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HEMATOLOGICAL DIVERSITY IN PANCYTOPENIA: A TERTIARY CARE HOSPITAL EXPERIENCE

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ABSTRACT: **BACKGROUND:** Pancytopenia is defined as the reduction of all three formed elements of blood (Erythrocytes, leucocytes and platelets) below the normal reference range leading to anaemia, leucopoenia & thrombocytopenia. **AIM:** To evaluate the etiological spectrum of pancytopenia on the basis of hematological parameters including Bone marrow aspiration, trephine biopsy and flow cytometry. **MATERIALS AND METHOD:** All the patients with pancytopenia admitted at S.C.B Medical college & Hospital, Cuttack, Odisha, over a period of 2 years duration from September 2012 to September 2014 were studied prospectively after their written informed consent Patients of all ages & both sexes and those fulfilling the hematological criteria like Hb<13.5g% in Male, <11.5g% in Female, TLC<4000/cmm, TPC<1.5lacs/cmm were included in the study. Patient on myelotoxic chemotherapy and/or with a history of recent blood transfusion were excluded from the study. **RESULTS**: Most common cause of pancytopenia in the pediatric age group is Acute lymphoblastic leukemia (78.37%) out of which 73.33% are B-ALL, 23.33% are T-ALL and 3.33% of Mixed phenotypic acute leukemia (MPAL).Among the adults aplastic anemia (78.04%) is the most common cause of pancytopenia followed by megaloblastic anemia (9.75%). **CONCLUSION:** The causes of pancytopenia varied from infections like malaria to diseases like aplastic anemia where the marrow production of the blood cells was primarily affected. Hence identification of the cause is important for right intervention.
KEYWORDS: aplastic anemia, flow cytometry, pancytopenia.

INTRODUCTION: Pancytopenia is defined as reduction of all the three formed elements of blood (Erythrocytes, leucocytes and platelets) below the normal reference range leading to anaemia, leucopoenia and thrombocytopenia**.** It is not a disease entity but a triad of findings that may result from a number of disease processes. Variety of disorders, hematological and non-hematological can affect the bone marrow primarily or secondarily, resulting in the manifestation of pancytopenia.[1]

Early diagnosis of various causes of pancytopenia is very crucial and requires prompt clinical examination and investigations like complete blood count, peripheral smear and bone marrow examination as marrow cellularity and composition in cases of pancytopenia differ in relationship to underlying pathologic conditions. Examination of bone marrow aspirates and trephine biopsy help in most cases to establish the cause of pancytopenia. Whenever these investigations do not establish the diagnosis further investigations are necessary according to the nature of provisional diagnosis. Considering the magnitude of the problem with its multiplicity of implications as regards to etiology, features, diagnosis and remedy the present study "Hematological Diversity in Pancytopenia" has been taken up.

MATERIAL & METHODS: All the patients with pancytopenia admitted at S.C.B Medical College & Hospital, Cuttack, Odisha, over a period of 2 years duration from September 2012 to September 2014

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were studied prospectively after their written informed consent Patients of all ages & both sexes and those fulfilling the hematological criteria like Hb<13.5g% in Male, <11.5g% in Female, TLC<4000/cmm, TPC<1.5lacs/cmm were included in the study. Patient on myelotoxic chemotherapy and/or with a history of recent blood transfusion were excluded from the study. The analysis was carried out on the basis of detailed history, physical examination, systemic examination, routine blood hemogram, bone marrow aspiration & biopsy. Flow cytometry was done in selected cases.

