Original Article

Megakaryocytes in Chronic Phase of Chronic Myeloid Leukemia: A Descriptive Case Series

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ABSTRACT

Background: Megakaryocytic proliferation and functional alterations are frequently observed in various myeloproliferative neoplasms (MPN). An analysis of these alterations provides clue to the diagnosis of MPN such as essential thrombocythemia and myelofibrosis. We in our descriptive study have tried to evaluate and identify the morphological features of the megakaryocytes seen in chronic myeloid leukemia- chronic phase (CML-CP).

Methods: Bone marrow aspirate and trephine biopsy from 31 newly diagnosed cases of CML-CP were evaluated for the morphological parameters including count, distribution, clustering, cytoplasmic granularity, nuclear lobes, micromegakaryocytes, fragmented nuclei, bare nuclei, and emperipolesis. All the cases were also evaluated for marrow reticulin fibrosis

Result: Megakaryocytic count was increased in 58% of cases (18 out of 31), 67.7% had parasinusoidal distribution, 67.7% had no megakaryocytic clusters. Hypolobation of nuclei and presence of micromegakaryocytes were consistent findings in all the cases. The megakaryocyte count showed a positive correlation with the grade of marrow reticulin fibrosis and peripheral blood platelet count.

Conclusion: Characteristic changes in megakaryocyte number, distribution and morphological features is seen in CML-CP and may help in differentiating it from other MPN's.

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Introduction

The myeloproliferative neoplasms (MPNs) being clonal hematopoietic stem cell disorders are characterised by alterations in one or more myeloid lineages which include megakaryocytes. Megakaryocytic alterations play key diagnostic role in recognition of entities such as Primary myelofibosis (PMF) and Essential thrombocythaemia (ET). [1] These alterations also affect the clinical presentation, progression to fibrosis, prognosis and treatment. [2] The early phases of MPNs may have overlapping clinical and laboratory features such as cases of chronic myeloid leukemia in chronic phase (CML-CP) and Polycythemia vera (PV) with markedly raised megakaryocyte count. An analysis of changes in the megakaryocyte lineage can help in differentiating various MPN disorders in such situations. In our work we extensively studied the megakaryocyte alterations (numerical and morphological) in 31 cases of chronic myeloid leukemia- chronic phase (CML-CP).

Materials and Methods

In this prospective descriptive study 31 newly diagnosed cases of CML-CP were selected as the study group. To compare the megakaryocyte counts, 25 aspiration smears and trephine sections which were reported as normal haematopoiesis with normal peripheral blood parameters, were selected at random. Morphological assessment of megakaryocyte parameters was done on bone marrow aspirate smears, touch and roll imprints of biopsies and biopsy tissue sections. After getting an informed consent, the bone marrow aspiration and biopsy were done from the posterior superior iliac spine with the patient in corresponding lateral decubitus position. [3] The aspirate, touch and roll imprints were stained with Leishmann stain and the biopsy sections were stained with hematoxylin and eosin. [4] Silver stain was used for detection of reticulin fibrosis. [5] The slides were examined independently by two observers and the final observations were made subsequently. The diagnosis in all cases was confirmed as CML after demonstration of BCR-ABL fusion using fluorescence in situ hybridization (FISH) or Reverse transcriptase polymerase chain reaction (RT-PCR).

A minimum of 40 megakaryocytes were assessed in each case. The megakaryocyte parameters assessed were as follows:

Megakaryocyte Count, Distribution and Clustering: The number of megakaryocyte per 10 HPFs was estimated. The distribution of megakaryocytes in the trephine sections was categorized into parasinusoidal, paratrabecular, diffuse and parasinusoidal with occasional paratrabecular distribution. Presence of groups of five or more megakaryocyte was considered as megakaryocytic cluster.

Cytoplasmic Granularity: Megakaryocyte with pale grey or water clear cytoplasm with sparse or no granules were noted as hypo granular megakaryocyte while increased granularity which obscures rest of the cytoplasmic details were categorized as hypergranular forms. [6]

Nuclear Lobes: The number of nuclear lobes in each megakaryocyte was noted based on which the megakaryocytes were segregated into four groups: Megakaryocyte with a single nuclear lobe, 2-3 nuclear lobes, 4-5 nuclear lobes and megakaryocyte with >5 nuclear lobes.

Dysplastic Features i.e Micromegakaryocyte and Fragmented Nuclei: Megakaryocyte with size equivalent to a large lymphocyte/monocyte, were categorized under micromegakaryocyte. [6] Megakaryocyte where the nuclear lobes are disjointed resulting in two or more segments of nucleus containing one or more nuclear lobe(s) within a single megakaryocyte were categorized under megakaryocytes with fragmented nuclei.

Miscellaneous Findings i.e. Bare Nuclei and Emperipolesis: Megakaryocytes with naked nucleus (either normal or dwarf) not surrounded by cytoplasm were noted as bare nuclei. Presence of marrow elements of either granulocytic or erythroid series within the megakaryocyte cytoplasm were noted as emperipolesis.

