ORIGINAL RESEARCH

Correlation of bone marrow morphology and molecular studies in myeloproliferative neoplasms

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Received: 04 September, 2022 Received: 16 September, 2022 Accepted: 25 October, 2022

ABSTRACT

Background: Myeloproliferative neoplasms (MPNs) are clonal-origin hematopoietic stem cell disorders. Molecular studies in case of BCR-ABL negative MPN (JAK2v617F, CALR and MPL) have revolutionised the diagnostic approach of MPNs. PV is expected to be almost always accompanied by a JAK2 mutation, whereas the specific driver mutation cannot otherwise distinguish one MPN from another; however, in distinguishing ET from pre -PMF or PV, a higher JAK2V617F allele burden favours the diagnosis of the latter rather than the former.

Method: This was a cross – sectional study, for 18 months from from 1st November 2019 to 30th May2021 including review of clinical presentation, peripheral blood, BMA, and BMB along with clinical features in cases with BCR- ABLand JAK2V617F CALR and MPL mutation analysis.

Result: There was 100% level of agreement between Bone Marrow Morphology and molecular studies in case of 16 CML patients diagnosed on bone marrow morphology (BCR-ABL positive). But in case of BCR-ABL negative MPNs out of 16 cases only 13 showed genetic mutation which included JAK2V617F and CALR gene mutation.

Conclusion: Multimodal approach is needed for classifying MPNs .Increased knowledge about molecular alteration has led to drastic shift in classification of MPNs, with molecular features gaining importance and resulting in entities defined in part or even exclusively, by recurrent genetic alterations.

Keywords: Bone marrow, mutations, myeloproliferative neoplasms

INTRODUCTION

Myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal- origin haematopoieticstem cells alterations, characterised by excessive production of myeloid-lineage cells (i.e. granulocytic, erythroid and megakaryocytic), which is reflected in increased cellularity in peripheral blood and bone marrow.[23,24]

They primarily occur in adults, with incidence peaking in the fifth to seventh decades of life, but some subtypes are also reported in children. The annual incidence of all subtypes combined is 6 cases per 100000 population.[25,26,27,28]

The common pathogenic feature of the myeloproliferative neoplasm (MPN) is the presence of mutated, constitutively activated tyrosine kinase or other acquired aberrations in signalling pathway that lead to growth factor independence. Hematopoietic growth factor act on normal

progenitors by binding to surface receptors and activating tyrosine, which turn on pathways that promote growth survival. The mutated tyrosine kinases found in the MPN circumvent normal controls and lead to the growth factor independent proliferation and survival of marrow progenitors. Because the tyrosine kinase mutations underlying the various MPN do not impair differentiation, the most common consequence is an increase in the production of one or more mature blood element.[29]

Our study was an attempt to compare the frequencies of different gene mutations in myeloproliferative neoplasms and to assess the diagnostic accuracy of bone marrow morphology in diagnosis of myeloproliferative neoplasm.

MATERIAL AND METHODS

This was a cross – sectional study, for 18 months from from 1st November 2019 to 30th May2021 including review of clinical presentation, peripheral blood, BMA, and BMB along with clinical features in cases with BCR- ABL. The study was conducted in a tertiary care hospital. Total of 40 suspected patients of MPNs were observed in the study group.Out of 40 of patients in our study there were 15 males and 25 females with a male/female ratio of 1.2:2 Bone marrow morphology after obtaining bone marrow aspirate and biopsy was examined along with the molecular studies. Complete Blood counts, peripheral blood filmsmears were stained by Leishman stain,BMB smears were stained by routine hematoxylin& eosin (H&E).Reticulin staining was performed by Gomori'smethod, erythropoietin levels, USG for organomegaly and other ancillary tests were also performed on this study group.

Proportions, graphs and tables were used to analyse data. Data obtained was entered in Microsoft excel spreadsheet and exported to editor of statistical package for social Sciences (SPSS Ver. 23). Categorical variables were described as frequencies and percentages. Continuous variables were described as means. Appropriate graphs like Pie charts and Bar chats were used to describe and present the data. Cohen's kappa was calculated to assess the extent of agreement between two measurements. Diagnostic accuracy was calculated through sensitivity, specificity, positive predictive values and negative predictive values.Analysis was done using SPSS and EPI info online.

