Research Paper

Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

Diagnostic Utility of Fibrinogen Estimation by Clauss & PT Derived Method in an Oncology Setup

Medical Science

KEYWORDS : Fibrinogen, Fibrinogen Assays, Clauss method, Solid malignancy, Hematological malignancy

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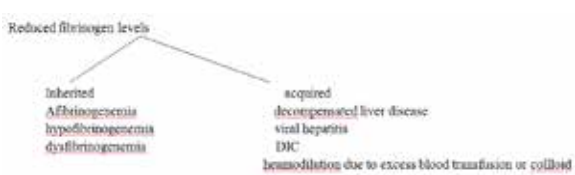
ABSTRACT

Aim: Fibrinogen assay is significant in area of thrombosis research since it is affected by various physiological & pathological conditions such inherited/acquired coagulopathies indicated by low levels of fibrinogen whereas increased levels is a strong independent factor for development of arterial thromboembolism. We compare PT derived method & clauss method of quantitative fibrinogen estimation in 2 groups of patients. Group 1:with Normal coagulation parameters and liver function tests and Group 2:With Deranged coagulation parameters &/or liver function tests. Materials & methods: Reagents for quantitative fibrinogen determination on human citrated plasma using IL coagulation system based on absorbance changes at 450 nm during clotting process of PT were used.Normal range of fibrinogen levels were determined from samples of 20 healthy subjects. Sample size of 64 patients is considered out of whom 15 are cases of solid malignancy, 45 of heamatological malignancy(20 of which are APML) & 4 patients are on oral anticoagulants. They are segregated in 2 groups based on their coagulation parameters & LFT's. Result: In patients with normal coagulation profile ie group 1 ,clauss method was not statistically significant (p value >0.05) whereas In patients with deranged coagulation profile ie group 2, clauss method was statistically significant (p value <0.05). Conclusion: Clauss method is preferred in oncology patients for quantitative fibrinogen estimation as compared to PT derived method.

Introduction:

Fibrinogen is a major plasma protein(normal conc. 1.5-4g/l which is synthesized in hepatocytes predominantly. It comprises each of 3 polypeptide chains (Aa, Bb & gamma) linked by disulphide bridges. Thrombin cleaves Aa & Bb chains to release fibrinopeptides A & B resulting in formation of fibrin monomers that polymerize to form insoluble fibrin clot[1]. it is also an acute phase reactant[1] & can be raised in following conditions:

1. **Physiological**
a) Increasing age
b) Seasonal variation
c) Pregnancy & OCP's
d) Post menopause
2. Inflammatory states
3. Disseminated malignancy



Clinical utlity of fibrinogen assays[2]:

1) For general haemostatic screening in combination of PT,aPTT
2) Investigation of heamorrhagic states (afibrinogenemia, hypofibrinogenemia) ,acquired or congenital dysfibrinoenemia
3) Investigation of unexpected coagulation tests
4) Risk assesement profiling of arterial diseases.

A variety of different fibrinogen assays have been used for various clinical indications such as :

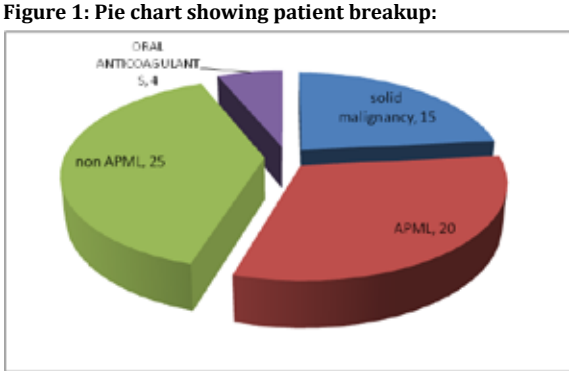
1. Clauss assay
2. PT derived method
3. Clottable protein assay
4. Immunological assay
5. Gravimetric assays
6. Sulphite pptn. Tests

Our study aims to assess applicability of clauss & PT derived method in quantitative fibrinogen estimation.

Materials/methods:

Our study group comprises of 64 oncology patients .

Figure 1: Pie chart showing patient breakup:



15- solid malignancy

45- heamatological malignancy. 25 APML, 20 non APML

4 – oral anticoagulants.

Total study group is futher divided into 2 subgroups based on coagulation parameters:

Group 1 : with normal coagulation profile

Group 2 : with deranged coagulation profile

Normal range of fibrinogen determined from blood samples of normal healthy subjects : 200-450 mg/dl.

Pre test variables:

1. Anticoagulant : 3.2% tri sodium citrate
2. Correct filling: 9 parts blood to 1 part cutrate
3. Haematocrit: adjust citrate for polycythemia
4. Venepuncture: clean, fast, minimal stasis
5. Blood & plasma inspection : clots, hemolysis, lipemia, icterus
6. Storage: 4 degree C for 48 hrs.(unless DIC)

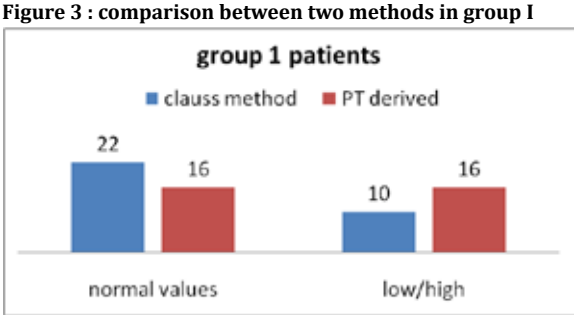
Coagulation system used in our set up for fibrinogen estimation is ACL- elite pro of Internal laboratories (IL) which works on photo optical principle utilizing a filter of wavelength 405 nm

