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BONE MARROW CHANGES IN MEGAKARYOCYTES IN THROMBOCYTOPENIA – A TERTIARY CARE CENTER STUDY

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ABSTRACT

BACKGROUND: Platelets play vital roles in repair of the minute vascular damages in day to day life, wound repair, the innate immune response and metastatic tumour cell biology.

AIMS AND OBJECTIVES: The aim of our study was to examine bone marrow aspiration smears for changes in megakaryocytes in patients with thrombocytopenia.

MATERIALS AND METHODS: It was a prospective study carried out from June 2022 to June 2023. A series of 53 bone marrow aspirates were examined in patients with thrombocytopenia to look for changes in megakaryocytes.

RESULTS: Out of the 53 cases megakaryocytes were suppressed in 20 cases (37%), inadequate in 9 cases (17%), hypoactive in 11 cases (21%), absent in 6 cases (11.5%) and adequate in 7cases (13.5%) of thrombocytopenia. In our study nutritional deficiency was the most common cause of thrombocytopenia.

CONCLUSION: Peripheral blood and bone marrow examination is crucial for patients with thrombocytopenia. Understanding the morphological changes of megakaryocytes in bone marrow aspirates can improve the diagnostic accuracy.

KEYWORDS: BONE MARROW ASPIRATION, MEGAKARYOCYTES, THROMBOCYTOPENIA

INTRODUCTION

Megakaryocytes arise from pluripotent hematopoietic stem cells that undergo lineage commitment, proliferation and differentiation under the influence of cytokines, in particular thrombopoietin (TPO) to produce 1000–3000 platelets. (1)

It has become apparent that megakaryocyte morphology plays an important role in thrombopoiesis and that large megakaryocytes produce more platelets than smaller ones.

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The higher the ploidy (nuclear lobulation), the higher the platelet production. The mechanisms that control platelet size are poorly understood but it seems that the body tries to maintain the total platelet mass and not the total platelet count.

The total platelet mass is the constant derived when multiplying the total platelet count by the mean platelet volume. When the platelet count decreases, the mean platelet volume increases. When megathrombocytes (giant platelets) are seen on the peripheral smear it usually indicates peripheral consumption of platelets.

Platelets play vital roles in repair of the minute vascular damages in day to day life, wound repair, the innate immune response and metastatic tumour cell biology. (2)

The average platelet count in humans ranges from 150 - 350 × 109 /L, although the level for any individual is maintained within fairly narrow limits. (3) Platelets are formed from cytoplasmic buddings from megakaryocytes. (4,5) Thrombocytopenia is a common hematological condition for which bone marrow aspiration is indicated. (3)

Normal megakaryocytes develops in the bone marrow and reaches cell sizes <50-100 microns in diameter. (6,7) The hallmarks of megakaryocyte maturation includes endoreduplication (polyploidisation) and expansion of cytoplasmic mass.(8)

AIM

The aim of this study was to examine bone marrow aspiration smears for changes in megakaryocytes in patients with thrombocytopenia.

MATERIAL AND METHOD

This was a prospective study over a period of 1 year (June 2022 to June 2023) in the Central Pathology Lab, Department of Pathology at Gajra Raja Medical College, Gwalior.

All the cases of thrombocytopenia which were diagnosed on haematology analyser (platelet count < 1,50,000); confirmed subsequently by peripheral smears were taken up for the study.

Bone marrow aspiration was done using Salah needle. Bone marrow examination was carried out after taking informed written consent by trained and competent medical officers under aseptic conditions according to the standard operative procedure from the posterior superior iliac crest using local anaesthesia. 0.5 to 1 ml bone marrow aspirations were done and smear were made.

Bone marrow was evaluated after taking in detailed clinical history with features like pallor, liver and spleen size, lymphadenopathy, history of drug intake, bone pains, bleeding etc. General anaesthesia was used in children. No immediate or long-term complications were reported.

Films of aspirated marrow made at the bedside and fixed once they were thoroughly dry. Leishman stain and Giemsa stain were used routinely.