RESULT: The observations are based on 102 cases of pancytopenia reported on routine hematological investigations in the Department of Pathology, S. C. B. Medical College, Cuttack. The age of presentation in the present study ranged from 1 year to 65 years. Maximum number of cases were observed between 30-65 years [40.20%], followed by 1-14 yr [34.31%]. The M:F ratio is 2.64:1. Diseases causing pancytopenia were categorised into three groups. Group - I includes aplastic anaemia. Aplastic anaemia was found to be the commonest cause of pancytopenia, 44 cases [43.13%]. Group - II includes acute lymphoblastic leukemia32 cases, acute promyelocytic leukemia 2 cases, JMML 1 case, hypocellular ALL 2 cases, MDS 3 cases and metastatic deposit in marrow 2 cases together constitute [41.17%] (Fig-I). Acute lymphoblastic leukemia is the second most common cause of pancytopenia 32 cases [31%]. Hypocellular ALL constitutes 2 cases (1.96%) in our study. Group - III includes the miscellaneous causes like megaloblastic anaemia, hypersplenism, disseminated histoplasmosis with hemophagocytosis and malaria. All these minor causes of pancytopenia together constitute 15.68% of cases. In our study ALL constitute the most common cause of pancytopenia in pediatric patients and aplastic anemia is the most common cause of pancytopenia among adult cases.

DISCUSSION: Table - I shows age wise distribution of cases of pancytopenia most common cause of pancytopenia in the pediatric age group is acute lymphoblastic leukemia that includes 29 cases (78.37%) out of 37 pediatric cases**.** In the present study, among young adults, aplastic anemia 11 cases (45.83%) out of 24 cases constitute majority of cases of pancytopenia followed by megaloblastic anemia 8 cases (33.33%).Our finding coincide with Adit et al [2001] and Santra G et al [2010].[2,3] In our study no drugs or secondary causes of aplastic anaemia could be traced out in 29 cases(65.90%) which were grouped under idiopathic [primary] aplastic anaemia. In 15cases (34%) history of exposure to drugs, chemicals and viral infection was traced out that were categorized as secondary aplastic anemia. Our observation is close to Welch et al [1954][4] reported 49.2%, Wolff et al [1957] 57.2% and C. S. Sahu et al [1982] 53.6% of idiopathic aplastic anemia. The bone marrow biopsy in cases of aplastic anaemia reveals increased fat space, marked reduction of myeloid and erythroid cells similar to study by Saitoh et al [2002]. Megakaryocyte were not found in our series (Fig. II).

Table II shows the association of PNH Clone in Aplastic anemia cases. Among all aplastic anemia cases those diagnosed as idiopathic aplastic anemia were advised for flow cytometry to rule out association of PNH Clone. One case was lost for follow up and in rest 28 cases flow cytometry for CD55 and CD59 was done. Out of 28 cases, 3 cases (10.7%) showed marked decrease in the expression of CD55 and CD59 on the surface of red blood cells (Fig-IV).

Analysis of glycosylphosphatidyl inositol (GPI)-anchored proteins, such as CD55 and CD59 by flow cytometry, is a sensitive and quantitative test for PNH screening enabling the detection of

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small PNH clones which occur in up to 50% of patients with aplastic anaemia, the proportion depending on the sensitivity of the flow cytometric analysis used (Dunn et al, 1999; Socie et al, 2000; Sugimori et al, 2005).[5] The morphological subtype of acute leukemia observed were, ALL LI, L2and L3, APML and JMML. In the present study majority of cases come under ALL L1 subtype 27cases (64.28%).

ALL L2 constitute 4 cases (9.52%).One case (2.38%) each of ALL L3 and JMML was found. APML 2 cases (4.76%) and MDS 3 cases (7.14%) were noted. Chessells J M. 2001[6] reported Hypocellular acute lymphoblasticleukemia (ALL) occurs in 1–2% cases of childhood ALL. There were 2 cases (4.76%) of metastatic deposit in marrow, one was a two years old male child with metastatic neuroblastoma and other one a sixty five year old male with metastatic adenocarcinoma. One case of Histoplasmosis with SLE was there. In the bone marrow there was suppression of all the three lineages of cells with marked increase in reticulum cells showing features of Hemophagocytosis (Fig. IIIA, B).