RESULT

Total of 40 suspected patients of MPNs were observed in study group.Bone marrow morphology after obtaining bone marrow aspirate and biopsy was examined along with the molecular studies. Complete Blood counts, peripheral blood film, reticulin stain, erythropoietin levels, USG for organomegaly and other ancillary tests were also performed on this study group. Out of 40 of patients in our study there were 15 males and 25 females with a male/female ratio of 1.2:2. Among the total no of patients studied 45.1 to 60 year (40%) age group was most common age group followed by > 60 year (35%) age group with a mean age of 53.28 years as depicted in table 1&2

There was a wide range of age distribution between 15 years and 90 years with a mean age of 53.28 years. These constituted 16 (51.6%) cases of CML, 15 (37.5%) cases of BCR-ABL negative cases, among these ET constituted of 5 (16.1%) cases, PV 4 (12.1%), PMF 3 (9.6%) and 1(0.9%) was MPN-UC .9 patients (22.5%) were negative for MPN as depicted in Table 3

The most common presentation of our study group was leukocytosis (75%) followed by anaemia (60%) and thrombocytosis (45%). Thrombocytopenia was the least common presentation.

On complete blood count leukocytosis was seen in 16 (100%) followed by anaemia in 15(93%) cases from 16 diagnosed BCR-ABL positive CML patients. On the other hand, thrombocytosis in 11 (73%) cases followed by erythrocytosis in 5 (34%) of the cases was the most common presentation in BCR ABL negative patients. Among these BCR-ABL negative

patients thrombocytosis was seen in all the 6 (100%) cases of ET and 3 (75%) of the PV and all the cases (100%) of PMF cases. Among 5 erythrocytosis presentation of BCR-ABL negative patients ET was diagnosed in almost all the cases (100%), PV was diagnosed in all (100%) the presentations of erythrocytosis and PMF was diagnosed in only one cases as depicted in Table 4

Splenomegaly (70%) was the most common organomegaly presentation in our study group of 40 patients followed by hepato-splenomegaly (20%) and hepatomegaly (10%) as depicted in Table 5

With respect to BCR-ABL positive CML, splenomegaly was the most common organomegaly seen in 13(81.25%) and hepatomegaly in 3(18.75%) out of the 16 cases of BCR-ABL positive CML. Among 15 cases of BCR-ABL negative cases Splenomegaly was present in (67%) cases of ET, (75%) cases of PV and (60%) cases of PMF. 5 of the patients presenting with organomegaly were not diagnosed as MPNs as depicted in Table 6

Out of 40 patients those patients who were having erythrocytosis and thrombocytosis.EPO level was normal in 40% of cases and decreased in 10% of cases (diagnosed as PV) and 50% cases it was not done as depicted in Table 7

Reticulin stain was applied to all 40 patients among which 5 were diagnosed as PMF on morphology but 3 of them showed Grade 2 changes and 2 out of 5 PMF cases showed Grade 2 changes, Grade 1 change was also present in one of the cases of MPN-UC. Grade 0 change was seen in 3 CML cases .36 out of 40 cases were those patients were Reticulin stain was not applied as depicted in table 8

On bone marrow aspirate examination CML was diagnosed in 15 (37.5%) patients out of 16 diagnosed on BMB, as one of the samples aspirated in that case was inadequate. Out of 14 (35%) BCR-ABL negative cases diagnosed, 6 were of ET, 4 were of PV, 3 of PMF and one was of MPN-UC. Out of 40 patients 4 BMA samples were dilute and 7 of them didn't show any conclusive features of MPN. The association of bone marrow aspiration morphology with molecular studies was statistically highly significant with a P value of < 0.001 Chi-Square Test as depicted in Table 9

