

MEAN PLATELET VOLUME AS AN INDICATOR OF ASCITIC FLUID INFECTIONS IN CIRRHOTIC PATIENTS

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Abstract

Background: Patients with cirrhosis are usually prone to develop bacterial infections, primarily ascitic fluid infection (15 % -25 %.) Early antibiotic prophylaxis prevents the infection; and hence predicting AFI is important. Mean Platelet volume is proposed to be an indicator of platelet function. Hence the study is planned to assess the predictability of AFI with the help of MPV. **Methodology:** Hospital based cross-sectional study design was adopted. All the patients presenting to Department of General Medicine of adichunchungiri institute of medical sciences with ascites due to liver cirrhosis formed the study population. Data collection period was the calendar year of 2021. Purposive type of sampling technique was used. Based on type of variable, percentages, mean with SD, graphs and tests of statistical significance were used. **Results:** A total of 61 DCLD patients were included in the study. Mean age of the study participants was 45.4 years. 93% of the study participants were males. Prevalence of Ascitic Fluid Infection (AFI) among DCLD patients is 33%, Common presentation of AFI is fever (26.2%) followed by Pain abdomen (23%). mean MPV among study participants / AFI suspects is 8.97 fL. Mean MPV among cirrhotic AFI patients and cirrhotic non-AFI patients is 10.2 fL and 8.42 fL. **Conclusion:** The study has concluded that MPV is a simpler alternative to diagnose AFI. However further studies are required to confirm the role MPV in diagnosis of AFI.

INTRODUCTION

Cirrhosis is a diffuse pathological process, characterized by fibrosis and conversion of normal liver architecture to structurally abnormal nodules known as regenerative nodules.^[1] It can arise from a variety of causes and is the final stage of any chronic liver disease i.e. longstanding injury proceeds to progressive injury to the liver resulting in cirrhosis.

Cirrhosis is a leading cause of mortality and morbidity across the world. It is 15th leading cause of morbidity and 11th leading cause of death, accounting for 1.5% of disability-adjusted life years and 2.2% of deaths and worldwide in 2016.^[2] CLD caused 1.32 million deaths in 2017, approximately two-thirds among men and one-third among women.^[3]

For clinical manifestation to occur, at least 80-90% of liver parenchyma should be destroyed. Cirrhosis is an indolent disease with silent course and the

patient remain asymptomatic until they reach the stage of decomposition. It can lead to portal hypertension, liver failure, and hepatocellular carcinoma. In general, it is considered to be irreversible in its advanced stages, although there can be significant recovery if the underlying cause is treated.

Alcoholic Liver Disease (ALD) is the most important risk factor for the development of cirrhosis. It is caused by chronic heavy alcohol ingestion. About 40 to 80 g/day in men and 20 to 40 g/day in women for 10 to 12 years is sufficient to cause liver damage in the absence of other liver diseases.⁴ Women are more prone to develop alcoholic liver disease due to decreased activity of alcohol dehydrogenase. Also women have a lean body mass and low threshold for toxic dose compared to men.^[4]

Chronic heavy alcohol consumption progresses to steatosis of liver in 92% of people. Steatosis can

progress to alcoholic hepatitis in 12-37% of people and to cirrhosis in 5-18% of people.^[5]

Clinical presentations are highly variable. There is no specific laboratory test to identify alcohol as a cause of liver damage. Liver biopsy, in the context of a history of alcohol abuse, is diagnostic but is not absolutely indicated in all patients. Complications include esophageal or gastric variceal bleeding, ascites, coagulopathy, hepatic encephalopathy, and liver cancer.^[5]

Ascitic fluid infections (AFI)

The most common complication of cirrhosis with portal hypertension is the ascitic fluid infection (31%).

AFIs have been classified into five variants based on analysis of the following parameters:^[6]

- Polymorphonuclear leukocyte (PMN) count.
- Culture growth.
- Mode of entry of organism into the fluid.

Classification Of Ascitic Fluid Infection:^[7]

- Spontaneous Bacterial Peritonitis
- Monomicrobial Non-Neutrocytic Ascites
- Culture Negative Neutrocytic Ascites
- Polymicrobial Bacterascites
- Secondary Bacterial Peritonitis

Criteria for diagnosing Spontaneous Bacterial Peritonitis:^[7]

- PMN count >250cells/mm³.
- A positive ascitic fluid culture.