Table III shows the subtype of ALL from flow cytometry. In our study ALL constitute the most common cause of pancytopenia in pediatric patients and the treatment decision of these patients depends on immunophenotype. So flow cytometry was advised in all ALL Cases and follow up was done. Two cases were lost for follow up. Out of 30 cases of ALL, 22 cases (73.33%) are B-ALL (Fig-V), 7 cases (23.33%) are T-ALL (Fig-VI) and 1 case (3.33%) of mixed phenotypic acute leukemia (MPAL) was diagnosed. The immunological markers CD19, CD20, CD22, and CD79 a belong to B cells, while CD2, CD3, CD5, CD7 are found on the surface of the T cells. Except for M0 and M7 (CD41, CD61), the myeloid markers are CD13, CD33, CD117 and also the cytoplasmic myeloperoxidase (MPO). MPAL accounts for approximately less than 5% of all acute leukemia and represents a diagnostic and therapeutic dilemma.[7]

CONCLUSION: Pancytopenia is a common hematological problem encountered in clinical practice. The present study concludes that detailed primary hematological investigations along with bone marrow aspiration, trephine biopsy and flow cytometry are helpful for understanding the disease process to diagnose, or to rule out the causes of pancytopenia and in planning further management of cytopenic patients.

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AGE	CAUSES	NUMBER	PERCENTAGE OF CASES
1-14 yrs	ALL	29	78.37%
	HYPOCELLULAR ALL	2	5.40%
	APML	2	5.40%
	APLASTIC ANEMIA	1	2.70%
	JMML	1	2.70%
	HYPERSPLENISM	1	2.70%
	METASTATIC NEUROBLASTOMA	1	2.70%
	TOTAL	**37**	**36.27%**
15-30 yrs	APLASTIC ANEMIA	11	45.83%
	MEGALOBLASTIC ANEMIA	8	33.33%
	ALL	3	12.5%
	MALARIA	1	4.17%
	HYPERSPLENISM	1	4.17%
	TOTAL	**24**	**23.52%**
31-65 yrs	APLASTIC ANEMIA	32	78.04%
	MEGALOBLASTIC ANEMIA	4	9.75%
	MDS	3	7.31%
	METASTATIC ADENOCARCINOMATOUS DEPOSIT	1	2.43%
	HISTOPLASMOSIS WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS	1	2.43%
	TOTAL	**41**	**40.20%**
1-65 yrs	**GRAND TOTAL**	**102**	**100%**

Table I: Age Wise Distribution of cases

PNH Clone	No. of cases	%
PNH positive	03	10.7
PNH negative	25	89.3
Total	**28**	**100**

Table II: Association of PNH Clone in Aplastic anemia

| Subtypes of ALL	Number	Percentage
B-ALL	22	73.3%
T-ALL	7	23.33%
Mixed Phenotype Acute leukemia (MPAL)	1	3.33%

