mixing tests forms a great diagnostic tool. On Mixing PP 1:1 NPP, if there is correction it indicates Factor deficiency and if mixing results shows non correction then it indicates inhibitors (Figure 2).

The principle of the mixing study seems simple but results often are difficult to interpret in practice. There is no uniform agreement as to what criteria should be used to judge correction.

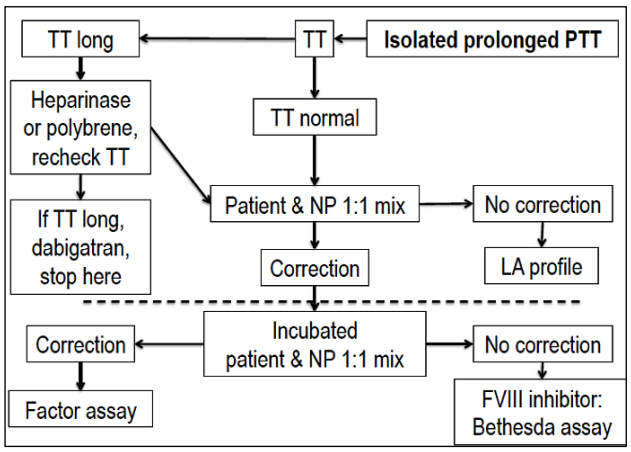

Figure 2: APTT mixing study algorithm.[3,4]

The normal range usually can be used as the guide for correction but the draw back is weak LAC can show correction with a 1:1 mix and in contrast, factor deficiencies with a markedly prolonged PT or aPTT may not be corrected to normal in a 1:1 mix of PP with PNP.[7,8]

The Current 3 major LAC guidelines (BSH, ISTH and CLSI) recommends mixing tests for detection of LAC, even though these test order/sequence vary and there are certain limitations, but still these guidelines advocates mixing test so as to maximize the diagnostic performance.[9,10]

Interpretation of mixing studies results[4]

- If results of Mixing study show correction for both the immediate and incubated APTT, the patient most likely has a single/multiple factor deficiency.
- If Mixing study results shows no correction in either immediate or incubated APTT, the patient may have a coagulation inhibitor most likely LAC.
- If mixing test results shows correction for immediate APTT, but no correction for incubated APTT, the patient may have a slow acting inhibitor such as factor VIII (Table 1).

This study on interpretation of mixing studies as a screening test, shows RI with a cut off value of <10 is 92.5% sensitive in diagnosing Factor deficiency and a cut off value of >15 is 91.1% sensitive for inhibitor diagnosis and it could not categories, 8% of total cases into factor deficiency /inhibitor.[11-13]

Changs % correction with a cut off value of >70% is 85 % sensitive in diagnosing factor deficiency and a cut off value of <58 is 82.2% sensitive for inhibitor diagnosis and it could not categories, 16.5% of total cases into

factor deficiency /inhibitor.[7,8,14] This has prompted me to undertake this study as an extension and supplementation to my previous study.

For factor deficiency RI shows a sensitivity of 91% and 92% for APTT (1:1) and (4:1) mix respectively. For Inhibitors, RI shows a sensitivity of 79% and 89 % for APTT (1:1) and (4:1) mix, respectively.

These results show that for LAC, RI sensitivity increased from 78% for APTT (1:1) mix to 92% with (4:1) mix. These results clearly indicate that for weak LAC, RI was negative with 1:1 mix and showed more sensitivity in detection of LAC with (4:1) mixing study.

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These results show that for LAC determined by Changs %, the sensitivity increased from 68% for APTT (1:1) mix to 80% with (4:1) mix. These results clearly indicate that for weak LAC, Changs % was negative with 1:1 mix and showed more sensitivity in detection of LAC with (4:1) mixing study.

Brandt correction using upper limit of normal reference range criteria shows 70% and 45% sensitivity for factor deficiency and Inhibitor respectively. Brandt correction PNPaPTT + 5 secs criteria shows 75% and 50% sensitivity for factor deficiency and Inhibitor respectively. Based on this result as the APTT 4:1 mix is more sensitive for inhibitor identification, immediate % correction and incubated % correction was studied by using the above criteria % correction Sensitivity for detection of Factor deficiency = 92% and for Inhibitor = 86%.

CONCLUSION

For evaluation of unexplained prolongation of PT and PTT, mixing test should be used as a routine screening procedure for interpretation. Mixing tests forms an important diagnostic tool in differentiating factor deficiency from inhibitors especially in LAC patients. This study recommends mandatory use of mixing tests in LAC cases as also advocated by BSH, ISTH and CLSI. Rosners Index is more sensitive than changes % and BRANDT correction in the interpretation of mixing studies. RI, Changs % and BRANDT correction are more sensitive in detecting weak LAC at APTT; PP 4:1NPP mix in comparison to PP 1:1NPP mix. It can be safely concluded that RI can be used as a reference method for evaluation of mixing studies and its sensitivity is greatly increased by using PP4:1NPP mix.

It's a matter of debate that whether these indices can be effective with other analyzers and reagents.

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