Criteria for Patient Selection:

1. Inclusion Criteria: Patient of all group and gender who presented with platelet count < 1.5 lakh/ cubic mm.

2. Exclusion Criteria: Patient who were unable to tolerate the bone marrow aspiration procedure.

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RESULTS:

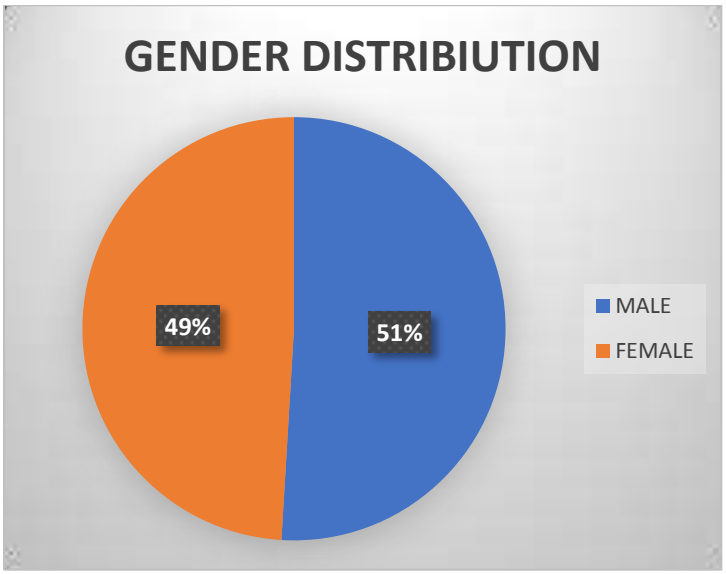
Demographic Distribution

AGE GROUP (IN YEARS)	NO. OF CASES	PERCENTAGE
<10	23	43%
11-20	19	35%
21-30	03	06%
31-40	01	02%
41-50	01	02%
51-60	04	08%
>60	02	04%
TOTAL	53	100%

Table 1: AGE WISE DISTRIBUTION

- Majority belonged to the age group of < 20 years.
- >20 years had only 21 % cases were as 79 % cases were in < 20 years.

- Figure 1: GENDER DISTRIBUTION



- Male patients showed predominance with male to female ratio of 1.03:1

Table 2: CONDITIONS ASSOCIATED WITH THROMBOCYTOPENIA

S.NO	CONDITIONS	NUMBER OF CASES	PERCENTAGE
1	ACUTE LEUKEMIA	12	22%
2	MEGALOBLASTIC ANEMIA	08	15%
3	IRON DEFICIENCY ANEMIA	03	5.5%
4	MIXED NUTRITIONAL DEFICIENCY	06	11%
5	MDS	02	3.5%
6	CHRONIC INFECTIOUS ETIOLOGY	03	5.5%
7	IDIOPATHIC	04	7.5%
8	HYPOPLASTIC MARROW	03	5.5%

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9	INADEQUATE/NON-DIAGNOSTIC	09	17.0%
10	APLASTIC ANEMIA	02	3.5%
11	ITP	01	02%
	TOTAL	53	100%

- In our study among the conditions associated with thrombocytopenia the most common cause was nutritional deficiency which predominantly included 8 cases of megaloblastic anemia and 6 cases of mixed nutritional deficiency
- 12 cases of acute leukemia i.e. 22% cases had thrombocytopenia.
- Chronic infectious etiology and hypoplastic marrow had 3 cases each were as in MDS and aplastic anemia each had 3.5% cases.
- Aplastic anemia and ITP had 3.5% and 2% cases respectively.
- 9 cases were inadequate and 4 cases had unknown etiology.

Table 3: RANGE WISE DISTRIBUTION

RANGE OF THROMBOCYTOPENIA	NO. OF CASES	PERCENTAGE
MILD	08	15%
MODERATE	11	20%
SEVERE	34	65%
TOTAL	53	100%

- Severe thrombocytopenia was seen in majority of cases i.e. 65%
- Were as mild and moderate thrombocytopenia was seen in 15% and 20% cases respectively.

CHANGES IN MEGAKARYOCYTES:

In our study, megakaryocyte alterations were divided into 5 major categories: suppressed, absent, adequate, inadequate or nondiagnostic and hypoactive. (table 4)

Supressed category was based on the number of megakaryocytes per high power field. In this study, criteria for suppression was taken as less than 2 megakaryocytes per high power field.