Presence of Philadelphia chromosome (9:22) was present in all the 16 cases of CML. Rest of the 24 patients out of 40 number of cases were negative for Philadelphia chromosome translocation. The association of bone marrow aspiration morphology with molecular studies was statistically highly significant with a P value of < 0.001 Chi-Square Test as depicted in Table 10

On molecular study as depicted by the above table number 11 BCR- ABL positive gene mutation was seen in all the 16 (40%) cases which were diagnosed as MPN on morphological study. Among the 15(38%) JAK2V617F (Exon 14) gene mutation was seen in 13 (87%) patients. This mutation was common among the sub groups of BCR-ABL negative cases i.e., 3(20%) ET, 4(27%) PV, 3 PMF (20%) and one MPN-UC. CALR (Exon 9) mutation was present in case of ET in isolation and also along with JAK2V617F mutation in another case of ET. 9(23%) of the molecular study done were negative for MPN as depicted in Table 11

There was 100% level of agreement between Bone Marrow Morphology and molecular studies in case of 16 CML patients diagnosed on bone marrow morphology (BCR-ABL positive). But in case of BCR-ABL negative MPNs out of 16 cases only 13 showed genetic mutation which included JAK2V617F and CALR gene mutation and 3 of the cases which showed features of BCR-ABL negative mutation on bone marrow morphology, didn't show any of the mutations (negative on molecular study). On the other hand, 2 cases in which no conclusion was drawn on bone marrow morphology came to be positive for genetic mutation (JAK2V617F). Its pertinent to mention here that 6 cases which didn't show features of MPN on morphology where in agreement with molecular studies which turned out to be negative for MPN. The association of bone marrow aspiration morphology with molecular studies was

statistically highly significant with a P value of < 0.001 Chi-Square Test as depicted in Table 12

Table 1: Gender distribution of MPNs

Gender	Frequency	Percent
Male	15	37.5
Female	25	62.5
Total	40	100.0

Table2: Frequency of different age groups in suspected cases of MPN

Age group	Frequency	Percent
0 to 15	1	2.5
15.1 to 30	0	0
30.1 to 45	9	22.5
45.1 to 60	16	40
60.1 and above	14	35.0
Total	40	100

Table3: Age groups involved on basis of molecular study in MPNs

Age group	Molecular study			Total
	BCR ABL positive MPN(CML)	BCR ABL negative MPNs	Molecular study negative	
0 to 15	0	0	1	1
15.1 to 30	0	0	0	0
30.1 to 45	6	2	1	9
45.1 to 60	4	8	4	16
60.1 onwards	6	5	3	14
Total	16	15	9	40

Table 4: Frequency of different parameters on PBF in suspected cases of MPNS

PBF Findings	Type	Frequency	Percent
Anaemia	Present	24	60.0
	Absent	16	40.0
Erythrocytosis	Present	9	22.5
	Absent	31	77.5
Leukocytosis	Present	30	75.0
	Absent	10	25.0
Thrombocytosis	Present	18	45.0
	Absent	22	55.0
Thrombocytopenia	Present	1	2.5
	Absent	39	97.5
	Absent	38	95.0

Table5: Frequency of organomegalyon USG in suspected patients of MPN

USG	Frequency	Percent
Hepatomegaly	4	10.0
Splenomegaly	28	70.0
Hepatosplenomegaly	8	20.0
Total	40	100.00

Table 6: Organomegaly on USG in relation to different molecular studies in MPNs

USG	Molecular study			Total
	BCR ABL positive	JAK2V617F (Exon 14)/ CALR (Exon 9)/ MPL (Exon 10)	Molecular study negative	
Hepatomegaly	0	0	4	4
Splenomegaly	13	11	4	28
Hepato- Splenomegaly	3	4	1	8
Total	16	15	9	40

Table 7: Frequency of EPO levels especially in case of PV

EPO levels	Frequency	Percent
Normal	16	40
Decreased	4	10
Not done	20	50
Total	40	100

Table 8: Frequency of Reticulin stain in different MPNS

Reticulin stain	Frequency	Percent
Grade 1	36	90
Grade 2	1	2.5
Grade 3	3	7.5
Total	40	100.0