Criteria for diagnosing Monomicrobial Non-Neutrocytic Ascites:

- PMN count < 250cells/mm³.
- A positive ascitic fluid culture for a single organism.

Criteria for diagnosing Culture Negative Neutrocytic Ascites:

- PMN count is 250cells/mm³.
- Ascitic fluid culture: no organism.

Criteria for diagnosing Polymicrobial Bacterascites:

- PMN count < 250cells/mm³.
- Ascitic fluid culture: multiple organisms.

Criteria for diagnosing Secondary Bacterial Peritonitis:

- PMN count is 250cells/mm³.
- Ascitic fluid culture: multiple organisms.
- Intra-abdominal surgically treatable primary infection.

Platelets

Platelets are small anucleate cells playing a major role in haemostasis and Thrombosis. Platelets were described by Addison in 1841 as extremely minute granules in clotting blood and were termed platelets by Bizzozero, who observed their adhesive qualities as increased stickiness when vessel is damaged.

Mean platelet volume (MPV):

Measurement of peripheral blood platelet count tells little about platelet related haemostatic function. However, most haematology analysers, measure another platelet parameter, the mean platelet volume which can give useful clinical and patho-

physiological information about patients and vascular diseases.

Measurement of platelet volume:

The optimal method for measuring platelet volume

- Electrical impedance (Coulter haematology analyser).
- Light diffraction (Technicon).

Alternative and less satisfactory methods includes

- Semi-quantitative measurement of diameter on platelet smears.
- Flow cytometry.

MPV is calculated from the curve by a formula

$$\text{MPV (fL)} = [\text{Pct (\%)} \times 1000] \div [\text{Plt (x10}^3/\mu\text{L)}]$$

Role of mean platelet volume:

Some studies have shown that MPV has increased in myocardial infarction, cerebrovascular accident, Alzheimer disease, hypertension and celiac diseases. In contrast it has been shown that MPV decreases in various inflammatory diseases like Rheumatoid arthritis, Ankylosing spondylitis, Ulcerative colitis and acute pancreatitis. It has been suggested that the dual role of this marker largely depend upon the intensity of inflammation.

Circulating platelets are abundant source of various pro-inflammatory mediators and pro thrombotic factors. They play a key role in the initiation and propagation of inflammatory and vascular events. Platelets are anucleate cells and their size mostly depends on the fragmentation of megakaryocytes. Studies have shown that cytokines such as interleukin 3 and interleukin 6 influence megakaryocyte ploidy and can lead to the production of large and reactive platelets. Thus Mean Platelet Volume (MPV) have been proposed as an indirect marker of platelet reactivity.

MATERIALS AND METHODS

Study Setting: The study was conducted in Adichunchugiri Institute Of Medical Sciences, BG Nagar, Mandya

Study Design: Hospital based cross-sectional study design was adopted to fulfil the objectives. Eligible participants were enrolled in the study when they approached AIMS for the management of ascites.

Study period: Data collection period was the calendar year of 2020 i.e. from 1stJanuary, 2020 to 31stDecember, 2021

Study Population: Patients presenting to Department of General Medicine of AIMS with ascites due to liver cirrhosis formed the study population. They were screened for eligibility criteria.

Eligibility Criteria

Inclusion Criteria

- Age more than 18 years.
- All inpatients with decompensated liver disease, before the first dose of antibiotic administration.

Exclusion Criteria

- Patients who received antibiotics prior to hospitalization.
- Patients with hollow viscus perforation and secondary bacterial peritonitis.
- Systemic inflammatory diseases.
- Cerebrovascular accidents.
- Myocardial infarction.
- Other acute infections.
- Collagen vascular disorders.

Sampling:

➤ Sample Size Calculation

The sample size was calculated to be 53 using the formula,

$$N = \frac{z^2 pq}{d^2}$$
$$N = \frac{1.96 \times 1.96 \times 83 \times 17}{10 \times 10}$$

where N = Sample size,

z = Standard normal deviate for an α -error of 5% i.e. 1.96,

p = proportion of cirrhotic AFI patients with elevated MPV i.e. 83 %

q = 100 – p = 17 %

d = Maximum allowable error; here, 10% was considered.

Thus the minimum sample size to be attained was 54. However, the total number of eligible participants who were admitted in the hospital (i.e. 61) exceeded this number and all of them consented for the study; hence all were included for the study.