Absent category included total absence of megakaryocytes in the examined bone marrow smears.

Adequate category included more than or equal to 2 megakaryocytes per high power field

Inadequate or nondiagnostic category was assigned to those cases which had diluted smears or less cellularity to make adequate diagnosis.

Hypoactive category was characterised by megakaryocytes with increased nuclear to cytoplasmic ratio and hypo lobated or non-lobated nucleus. (2)

The above categories were made to identify the exact cause of thrombocytopenia in various cases.

Table 4: ALTERATIONS IN MEGAKARYOCYTES

SL.NO.	ALTERATION	NO. OF CASES	PERCENTAGE
1	SUPRESSED (<2/ hpf)	20	37%
2	ABSENT	06	11.5%

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3	ADEQUATE	07	13.5%
4	INADEQUATE/NONDIAGNOSTIC	09	17%
5	HYPOACTIVE	11	21%
	TOTAL	53	100%

- In our study, megakaryocytes were supressed in 20 cases, hypoactive in 11 cases and inadequate in 9 cases.
- We saw absence of megakaryocytes in 11.5% cases were as it was adequate in 7 cases of thrombocytopenia.

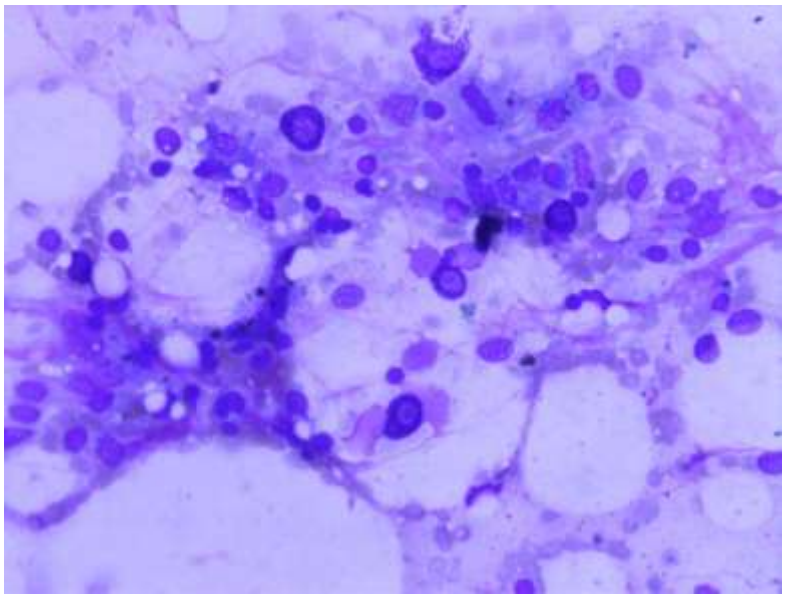

Figure 2 : Aplastic Anaemia (40x) showing fatty fragment with mast cells & absent Megakaryocyte

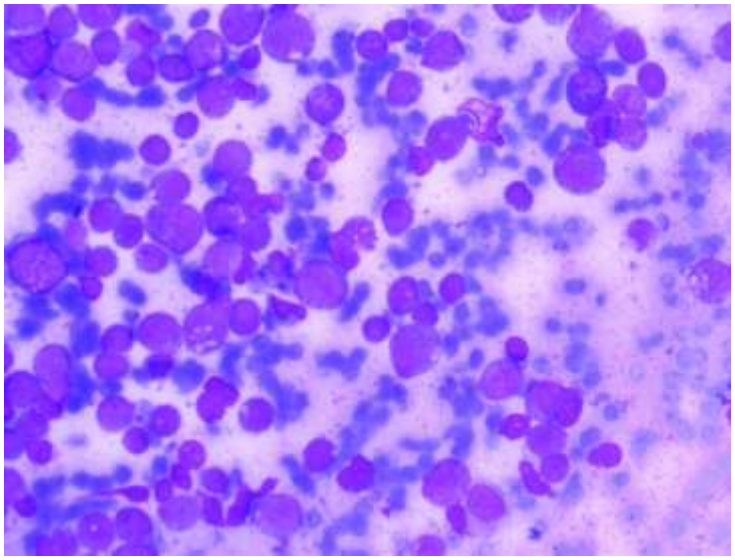

Figure 3: Acute leukemia (AML) 40x showing Immature Blast with Suppressed Megakaryocytes