Table 9: Frequency of recognition of MPNs on BMA

BMA	Frequency	Percent
Dilute	4	10
CML	15	37.5
ET/PV/PMF/MPN UC	14	35.0
NO MPN	7	17.5
Total	40	100.00

Table 10: Frequency of BCR-ABL in MPNs

Cytogenetics	Frequency	Percent
Present	16	40.00
Abscent	24	60.0
Total	40	100.00

Table 11: Frequency of molecular study in MPNs

Molecular study	Frequency	Percent
BCR ABL positive	16	40.0
JAK2V617(Exon14)/CALR(Exon9)/MPL(Exon10)	15	37.5
Molecular study negative	9	22.5
Total	40	100.0

Table 12: Level of agreement between bone marrow morphology and molecular studies in MPNS

BMB	Molecular study.			Total
	BCR ABL positive	BCR-ABL Negative JAK v617F (exon 14) /CALR (exon9)/MPL (exon 10)	Molecular study negative	
CML	16	0	0	16
ET/PV/PMF/MPN-UC	0	13	3	16
NO MPN	0	2	6	8
Total	16	15	9	40

Fig1: CML -CP.PBF showing leukocytosis with left shift and Basophilia (100X)
Figure2: Bone Marrow aspirate showing hyper cellular marrow with agranulocytosis Hyperplasia and Megakaryocyte hyperplasia (40x)

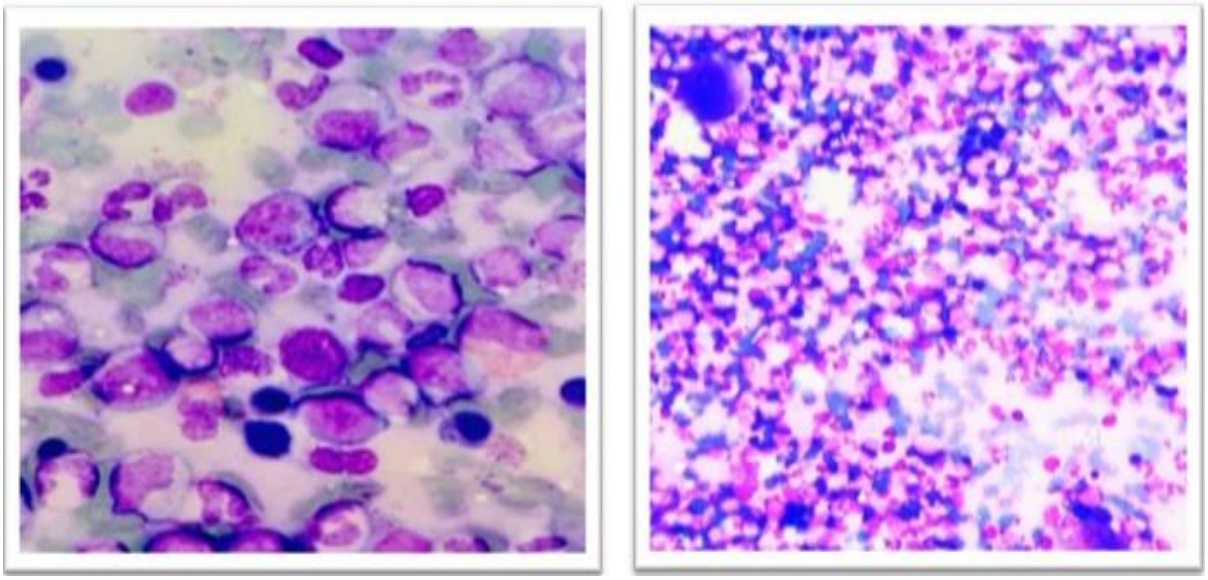


Fig3: CML-CP bone marrow biopsy with granule cystic and megakaryocytic hyperplasia with Dewarfmegakaryocytes (40x)
Fig 4: showing PBF of polycythemia vera

