Pathology

# Interpretation of Mixing Studies Done on Abnormal Screening Coagulation Test Results in a Tertiary Care Hospital and Correlation with Clinical Conditions: A Descriptive Observational Study

**Dr. Manisha[1], Dr. Kishore Khatri[2*], Dr. A R kalla[3], Dr. Chanda Khatri[4]**

[1]PG student, Department of pathology, Dr S N Medical College, Jodhpur India
[2]Assistant professor, Department of pathology, Dr S N Medical College, Jodhpur India
[3]Senior Professor and Head, Department of pathology, Dr S N Medical College, Jodhpur India
[4]Assistant professor, Department of Anesthesia and intensive care, Dr S N Medical College, Jodhpur India

**Abstract:** Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are common coagulation screening tests in any patient with bleeding or patients taking warfarin or heparin (for therapeutic purpose). Characteristic features of bleeding caused by coagulation pathways defects are large and few skin hemorrhages, deep hematomas and hemarthroses and delayed bleeding is more common. Mixing studies are done to differentiate between factor deficiency and presence of any circulating inhibitors. In present study 150 cases of abnormal coagulations screening results (INR>1.5 and aPTT > 45 sec.) were taken. Mixing studies on PT with 1:1 mix with pooled normal plasma are done and patients divided in three groups based on percentage correction in A, B and C. Various demographic details, clinical profiles and history correlated with three groups and we reviewed the literature. The age range of patients was from 1 day to 78 years. Male to female ratio was 2.06:1. The ratio of patients from medicine side to surgical side was 1.35:1 (8:7). Out of 80 patients from medical side 62.5% showed >75% correction, 32.5% showed <70% correction and 5% showed 70-75% correction. Out of 70 patients from surgery side, 65.7% showed >75% correction, 22.8% showed <70% correction, and 11.5% showed borderline correction. Most patients of DIC or patients taking warfarin or heparin showed >75% correction. Few patients didn't show expected correction that might be caused by associated metabolic disturbances like acidosis, sepsis, and severe factor deficiencies etc. In the study we conclude that mixing studies may help us to decide that whether the case belongs to factor deficiency or presence of circulating anticoagulant, therefore help to rationalized use of FFP and Vit K. Non correction with known causes/etiologies of factor deficiency needs correction of hidden comorbidities likes acidosis & other metabolic disturbances. Further extensive studies are needed to standardize the cut off criteria for correction so that further treatment plan can be decided in centers where factor assays are not available.
**Keywords:** Prothrombin time (PT), activated partial thromboplastin time (aPTT), mixing studies.

## INTRODUCTION

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are common coagulation screening tests in any patient with bleeding or patients taking warfarin or heparin. This helps to differentiate the bleeding due to platelet related causes and coagulation cascade pathway. Prothrombin time (PT) assesses coagulation factors in extrinsic and common pathway while activated partial thromboplastin time (aPTT) assesses coagulation factors in intrinsic and common pathway. The bleeding caused by thrombocytopenia or platelet function defects usually present with petechial, bleeding gums, nose bleeds etc. Skin petechial is more in number and small, deep hematomas and delayed bleeding is not common. Characteristic features of bleeding caused by coagulation pathways defects are large and few skin hemorrhages, deep hematomas and hem arthroses and delayed bleeding is more common. Mixing studies are done to differentiate between factor deficiency and presence of any circulating inhibitors. The common circulating inhibitor is lupus anticoagulant which is an antibody that binds to phospholipids and proteins associated with the cell membrane. It is actually a prothrombotic agent. These antibodies interfere with phospholipids used to induce in vitro coagulation.

Wide variety of etiologies is associated with factor deficiency apart from some uncommon isolated genetic factor deficiency. The most common cause is DIC, caused by a wide variety of conditions and other large group is therapeutic anticoagulation by warfarin or heparin.

The method of doing mixing studies using 1:1 mix or 4:1 mix with pooled normal plasma and then cut-off criteria for correction are differently documented by studies from many authors. In the present study which is basically a descriptive observational study, we have tried to classify the patients of abnormal coagulation screening test results in three groups based on: correction >75% (group A), <70% (group B) and 70-75% (group C, borderline cases) and correlated the various demographic data and etiologies to these groups and reviewed the literature to find the pathogenic basis and mechanisms associated with different etiologies.