Sampling technique: Purposive type of sampling technique was adopted as it was the most feasible technique with respect to the study. Purposive sampling is a specific type of non-probability sampling method. Here the subjects are selected based on the purpose of the sample. In the current study, purpose was to study the Ascitic Fluid

Infections and the role of Mean Platelet Volume in prediction of the infection.

Method of data collection: Data was collected after obtaining written informed consent. The data regarding hospital number, name, age, sex etc. were noted in the proforma.

Investigations

- Complete Blood Profile: Haemoglobin, Total Leucocyte Count, Differential Leucocyte Count, Platelet count.
- ESR (Erythrocyte Sedimentation Rate).
- Mean Platelet Volume.
- Liver profile: Enzymes (AST / ALT), Proteins (Total proteins, S. Albumin, S. Globulin), S. Bilirubin, PT INR.
- Renal profile: B. urea, S. creatinine.
- Ascitic Fluid: PMN in AF, AF culture (Paracentesis)

Statistical Analysis

Categorical data were compiled and represented in frequencies, proportions and percentages. Numerical data were averaged and represented as mean along with standard deviation. Also, numerical data were grouped into various categories and represented as percentages. Appropriate graphical representations were utilized for representation of the data. Suitable tests of significance (Chi-square test, independent samples t-test) were applied depending on the type of the variable.

RESULTS

A total of 61 patients with DCLD were enrolled for the study. Observations in these patients are summarised below

Characteristics of the Patients

All the 61 study participants required admission and Inpatient care. Their distribution as per age, gender and other characteristics is described in the following pages.

Table 1: Age distribution of the study participants

Age group (years)	Frequency	Percentage
Less than 40 years	17	27.9 %
40 to 49 years	24	39.3 %
50 to 59 years	15	24.6 %
60 to 69 years	4	6.6 %
More than 70 years	1	1.6 %
Total	61	100.0 %

Age of the study participants was distributed across the age of 27 years to 86 years. Mean (Standard deviation) age was 45.4years

Table 2: Sex distribution of the study participants

Gender	Frequency	Percentage
Male	57	93.4 %
Female	4	6.6 %
Total	61	100.0 %

Among the study participants, majority of them i.e. 93% were males.

Table 3: Distribution of study participants by age and gender

Age group (years)	MaleN, (Percentage)	FemaleN, (Percentage)	TotalN, (Percentage)
Less than 40 years	16 (28.1 %)	1 (25.0 %)	17 (27.9 %)

40 to 49 years	21 (36.8 %)	3 (75.0 %)	24 (39.3 %)
50 to 59 years	15 (26.3 %)	0	15 (24.6 %)
60 to 69 years	4 (7.0 %)	0	4 (6.6 %)
More than 70 years	1 (1.8 %)	0	1 (1.6 %)
Total	57 (100 %)	4 (100 %)	61 (100 %)

As per age and gender distribution, most of the study participants were males belonging to the age group of 40 to 49 years. Almost equal number of males i.e. 16 and 15 belonged to the age group of < 40 years and 50 - 59 years.

Table 4: Causes of Decompensated Liver Disease in the study participants

Probable causes	Frequency*	Percentage
Chronic Alcoholism	29	47.5
Hepatitis B Virus infection	8	13.1
Hepatitis C Virus infection	2	3.3
Malignancy	1	1.6
None of the above causes	22	36.1

*Frequency does not add up to 61 as the categories are not mutually exclusive.

Most common cause of DCLD was chronic alcoholism seen in 29 patients i.e. in 47.5% patients. Among the viral infections, HBV (positive in 8 among 61 i.e. 13.1%) was more common than HCV (positive in 2 among 61 i.e. 3.3%). Malignancy was the cause in 1 of the patients. No causative factor for DCLD could be identified in 22 patients (36%).

Table 5: Presenting symptoms in the study participants

Presenting complaints	Frequency*	Percentage
Pain abdomen	14	23.0
Fever	16	26.2
Upper GI bleed	4	6.6
Hepatic Encephalopathy	6	9.8
Diarrhoea	3	4.9

*Frequency does not add up to 61 as the categories are not mutually exclusive.