Table III: Subtyping of ALL from Flow cytometry

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Fig. I: Morphological classification of neoplastic group of patients after bonemarrow examination.
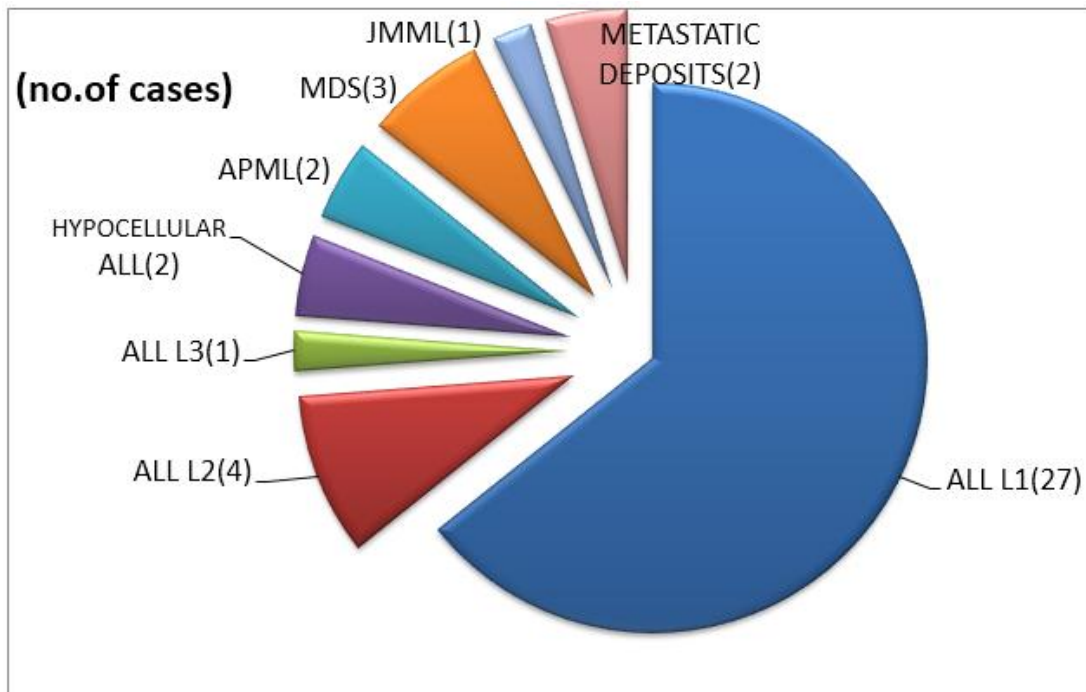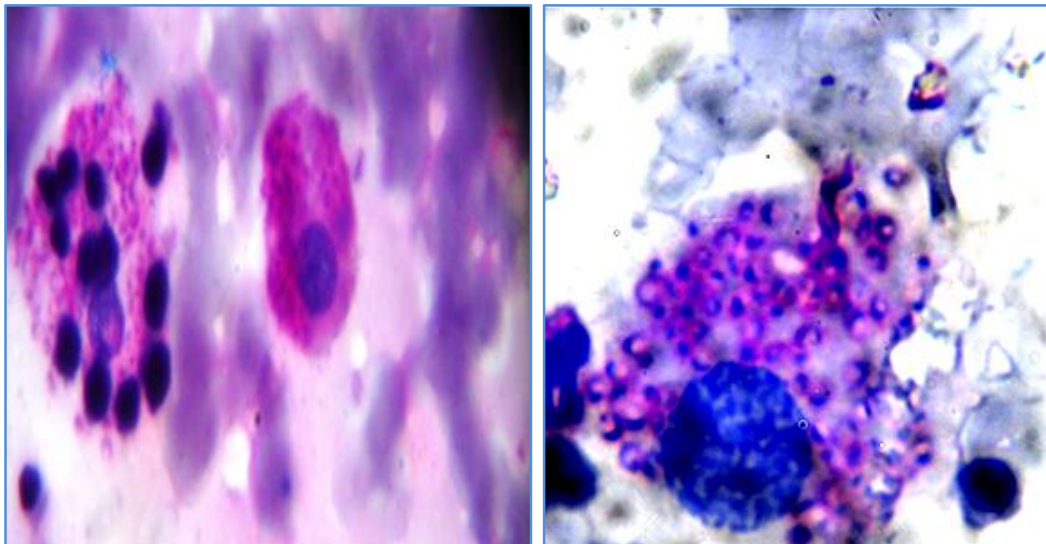