**MATERIALS AND METHODS**

The present study was conducted in MG hospital and MDM hospital, attached to Dr S N medical college Jodhpur. We selected all cases with abnormal PT and aPTT results (INR more than 1.5 and aPTT more than 45 seconds).

All demographic and clinical data related to them are collected. A total of 150 cases are included in the study.

**Instrument**

All tests are done on automated coagulation analyser Stago ST-art4. Reagent used for PT includes Neoplastin (tissue thromboplastin CL plus), lot no 251133. For aPTT , CK Prest, Lot no 251776, expiry date 02/2019 and $CaCl_2$ 0.025M, lot no 251137, expiry 09/2018. Controls for coagulation used are coag control N (normal) and P (pathological), lot no 250835, expiry 06-2018.

**Method**

Patients sample taken in 3.2% sodium citrate vacutainer. Sample centrifuged at 2000 RPM for. 15 minutes. For control of centrifuge speed, platelet poor plasma is made and validated only when platelet counts are below 10,000 /cumm. The 100 µl plasma is taken in fresh cuvette and incubated in on-board incubator. Reagent also is incubated in on-board incubator of instrument. After that the cuvette is placed in test compartment of instrument and a magnetic ball is added. Then 200 µl neoplastin is added. The time taken to clot plasma (stoppage of movement of magnetic ball) from addition of neoplastin is PT. Same is with aPTT, in that 100 µl reagent CK Prest and 100 µl $CaCl_2$ is added.

The normal range of PT is 11-16 seconds, in our centre MNPT (mean normal prothrombin time) is 12.3 seconds. The normal range for aPTT is 30-40 seconds.

**Mixing studies**

In patients with prolonged PT and aPTT, mixing is done by adding equal amount of pooled normal plasma and then this mixture is tested as sample and test re-run.

**Correction formula**

Percent correction= $\dfrac{\text{Patients PT( or aPTT)--1:1 mix PT( or aPTT)}}{\text{Patient PT (or aPTT)- Control normal plasma PT( or aPTT)}}$

For evaluation of correction, original criteria by Chang S *et al.* [1] were used. The cases were divided in three groups.

Group A: where correction of PT on 1:1 mixing study was more than 75%.

Group B: where correction less than 70%.

Group C: Correction between 70-75%.

**OBSERVATIONS AND RESULTS**

This 2 months study is carried out in a tertiary care hospital. Total 150 cases presented for coagulation profile in central lab where INR more than 1.5 and aPTT more than 45 seconds were selected in this study. Age range of patients was from 1 day to 78 years. Male to female ratio was 2.06:1 (Table 1).

**Table-1: Male to female ratio was 2.06:1**

| Male | Female | Total |
|------|--------|-------|
| 101(67.33%) | 49(32.67%) | 150(100%) |

**Table-2: Speciality wise distribution of patients**

| Patients from medical specialities | | | | Patients from surgical specialities | | | | Total |
|---|---|---|---|---|---|---|---|---|
| Group A | Group B | Group C | Total | Group A | Group B | Group C | Total | Total |
| 50(62.5%) | 26(32.5%) | 04(05%) | 80 | 46(65.7%) | 16(22.85%) | 08(11.4% | 70 | 150 |

In this study medicine and surgical speciality patient's distribution in group A and B is not very different but borderline correction is more seen with surgical patients.