Table 6: Mean (Standard deviation) platelet volume (in femtolitres) in DCLD

Diagnosis	Mean Platelet Volume (in femto-litres)		
	Range	Mean	Standard deviation
AFI present	7.8 - 10.9	10.2	± 0.46
AFI absent	7.0 - 9.5	8.42	± 0.44
Overall (DCLD) / AFI suspects	7.0 - 10.9	8.97	± 0.92

Overall, MPV was 8.97 fL among 61 DCLD patients. When seen separately among those with Ascitic Fluid Infection and those without AFI, MPV was 10.2 fL and 8.42 fL. Independent samples t-test was applied and it was found that the difference between these 2 means was statistically significant (p value <0.001.)

Table 7: Proportion of study participants with AFI as per diagnostic criteria

Diagnosis	Frequency	Percentage
AFI present	20	32.8 %
AFI absent	41	67.2 %
Total	61	100.0 %

There are 5 types of AFI as discussed earlier. 20 out of 61 study participants satisfied the criteria for diagnosis i.e. 33% of DCLD patients with ascites have AFI.

Table 8: Proportion of various subtypes of AFI among study participants [N=20]

Diagnosis	Frequency	Percentage
Spontaneous Bacterial Peritonitis	7	35.0 %
Culture Negative Neutrocytic Ascites	10	50.0 %
Monomicrobial Non-Neutrocytic Ascites	3	15.0 %
Total	20	100.0 %

There was no poly-microbial growth on culture and hence there are only 3 types of AFI in our study. Half of those who suffered from AFI had CNNA. 7 of the 20 AFI patients i.e. 35% had SBP while the remaining 3 i.e. 15% had MNNA.

Table 9: Culture growth from Ascitic Fluid of the patients

Organisms	Frequency	Percentage
Escherichia coli	7	11.5 %
Enterococcus faecalis	2	3.3 %
Klebsiella sp.	1	1.6 %
No growth	51	83.6 %
Total	61	100.0 %

Paracentesis was done for all the AFI suspects and culture was done to look for the micro-organisms. Most of the patients i.e. 84% had no growth. Among those who had monomicrobial infection, *Escherichia coli* infection (i.e. 11%) was the most common. Another 2% patients were infected with *Klebsiella sp.* And 3% were infected with *Enterococcus faecalis*.

Table 10: Mean 'Platelet Count' among DCLD patients

Diagnosis	Platelet Count (in X 105 cells/cubic mm)		
	Range	Mean	Standard deviation
AFI present (N=20)	0.5 - 1.45	0.85	± 0.29
AFI absent (N=41)	0.6 - 1.50	0.84	± 0.36
Overall (DCLD) / AFI suspects	0.34 - 1.50	0.84	± 0.34

[Independent samples t-test; p value: 0.910]

All the 61 DCLD patients have their platelet count within upper normal limit. Mean [Standard deviation] platelet count among those who had AFI and those who did not have AFI was 0.85 [0.29] and 0.84[0.36] X 105 cells/cubic mm. Independent samples t-test was done to test the significance; and it was found that p value was >0.05. So, not significant.

Table 11: Correlation of Mean (Standard deviation)of 'mean platelet volume'(in femto-litres) in decompensated chronic liver disease with Ascitic Fluid Infection

Diagnosis	Mean Platelet Volume (in femto-litres)		
	Range	Mean	SD
AFI: Spontaneous Bacterial Peritonitis	9.4 - 10.7	10.16	± 0.49
AFI: Culture-Negative Neutrocytic Ascites	7.8 - 10.8	10.07	± 0.35
AFI: Monomicrobial Non-Neutrocytic Ascites	9.2 - 10.9	10.17	± 0.87
AFI absent	7.0 - 9.5	8.42	± 0.44
Overall (DCLD) / AFI suspects	7.0 - 10.9	8.97	± 0.92

[One way ANOVA statistic / F statistic: 63.64; p value: < 0.001]

Mean (SD) of 'Mean Platelet Volume' was calculated for 61 patients based on their type of AFI. Values are as given below:

- Mean (SD) MPV among SBP group : 10.16 (± 0.49) fL
- Mean (SD) MPV among CNNA group : 10.07 (± 0.35) fL
- Mean (SD) MPV among MNNA group : 10.17 (± 0.87) fL

Mean (SD) MPV among those with no AFI among DCLD: 8.42 (± 0.44) fL

To test if these means are significantly different from each other, One-way ANOVA test was applied. F statistic: 63.64 and p value of < 0.001 was obtained, indicating that the difference between the mean MPVs among AFI status of the patients is not statistically significant.