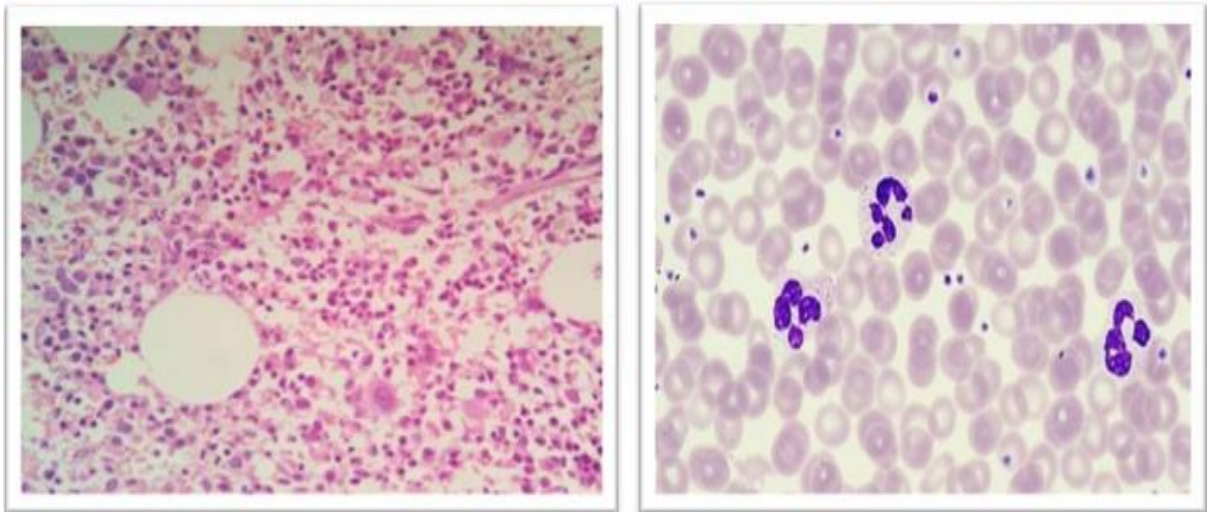


Fig5: polycythemiaVera: bone marrow showing trilineagehyperplasia (100x)
Fig 6: Polycythemia vera with bone marrow aspirate showing pleomorphism Megakaryocyte

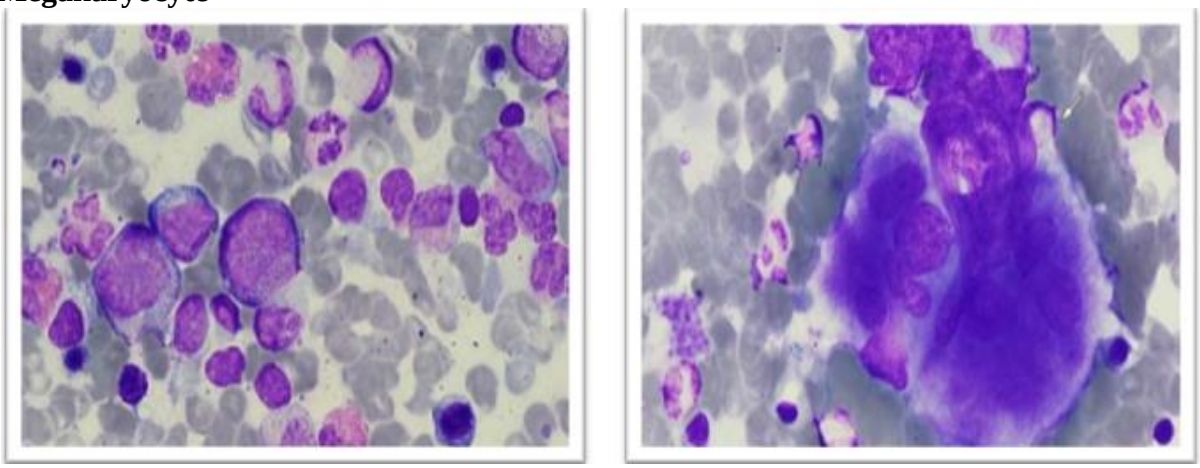


Fig7: Tear drop cells in case of primary Myelofibrosis on PBF (40x)
Fig 8: showing leukoerythroblasts on PBF in case of PMF (100x)