**Table-3: Groupwise distribution of associated etiology**

| Etiology | Group A | Group B | Group C | Total |
|---|---|---|---|---|
| Trauma | 18(51.4%) | 14(40%) | 3(8.6%) | 35(23.3%) |
| Cardiac surgery | 11 | 10 | 0 | 21(14%) |
| RHD | 1 | 0 | 0 | 1 |
| MI, CVA | 15 | 3 | 2 | 20(13.3%) |
| Malnutrition | 6 | 0 | 1 | 7 |
| DVT | 3 | 0 | 0 | 3 |
| DIC | 9 | 0 | 1 | 10 |
| CLD | 10 | 10 | 1 | 21(14%) |
| ICH | 2 | 0 | 1 | 3 |
| Acidosis | 3 | 0 | 1 | 4 |
| Hypothermia | 1 | 0 | 0 | 1 |
| Sepsis | 13 | 3 | 1 | 17(11.3%) |
| Embolism | 3 | 0 | 0 | 3 |
| Malignancy | 1 | 1 | 1 | 3 |
| Snake bite | 0 | 1 | 0 | 1 |
| Total | 96 | 42 | 12 | 150 |

In our study maximum 23.3% cases were from Trauma ICU, followed by cardiac surgery and chronic liver disease patients. Sepsis also contributed significantly.

## DISCUSSION

Mixing studies have been used for the evaluating prolongation of PT and aPTT. If a prolonged PT or aPTT of patient plasma is reduced to normal when mixed with normal pooled plasma, factor deficiency is indicated [1,2] and if it is not corrected, then a circulating anticoagulant is suspected. These inhibitors can be further divided into (a) Drug induced like Heparin, F II a inhibitor and Xa inhibitor, (b) Specific factor inhibitor like FVIII, FV and (c) Nonspecific inhibitor like lupus anticoagulant [20].

The principle of mixing studies is simple, but its result are difficult to interpret[3] because there is no uniform criteria by which the correction is to be judged, in addition, even if this strict criteria exists, there are cases where a mildly prolonged PT or aPTT was corrected in case of circulating anticoagulant with 1:1mix studies[3] and the cases with factor deficiency with markedly prolonged PT and aPTT were not corrected to normal 1:1 mix studies[4]. Various authors have also used a 4:1 mix with pooled normal plasma. Kaczor *et al*. [5] suggests 4:1 mix is more suggestive of detection of a lupus anticoagulant, but in contrast study by Brandt [4] found that 4:1 mix was unable to correct aPTT prolongation and it might then be misclassified as having circulating anticoagulant in factor deficiency cases.

A study by Shang-hsiung Chang *et al*. [6] found that a 4:1 mix with a correction cut off >50% on immediate testing and correction cut off at >10% on incubated mix show 100% sensitivity and 100% specificity in both factor deficiency group and anticoagulant group. However because of absence of well-defined and strict criteria, we have taken only the original criteria of 1:1 mix of PT and correction cut off >75% for factor deficiency and <70% for anticoagulant.

Following above criteria, thereupon we focused our study to classify our cases in three groups A, B, C and then review the literature for pathogenesis of different etiologies related to coagulopathy, so this study is basically a descriptive observational study.

### Coagulation abnormalities in chronic liver disease

Most coagulation factors are synthesized by liver parenchyma cells and liver reticuloendothelial system serves as important role in the clearance of activation products. The extent of coagulation abnormalities depends upon the degree of disturbed liver function, acute on chronic hepatocellular disease May results in reduction in Vit. K dependent factors (Factor 2, 7, 9, 10, Protein C & S) while fulminant hepatic failure may present with the pan-coagulation factor deficiencies. And Patients with liver cirrhosis have a wide spectrum of abnormalities Except for Factor 8 & VWF, all procoagulant and inhibitory proteins are decreased [19]. In present study 47.6% cases did not show correction, clinically also they did not respond to FFP in the initial days of their hospitalization, but gradually they started responding. This might be explained by the fact that at least 30 ml/kg FFP needed to raise the level of factors to 20-30 % to reduce the INR, but in these patient two units of FFP might not have raised the levels as more FFP could not be given due to portal hypertension.

### Trauma & coagulopathy

In patients with trauma of trunk and limbs, excessive blood loss & fluid resuscitation cause dilution of factors, but in case of head /brain injury which is the major concern in RTA, with less blood loss and restricted fluid resuscitation, dilution of factors is not important. Brain and spinal cord are rich source of PAF

than other tissues [7] and released during cerebral ischemia and tissue hypoxia. PAF acts on target cells through G-protein coupled seven transmembrane receptor proteins. It contributes to hypoxia induced breakdown of blood brain barrier [8], potentially resulting in release of other brain derived prothrombotic molecules to systemic circulation. In present study 51.4% trauma patient showed >75% correction while 40% cases showed <70% correction which could be explained by involved acidosis, hypo perfusion or mannitol infusion in these patients.