Fig. I

Fig. II: (A) Microphotograph of bonemarrow aspiration showing reticulum cells engulfing the erythroid precursors, Leishman's stain (X 400). (B) PAS Stain of marrow aspirate demonstrates PAS positive yeast form of organism within the cytoplasm of reticulum cell, PAS stain (x400).
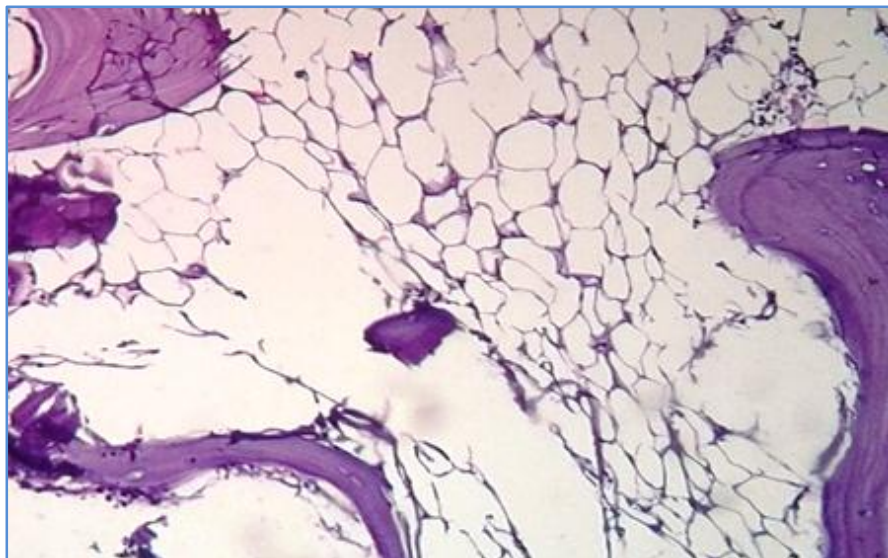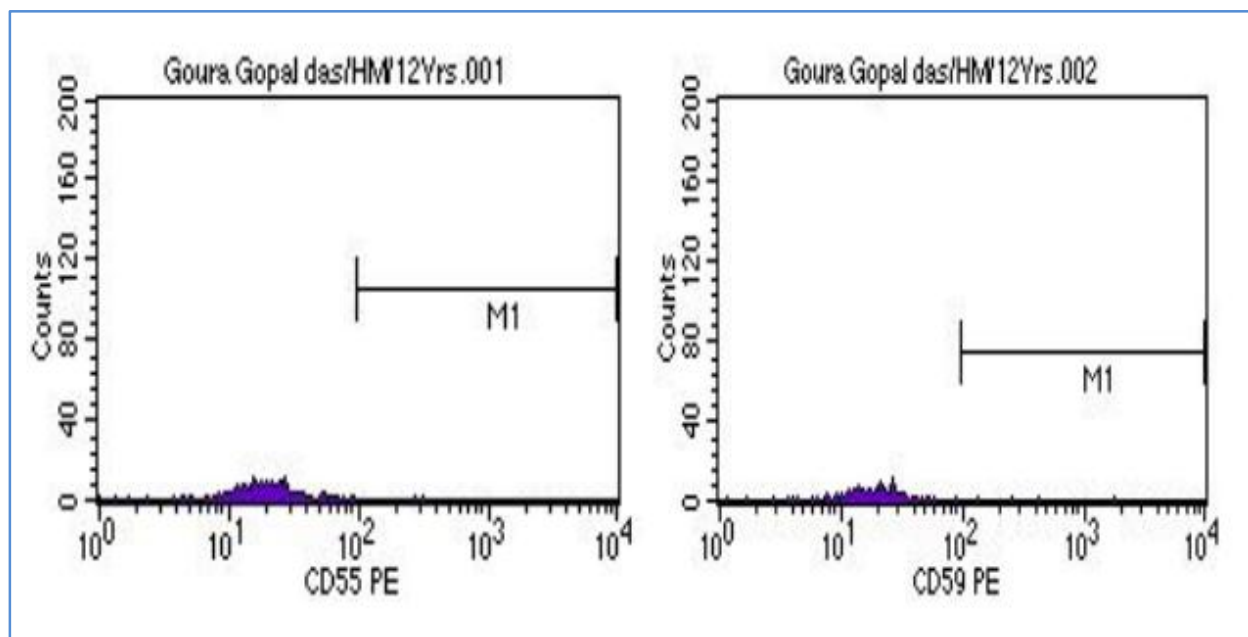