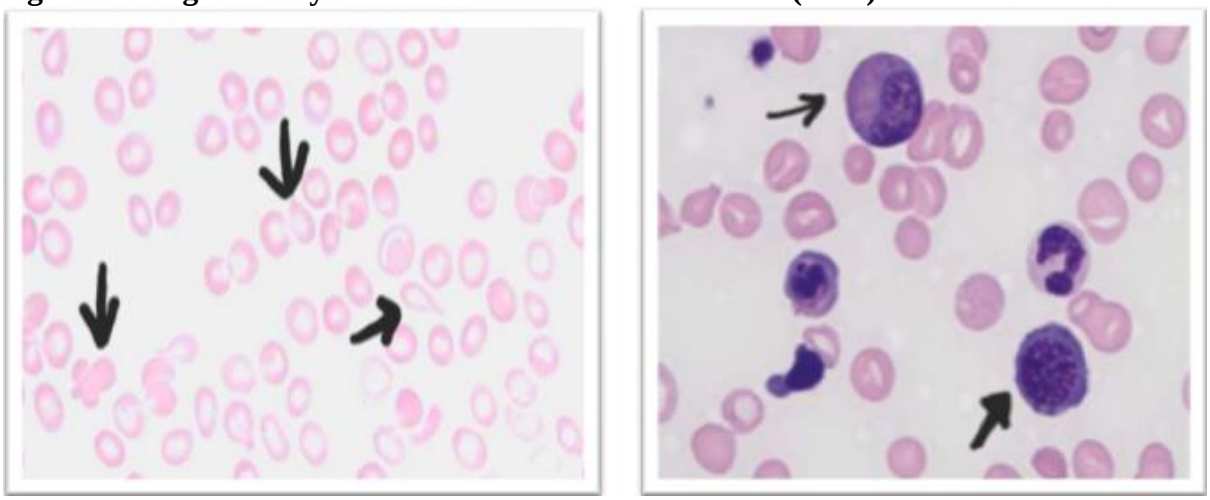


Fig9:Primary Myelofibrosis with Bone Marrow Biopsy showing Megakaryocytic hyperplasia with BizzareMegakaryocyte and Fibrosis (40x)
Fig10: Reticulin stain of primary Myelofibrosis