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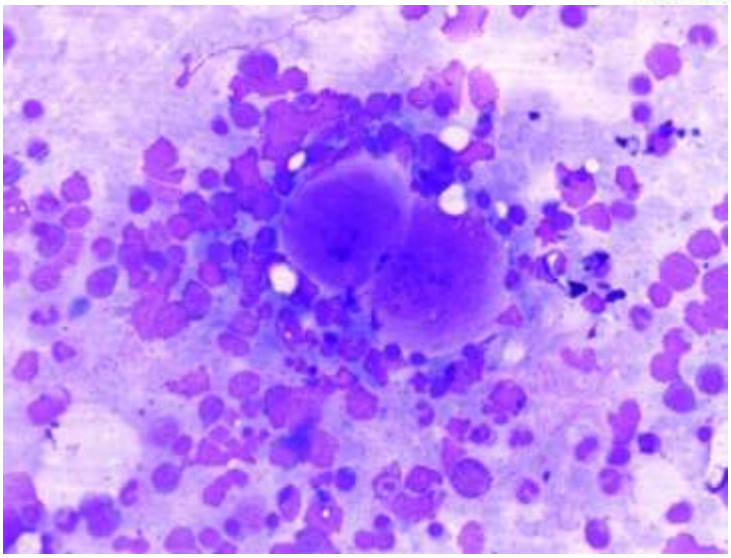

Figure 4 : IdiopathicThrombocytopenicPurpura(ITP) 40x showingimmature&hypolobatedplatelets