DISCUSSION

The study was conducted with the objectives of studying the ascetic fluid infection and estimating

the mean MPV among various subtypes of AFI. The study was conducted in a tertiary care Hospital and the data collection was done in the calendar year of 2021.

Findings of the study are discussed below

Table 12: Comparison of age distribution of the DCLD patients with other studies

Age categories	Our study	S.V. Sangeetha et al8
< 40 years	28 %	31.8 %
40 - 49 years	39 %	11.4 %
50 - 59 years	25 %	50.0 %
60 - 69 years	6 %	6.8 %
> 70 years	2 %	0.0 %

Age category wise, most of our patients belonged to age group of 40 – 49 years. However in another study conducted by Sangeetha et al8 in Tamil Nadu, most of the study participants belonged to the age group of 50 to 59 years.

Mean age of the patients in our study was 45.4 years (SD:± 9.8 years). In another study conducted by Suvak B et al.^[9] mean (SD) age was 58.1 (± 14.1) years. It was 58.5 (± 7.2) years as per the study conducted by Behiry M. E. et al.^[10] The disease tends to occur almost a decade earlier in our population. This is probably because of early exposure to risk factors and ignorance towards further preventive / treatment measures.

Table 13: Comparison of gender distribution of DCLD/AFI patients with various studies

Gender categories	Our study	Suvak B. et al, ^[9]	Martinez M.G. et al, ^[11]
Male	93 %	65 %	47 %
Female	7 %	35 %	53 %

Gender distribution of our study is as shown above. In other studies the proportion of females who have developed DCLD and / or AFI is comparable to that of the males. This is not the same in Indian scenario, since practice of high-risk behaviour and healthcare seeking behaviour is higher in males.

Table 14: Comparison of (hospital study based) prevalence of Ascitic fluid infection with other studies

Diagnosis	Our study	S.V. Sangeetha et al, ^[8]
AFI (Overall)	33 %	38.7 %
AFI: CNNA	16.5 %	-
AFI: SBP	11.5 %	24 %
AFI: MNNA	5 %	14.7 %

Overall prevalence of AFI among DCLD patients is comparable with another Indian study. However, CNNA cases were not recorded in that study. It is important to know the type of AFI prevalent in our study setting (or place of work) for better management of the condition. Also role of MPV in provisional diagnosis of AFI may differ with the type of AFI, as shown in some of the studies, where MPV is an important indicator when it comes to provisional diagnosis of SBP and CNNA.

Table 15: Comparison of Mean MPV of AFI patients with other studies

Mean Platelet Volume	Mean (fL)	Standard Deviation (fL)
Our study	8.97	± 0.92
Martinez M.G.et al, ^[11]	9.0	± 0.8
AmalA.etal, ^[12]	8.5	± 0.65
Suvak B. et al, ^[9]	8.37	± 0.98
Behiry M.E. et al, ^[10]	11.0	± 1.2

Mean MPV among cirrhotic patients who developed AFI in our study was 8.97fL. This is low estimate when compared to studies conducted by others in various parts of Egypt and United States in the last few years. Recommendation for MPV cut-off to diagnose AFI provisionally, as per Behiry M.E. et al, is 10.8fL. However none of the other studies show this high estimate of MPV.

Overall, MPV alone appears to be very insufficient to diagnose AFI. Further research is required in this regard. Other platelet indices can also be studied and a simple scoring system may be developed, which would provide better precision than that with MPV alone.

Strength of the study is that the study is conducted in a tertiary care hospital, which caters to a large population. Hence variety of cases can be covered.

Limitations of the study are relatively smaller sample size and absence of comparison / control groups.

CONCLUSION

- Commonly encountered causes of DCLD are Chronic Alcoholism and Viral infections (HBV and HCV).
- AFI is a common complication among DCLD patients. Prevalence of AFI in our study is 33%.

- AFI patients often present with Ascites, Pain Abdomen and Fever. Diarrhoea and Hepatic Encephalopathy are uncommon presentations.
- Commonly encountered organisms in AF-culture are: Escherichia coli, Klebsiella sp. and Enterococcus faecalis. However this demands an invasive procedure and a waiting period of 2 to 4 days.
- Mean Platelet Volume is a relatively rapid alternate; it definitely varies among different subtypes of AFI; and also from individuals with no AFI. The difference has been found to be statistically significant.
- Further studies are required to assess the role of MPV in presumptive diagnosis and management of AFI suspects.

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