## SEPSIS AND COAGULATION

In septicemia, toxin cause direct activation of coagulation via the effect of chemical mediators on the endothelium and monocytes leading to up regulation of tissue factor [9]. Tissue factor then activates Factor VII of extrinsic pathway & subsequently activated Factor VII of extrinsic pathway activates Factor IX. It leads to prothrombotic environment and ultimately DIC.

In our study 76.4% cases showed >75% correction on mixing studied while 17.6% cases showed <70% correction and 6% cases shoed borderline correction which can be explained by the associated anemia and pH abnormalities.

## ACIDOSIS AND COAGULATION

Mens *et al*. [10] reported that the activities of factor VIIa and FVIIa/TF complex on phospholipid vesicles decreased by more than 90% and 60% respectively, when pH was reduced from 7.4 to 7.1 in pigs, Martin *et al*. [11] showed that thrombin generation decreases to 47% of control values and fibrinogen concentration decreased by 18%.

## HYPOTHERMIA AND COAGULATION

In article by Kees H Poldermann mild hypothermia (upto 35 degree C)-No effect on any part of coagulation. Temp. Below 35 degree C induce mild platelet dysfunction & sometimes mild decrease in platelet count. Temp. Below 33 degree C, other steps in coagulation cascade such as synthesis and kinetics of clotting enzymes plasminogen activator inhibitor affected [12-16].

## Snake bite: vicc (venom induced consumption coagulopathy)

VICC results from activation of the clotting pathway by procoagulant toxins in the venom. The snake venom components that act on the coagulation system are classified according to the part of one coagulation pathway where they act and induce Factor V activators, Factor X activators, prothrombin activators and thrombin like fibrinogenases [17]. Almost all of these toxins cause activation of one or more clotting factors and lead to low or undetectable concentration of fibrinogen following envenoming [18]. In our study only 1 case registered showed <70% correction on mixing study which might be explained

by the fact that patient was brought to hospital 4hr after bite and also assumed that is factor level might be decreased to such an extent that upon mixing factor activities might not have reaches 20-30% so the correction was inadequate.

The patients of cardiac surgery, DVT, embolism, RHD, MI, CVA in our study was on therapeutic anticoagulation. So most patient i.e. 52.4% in cardiac surgery, 100% of RHD, 75% of MI and CVA, 100%DVT, 90% of DIC due to obstetric cases showed >75% correction on mixing studies. In rest of the patient it is assumed that often comorbid metabolic abnormalities might be cause for less than expected correction.

## ACQUIRED FVIII INHIBITORS

Collin PW *et al*. [21] and Knoebl P *et al*. in their study found that Factor VIII inhibitor can be acquired in number of conditions that include Idiopathic (51.9%), Malignancy(11.8%), autoimmune diseases(11.6%), Pregnancy(8.5%), Infections (3.8%), Drug induced(3.4%) and MGUS(2.6%). In our study, cases which show less than 70% correction which otherwise are expected to have factor deficiencies, might have developed these antibodies which interfere with clotting mechanism even after adding normal plasma as a source of factors.

## LIMITATION OF OUR STUDY

- Factor assays was not available to confirm the cases assigned as having factor deficiencies.
- No anticoagulant profile like for lupus available to confirm the presence of circulating anticoagulant.
- The study done and groups formed based on 1:1 mix study only.

## CONCLUSION

In the study we include that mixing studies may help us to decide that whether the case belongs to factor deficiency or presence of circulating anticoagulant, therefore help to rationalized use of FFP and Vitamin K. Non correction with known causes/etiologies of factor deficiency need correction of hidden comorbidities likes acidosis & other metabolic disturbances as well as acquired inhibitors which are associated with many conditions, also seen in our cases.. Further extensive studies are needed to standardize the cut off criteria for correction so that further treatment plan can be decided in centers where factor assays are not available.

## REFERENCES
1. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. New

England journal of medicine. 1995 Apr 6;332(14):912-7.