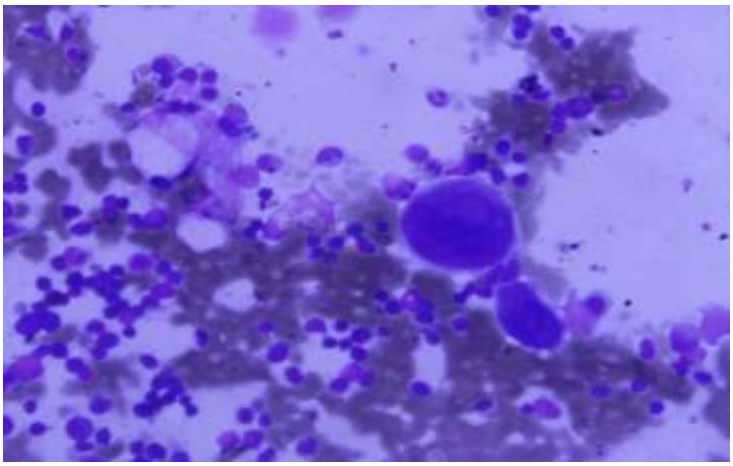

Figure 5 : MyelodysplasticSyndrome(MDS) 4 0 x showing Dysmegakaryopoiesis

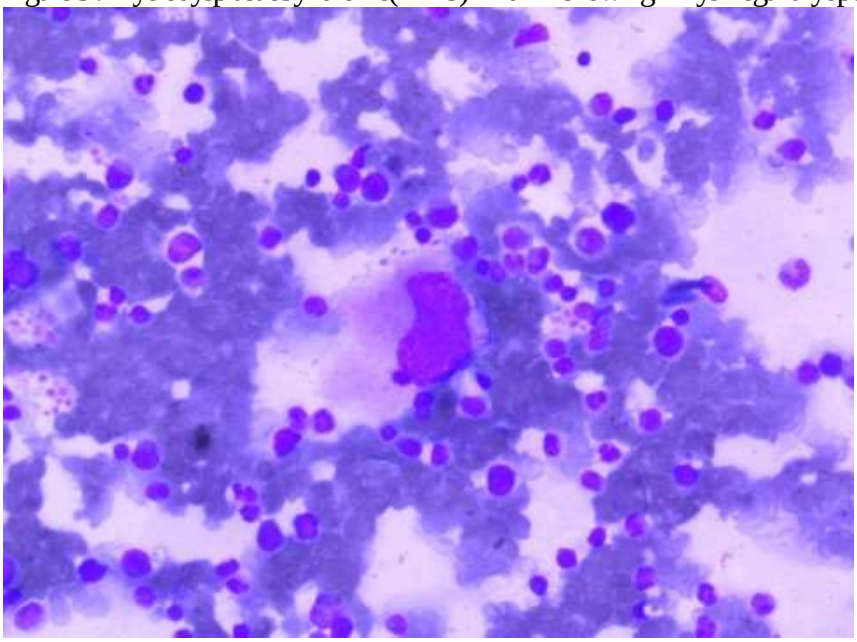

Figure 6 : MicromegakaryocytewithNonlobatedNuclei (10x)

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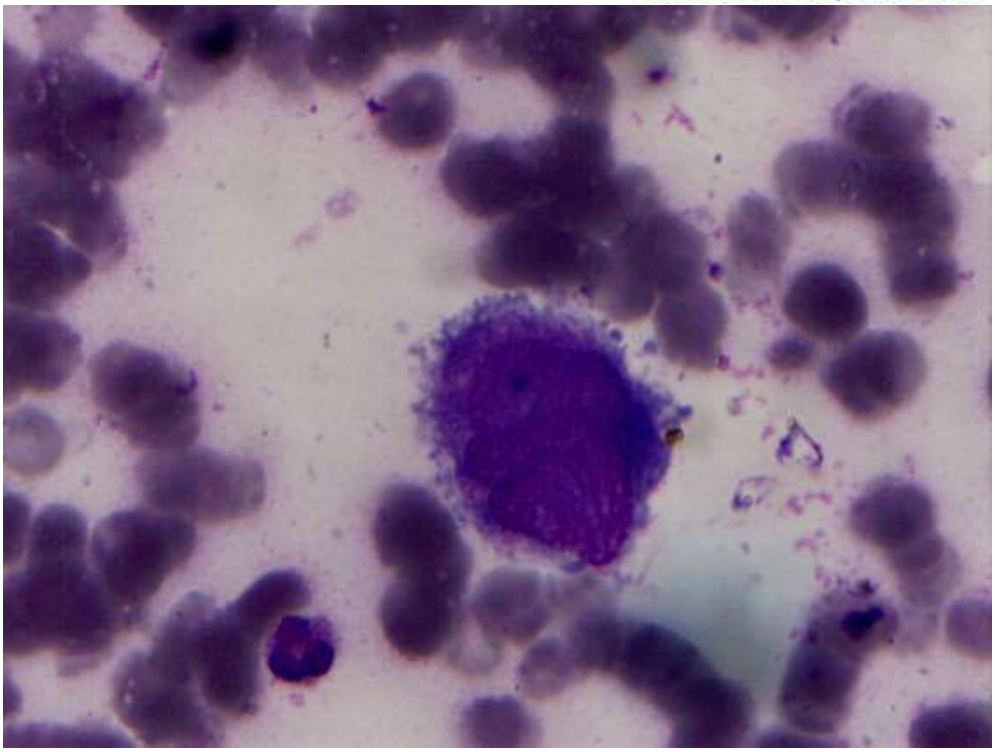

Figure 7 : Hypolobated Megakaryocytes(100x)