	Clauss method	PT derived method
Principle:	Fibrinogen estimation is done by photometry measuring time in sec. that it takes for absorbance to change by a pre defined amount. This time is further related to fibrinogen estimation[2].	It is based on clot monitoring measurements as it records the light scattered before & after formation of the clot & calculates difference between the 2 readings (LS)[3]
Calibration curve		
Kit insert:		ACL calculates fibrinogen value in mg/dl using a calibration curve which correlates fibrinogen conc. Of 3 callibrations & their LS ratios.
Conc. Used / test sample	log of 3 serial plasma dilutions (preferably 5) & clotting time.	Calibration done by performing PT on serial plasma dilutions of known fibrinogen conc. & by plotting a graph of optical change against fibrinogen conc.
	Fibrinogen C kit: Bovine thrombin reagent , with bovine albumin, calcium chloride , buffer & stablizers	Recombiplastin 2G kit: 1) lyophillised recombinant human tissue factor- reagent 2) diluents- calcium chloride, polybrene & preservative.

Results:

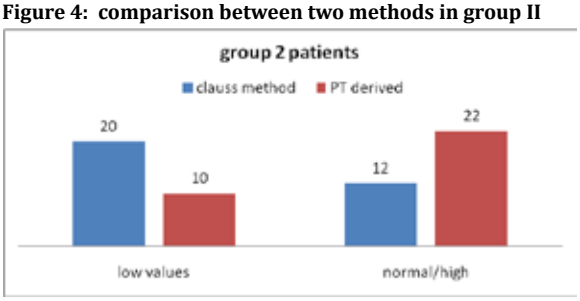
Group 1- pts. TOTAL = 32 With normal coagulation parameters

Figure 3 : comparison between two methods in group I



Group 2- pts. Total =32 with deranged coagulation profile

Figure 4: comparison between two methods in group II



Both methods are compared in 2 groups & results are interpreted as chi square test. They are as follows:

1) In patients with normal coagulation profile ie group 1 ,clauss method was not statistically significant at 95% confidence limit in comparison with PTderived method as p>0.05.
2) In patients with deranged coagulation profile ie group 2, clauss method was statistically significant at 95% confidence limit in comparison with PT derived method as p<0.05.

Mean +/- S.D for Clauss method in group 1 is 318+/-106 & for group 2 patients is 239+/-97

Mean +/- S.D for PT derived method in group 1 is 580+/-297 & for group 2 patients is 496+/-330

Correlation between 2 methods in group 1 & group 2 patients was 0.77 & 0.78 which means that they show *linear correlation.*

In group 2 sample:

Patients with increased PT & normal APTT – 19

CLAUSS PT derived

low	12	7
Normal	4	9
high	1	4

Patients with increased APTT & normal PT **– 7**

CLAUSS PT derived

low	4	1
Normal	3	5
high	0	1

Patients with increased PT & APTT **- 6**

CLAUSS PT derived

low	4	2
Normal	1	3
high	1	1

The recommended types of fibrinogen assays in different clinical situations affecting oncology patients:

Solid malignancies: clauss

APML: Clauss

Non APML heamatological malignancies: clauss

Patients on oral anticoagulants: clauss

Discussion:

Clauss method- merits & demerits:

- Suitable for detecting weak fibrin formation[1].
- Since it is a photooptical method, it is often affected by turbid & lipaemic samples or due to presence of bile or free heamoglobin[2].
- Potential for high carryover due to high conc. Of thrombin which may contaminate subsequent tests like PT APTT resulting in false shortening of clotting times.
- Selection of poor quality of standard plasma or calibrant may cause poor linearity in photo optical coagulometers & poor parallelism with serial dilutions of test plasma resulting in poor potency particularly for samples with low fibrinogen levels.

Clauss method – recommendations:

- Assays should not be performed within 4 hours of administration of unfractioned heparin or from heparin contaminated venous or arterial lines.
- Reagent should be checked for expiry or pH change.
- Quality control for reagants are mandatoryto check for temporal drift.

Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

- Reagent delivery system should be appropriately cleaned to avoid carry over.
- Calibration curve must be linear & should include atleast 3 (but preferably 5) dilutions of plasma
- Test samples must be rediluted if they are outside the linear range of assay.

PT derived method- merits & demerits:

- Used as a general haemostatic screening with PT & APTT in patients where normal fibrinogen values can be predicted such as epidemiological studies of normal populations.
- In thrombolytic therapy, FDP's interfere with clauss assay but not in PT derived method
- determinants like method of calibration, type of analyser, reagent, optical clarity & nature of coagulopathy can affect quantitative fibrinogen estimation.
- Precision is inadequate in PT derived method if either PT was prolonged (eg. As a result of anticoagulants) or fibrinogen levels was high

Conclusion:

Clauss method is standard diagnostic test for quantitative fibrinogen estimation. For routine use both methods are comparable and can be used in combination with PT & aPTT for general hemostatic screening.

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