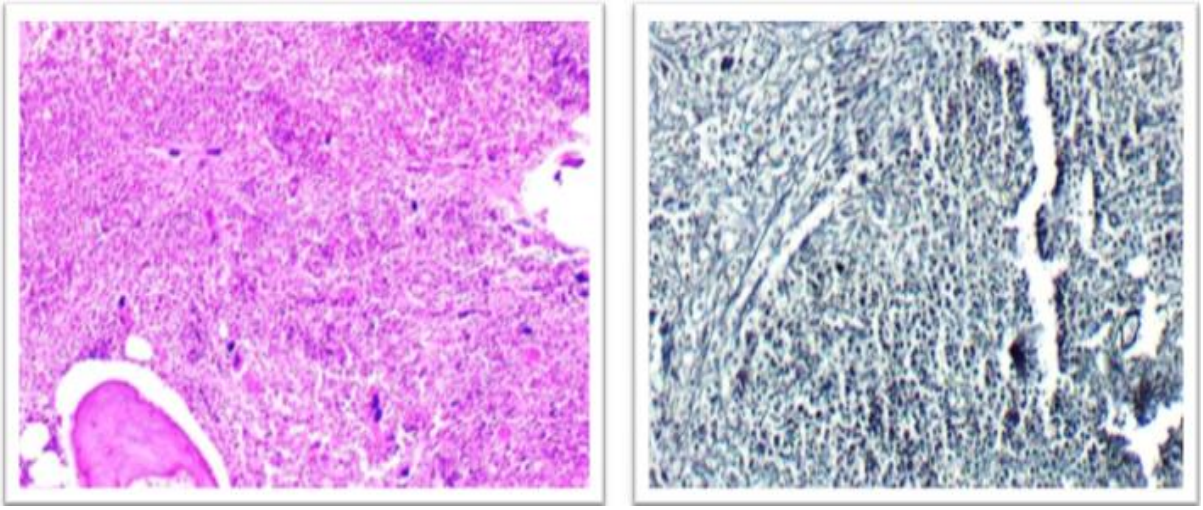


Fig11: PBF showing increased platelet count in case of Essential Thrombocytosis
Fig12: Essential Thrombocythemia: Bone marrow biopsy showing MegakaryocyteHyperplasia with HyperlobatedMegakaryocytes (40x)

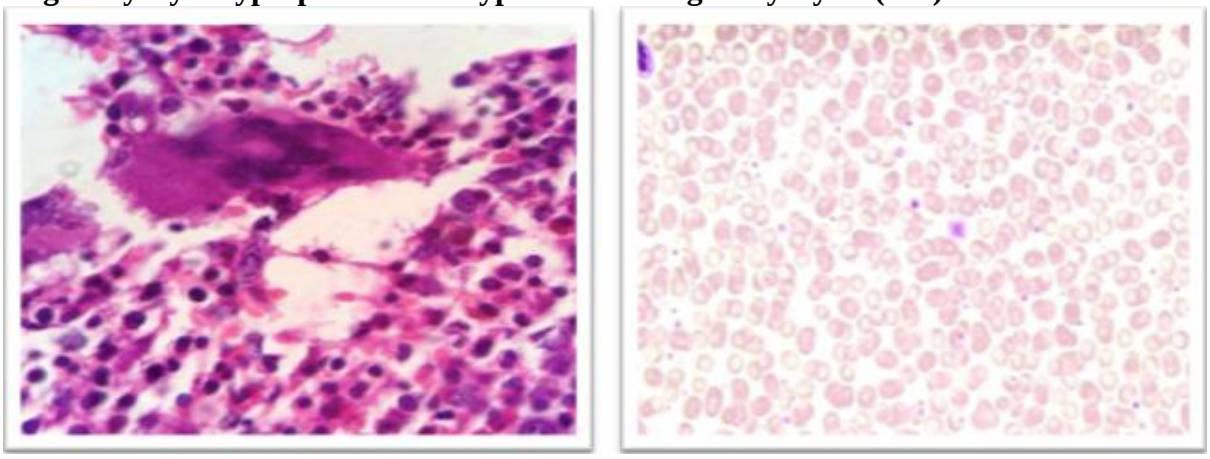
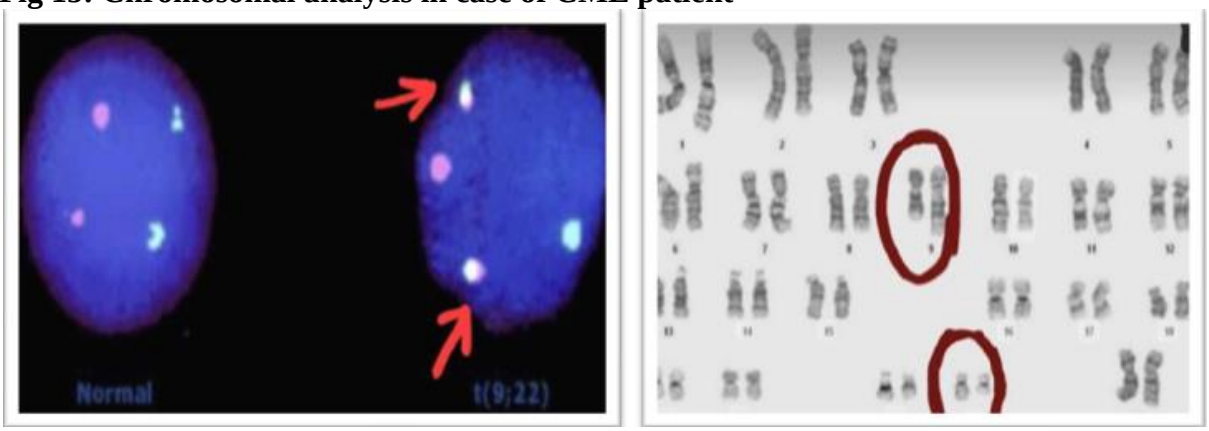