2. Bockenstedt PL. Laboratory methods in hemostasis. Thrombosis and hemorrhage,. 1998:517-80.

3. Triplett DA. Laboratory diagnosis of lupus anticoagulant. Semin Thromb Hemost. 1990; 16:182-192.

4. Brandt JT, Barna LK, Triplett DA. Laboratory identification of lipus anticoagulant: results of Second International Workshop for Identification of Lupus Anticoagulant. Thromb Hemost. 1995; 74:1597-1603.

5. Kaczor DA, Bickford NN, Triplett DA. Evaluation of different mixing study reagents and dilution effect in lupus anticoagulant testing. Am J Clin Pathol. 1991; 95:408-411.

6. Shang-hsiung Chang, Veronica T, Doris S. A percent correction formula for evaluation of mixing studies. Am J Clin Pathol 2002; 117:62-73.

7. Kumar R, Harvey SA, Kester M, Hanahan DJ, Olson MS. Production and effects of platelet-activating factor in the rat brain. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism. 1988 Nov 25;963(2):375-83.

8. Deng Y, Fang W, Li Y, Cen J, Fang F, Lv P, Gong S, Mao L. Blood-brain barrier breakdown by PAF and protection by XQ-1H due to antagonism of PAF effects. European journal of pharmacology. 2009 Aug 15;616(1-3):43-7.

9. Tapper H, Herwald H. Modulation of hemostatic mechanisms in bacterial infectious diseases. Blood. 2000 Oct 1;96(7):2329-37.

10. Meng ZH, Wolberg AS, Monroe III DM, Hoffman M. The effect of temperature and pH on the activity of factor VIIa: implications for the efficacy of high-dose factor VIIa in hypothermic and acidotic patients. Journal of Trauma and Acute Care Surgery. 2003 Nov 1;55(5):886-91.

11. Martini WZ, Pusateri AE, Uscilowicz JM, Delgado AV, Holcomb JB. Independent contributions of hypothermia and acidosis to coagulopathy in swine. Journal of Trauma and Acute Care Surgery. 2005 May 1;58(5):1002-10.

12. Valeri CR, Feingold HO, Cassidy GE, Ragno GI, Khuri S, Altschule MD. Hypothermia-induced reversible platelet dysfunction. Annals of surgery. 1987 Feb;205(2):175.

13. Watts DD, Trask A, Soeken K, Perdue P, Dols S, Kaufmann C. Hypothermic coagulopathy in trauma: effect of varying levels of hypothermia on enzyme speed, platelet function, and fibrinolytic activity. Journal of Trauma and Acute Care Surgery. 1998 May 1;44(5):846-54.

14. Patt A, McCroskey BL, Moore EE. Hypothermia-induced coagulopathies in trauma. The Surgical clinics of North America. 1988 Aug;68(4):775-85.

15. Ferrara A, MacArthur JD, Wright HK, Modlin IM, McMillen MA. Hypothermia and acidosis worsen coagulopathy in the patient requiring massive transfusion. The American journal of surgery. 1990 Nov 1;160(5):515-8.

16. Bracey JA, Hudson JD, Miller TA, Fischer RP. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. Circulatory shock. 1990 Oct;32(2):141-52.

17. Lu Q, Clemetson JM, Clemetson KJ. Snake venoms and hemostasis. Journal of Thrombosis and Haemostasis. 2005 Aug 1;3(8):1791-9.

18. Levi M, van der Poll T. Disseminated intravascular coagulation: a review for the internist. Internal and emergency medicine. 2013 Feb 1;8(1):23-32.

19. Mammen EF. Coagulation abnormalities in liver disease. Hematology/oncology clinics of North America. 1992 Dec;6(6):1247-57.

20. Kamal AH, Mayo Clinic Proc. 2007;82(7):864-873.

21. Collins PW, Hirsch S, Baglin TP, Dolan G, Hanley J, Makris M, Keeling DM, Liesner R, Brown SA, Hay CR, UK Haemophilia Centre Doctors' Organisation. Acquired hemophilia A in the United Kingdom: a 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. Blood. 2007 Mar 1;109(5):1870-7.