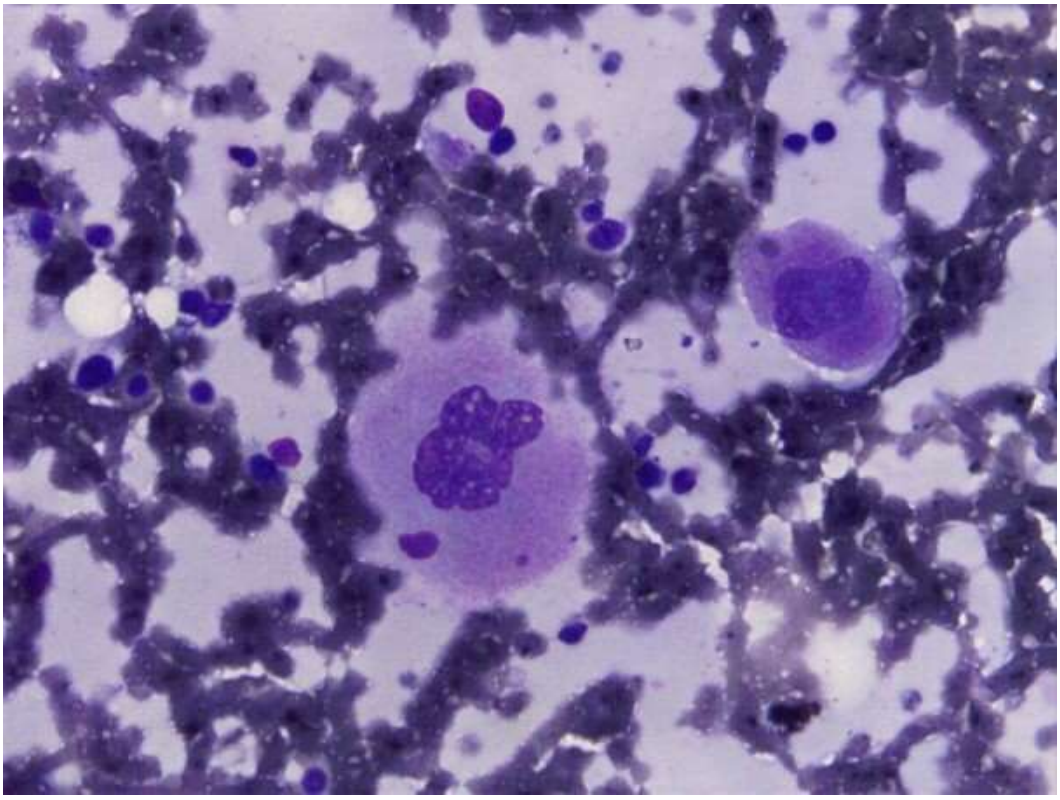

Figure 8 : AdequateMegakaryocytes (2/HPF)

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DISCUSSION

Megakaryocyte and platelets are highly specialized cells that participate in haemostatic and inflammatory functions.

Since each platelet lives only about 10 days, the platelet supply is continually renewed by production of new platelets from the maturation of megakaryocyte.

In present study 53 cases of bone marrow aspirations were done over a period of 1year. Out of the 53 cases, maximum number of cases was seen in <20 years of age similar to Pokharel S et al (9).

Least number of cases was seen in > 60 years of age which was in concordance with Muhury M et al (2).

There were more male cases than females in our study similar to studies conducted by Chaudhary et al (3) and Parul G et al (10). 17% of cases were inadequate to make opinion.

In our study, nutritional deficiency was the most common cause of thrombocytopenia i.e. 17 cases (32%) which includes 8 cases (15%) of megaloblastic anemia, 6 cases (11.5%) are of mixed nutritional deficiency anemia and 3 cases (5.5%) of iron deficiency anemia. Megaloblastic anemia was the most common cause of thrombocytopenia in patients with nutritional deficiency in our study.

However, megaloblastic anemia was the overall most common cause of thrombocytopenia in Chaudhary et al (3), Deepika et al (11) and Das et al (12).

Acute leukemia was the second most common cause of thrombocytopenia with 12 cases (22%) similar to Singh et al (13).

Megakaryocytes were decreased or absent in aplastic anaemia, which was also observed by Shadduck et al. (14)

Other causes were Hypoplastic marrow 3 cases (5.5%), ITP 1 case (2%), aplastic anemia 2 cases (3.5 %), MDS 2 cases (3.5%).

We also had 3 cases (5.5%) of chronic infectious etiology and 4 cases (7.5%) of unknown etiology.

Severe thrombocytopenia was seen in 65% cases with bone marrow showing suppressed and hypoactive megakaryocytes in majority of cases.

CONCLUSION

Peripheral blood and bone marrow examination is crucial for patients with thrombocytopenia. Understanding the morphological changes of megakaryocytes in bone marrow aspirates can improve the diagnostic accuracy of disorder associated with thrombocytopenia there by enabling appropriate treatment modalities.

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