Fig. II

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Fig III: Microphotograph of bone marrow biopsy showing marked increase in adipose tissue and depletion of hematopoietic cells in aplastic anemia. H & E staining (x100).
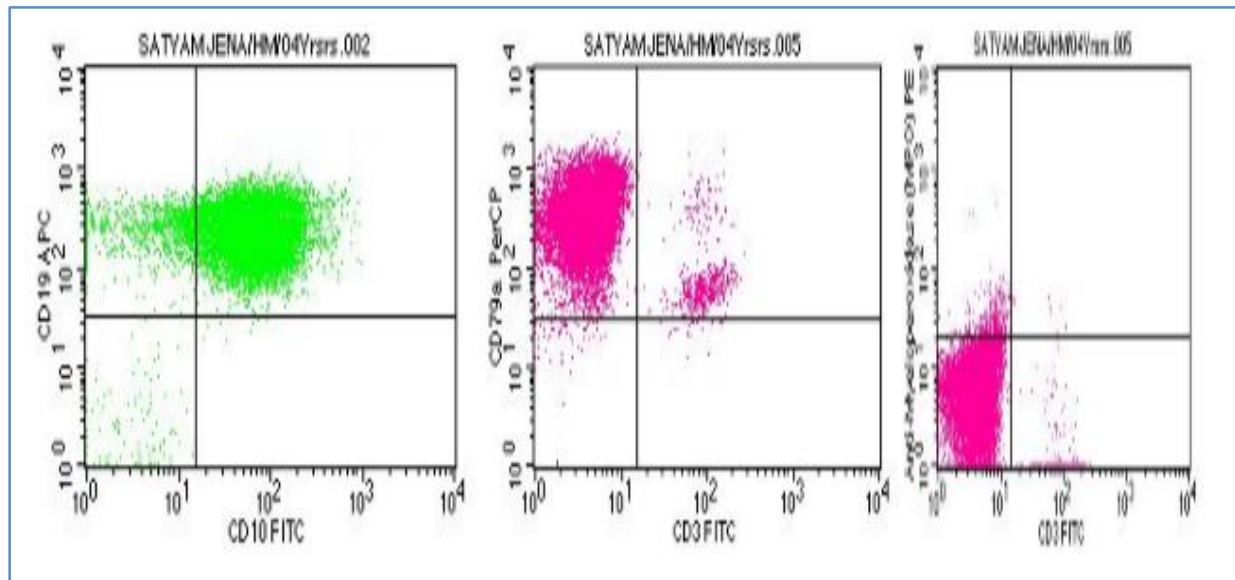

Fig. III

Fig. IV: Flow cytometry in PNH Positive aplastic anemia cases showing marked decrease in the expression of CD55 and CD59 on red blood cell surface.
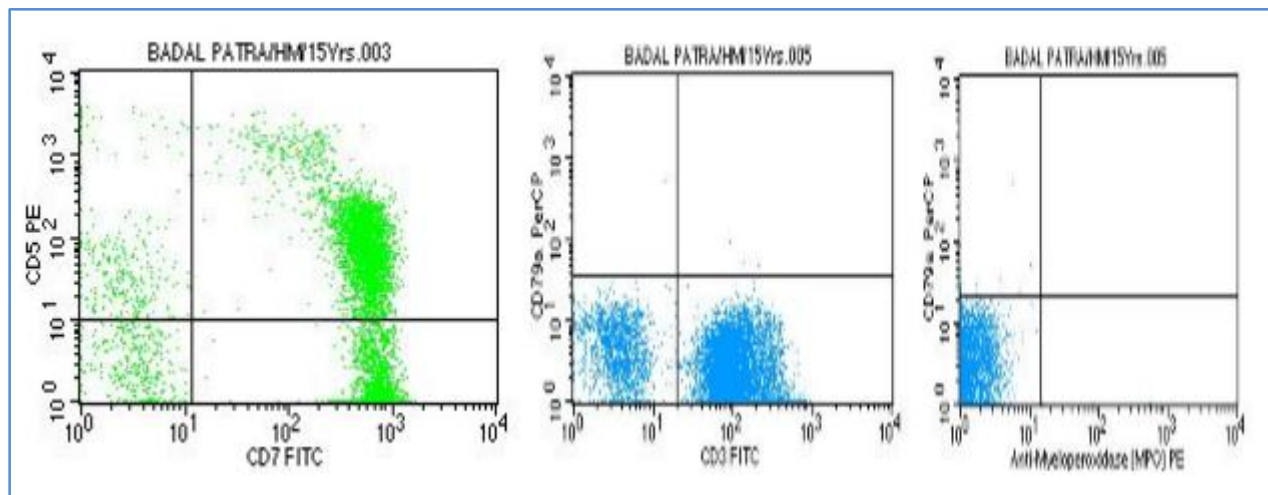

Fig. IV

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Fig. V: Flowcytometric findings showing bright positivity for CD19,CD79a and negativity for CD3 and cMPO in B-ALL.

Fig. V

Fig. VI: Flowcytometric findings showing bright positivity for CD3,CD5 ,CD7 and negativity for CD79a and cMPO in T-ALL.

Fig. VI

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