Fig14: FISH with Green showing ABL, Red showing BCR and yellow to white showing Fusion of the Gene
Fig 15: Chromosomal analysis in case of CML patient



DISCUSSION

The present study was conducted in the department of Pathology/ Haematology section Government Medical College Srinagar over a period of 18 months from 1st November 2019 to 30th May 2021. Total of 40 suspected patients for MPNs were observed in this study group.

MPN consists of diverse group of clonal disorders in which especially megakaryocytes exhibit distinctive features. The current WHO classification of tumours of hematopoietic and lymphoid tissue from 2017 classifies MPN by combination of clinical, morphological, immunophenotypic and genetic features. Given the good specificity and sensitive on morphology and reported high frequencies of the JAK2v617F, MPL, mutation in MPN, the diagnosis of these diseases should include clinical, haematological and genetic screening and not only clinical and haematological characteristics.

Thus an integrated multimodal approach is needed for classifying MPNs, although morphology of peripheral blood films and bone marrow aspirates, complemented by cytogenetics, flow cytometry and histology on bone marrow biopsy are the basis for diagnosis of MPNs, but increased knowledge about molecular alteration has led to drastic shift in classification of MPNs, with molecular features gaining importance and resulting in entities defined in part or even exclusively, by recurrent genetic alterations. However especially in case of MPNs, the bone marrow biopsy has a crucial role to provide additional

information about cellularity, histology of hematopoietic cells and their maturation, bone marrow stroma and bone structure.
Morphological assessment is enhanced by IHC staining for example for assessing blast count and highlighting atypical micro-megakaryocytes difficult to identify by routine stain. So, its strongly recommended to go for bone marrow examination at the time of diagnosis of MPN and encourage repeating the procedure during follow up, in case of progressive disease. In all cases, sufficient bone marrow aspiration should be taken to allow screening for molecular studies and cytogenetic
analysis. Establishing mutational status in patients of MPN is not only important in complementing morphology but also provides important prognostic information as specific targeted treatment modalities are now available against the molecular mutation.
In future it's expected that there will be increased role of other mutations in complementing morphological diagnosis in MPN and providing additional diagnostic and prognostic information.

CONCLUSION

MPN consists of diverse group of clonal disorders in which especially megakaryocytes exhibit distinctive features. The current WHO classification of tumours of hematopoietic and lymphoid tissue from 2017 classifies MPN by combination of clinical, morphological, immunophenotypic and genetic features. Given the good specificity and sensitive on morphology and reported high frequencies of the JAK2v617F, MPL, mutation in MPN, the diagnosis of these diseases should include clinical, haematological and genetic screening and not only clinical and haematological characteristics.
Thus an integrated multimodal approach is needed for classifying MPNs, although morphology of peripheral blood films and bone marrow aspirates, complemented by cytogenetics, flow cytometry and histology on bone marrow biopsy are the basis for diagnosis of MPNs, but increased knowledge about molecular alteration has led to drastic shift in classification of MPNs, with molecular features gaining importance and resulting in entities defined in part or even exclusively, by recurrent genetic alterations. However especially in case of MPNs, the bone marrow biopsy has a crucial role to provide additional information about cellularity, histology of hematopoietic cells and their maturation, bone marrow stroma and bone structure.
Morphological assessment is enhanced by IHC staining for example for assessing blast count and highlighting atypical micro-megakaryocytes difficult to identify by routine stain. So, its strongly recommended to go for bone marrow examination at the time of diagnosis of MPN and encourage repeating the procedure during follow up, in case of progressive disease. In all cases, sufficient bone marrow aspiration should be taken to allow screening for molecular studies and cytogeneticanalysis. Establishing mutational status in patients of MPN is not only important in complementing morphology but also provides important prognostic information as specific targeted treatment modalities are now available against the molecular mutation.

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