Grading of fibrosis on trephine biopsy was done based on the WHO grading of bone marrow fibrosis. [7]

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean ± SD.

Result

Megakaryocyte Count: The average megakaryocyte count in both aspirate smears and biopsy sections were higher in CML-CP when compared with the controls. 18 of the 31 CML-CP cases (58.0%) had an elevated megakaryocyte count, 10 (32.2%) had normal megakaryocyte count and 3(9.6%) had a lower megakaryocyte count. The other megakaryocyte parameters were analysed after dividing cases in three groups of normal, increased and decreased megakaryocyte count. [Table 1,2,3]

Peripheral Blood Platelet Count: On comparison of platelet count with megakaryocyte count it was seen that the mean platelet count was significantly (p value 0.00) higher (mean 6.11lac/mm3) in case with raised megakaryocytic count as compared to those with normal or low megakaryocytic count.

Splenomegaly: The three groups did not show any statistically significant difference in the grade of splenomegaly (p value 0.960)

Fibrosis: Reticulin fibrosis was detected in 29 of the 31 cases (93.5%). In the overall 31 cases, 2 cases (6.5%) had no fibrosis in the marrow, 11 cases (35.5%) had Grade I fibrosis, and 12 cases (38.6%) had Grade II fibrosis while 6 cases (19.4%) had Grade III fibrosis. [Figure 2 C, 2D, 2E] Of the 17 cases which had megakaryocytic count more than 20 per hpf 13 had either grade II or III fibrosis. The three groups did not show significant difference in the fibrosis grade (p value 0.927).

Distribution: The megakaryocyte in 21 (67.7%) cases had a parasinusoidal distribution, 4 (12.9%) cases had a diffuse distribution and 6 (19.3%) cases predominantly had parasinusoidal distribution with occasional paratrabecular megakaryocytes. Thus parasinusoidal was the predominant distribution pattern in majority of the cases. The paratrabecular or diffuse pattern of distribution was limited to the cases with a markedly elevated megakaryocyte count (p value 0.070)

Clusters: Megakaryocytic clusters were seen in 10 (32.3%) cases. Of these cases majority had increased megakaryocyte count (p value 0.04) [Figure 1A]

Granularity: Normal cytoplasmic granularity was seen in 77.4 %(n=24) of the cases. Hypogranular megakaryocytes were seen in 33.3% (n=6) and 10% (n=1) cases with increased and normal megakaryocytic count respectively. [Figure 1 B]

Nuclear Lobes: Hypolobation was prominent in all the cases with an average of 46.94% of the total megakaryocytes. The mean percentage of single lobed megakaryocytes was highest in cases with increased megakaryocytic count. The percentage of megakaryocytes with more than 5 nuclear lobes was significantly low with an average of 4.75% megakaryocytes. The mean percentage was lowest in cases with raised megakaryocytic count. [Figure 1C, 1D, 1E, 2B]

Micromegakaryocytes: Micromegakaryocyte was seen in all the cases. The mean percentage of micromegakaryocytes was 26.6, 39, and 48.6 in cases with decreased, normal, increased megakaryocytic count respectively. [Figure1F, 2A]

Nuclear Fragmentation: 48.4% of the cases showed occasional fragmentation in their megakaryocytic nuclei. [Figure 1G]

Bare Nuclei: Bare nuclei were detected in 71.0% of the cases.

Emperipolesis: Emperipolesis was observed in 29% of the cases. This was more frequently seen in cases with raised megakaryocytic count. [Figure 1H]

Table 1 : Comparision of Platelet count, splenomegaly, grade of bone marrow fibrosis in case groups according to megakaryocyte count

	Meg Count	N	Mean	Minimum	Maximum	p- value
No. in aspirate	Decreased	3	4.333	3.0	6.0	
	Normal	10	8.300	6.0	10.0	0.001
	Increased	18	21.944	7.0	43.0	
	Total	**31**	**15.839**	**3.0**	**43.0**	
No. In biopsy	Decreased	3	5.667	5.0	6.0	
	Normal	10	11.700	7.0	16.0	0.000
	Increased	18	36.611	18.0	89.0	
	Total	**31**	**25.581**	**5.0**	**89.0**	
PC	Decreased	3	1.740	1.2	2.4	
	Normal	10	2.345	1.2	3.4	0.000
	Increased	18	6.112	2.0	12.5	
	Total	**31**	**4.474**	**1.2**	**12.5**	
Splenomegaly	Decreased	3	2.33	2	3	
	Normal	10	2.20	0	3	0.960
	Increased	18	2.17	0	3	
	Total	**31**	**2.19**	**0**	**3**	
Fibrosis grade	Decreased	3	1.67	1	2	
	Normal	10	1.80	1	3	
	Increased	18	1.67	0	3	0.927
	Total	**31**	**1.71**	**0**	**3**	

Table 2 : Comparison of megakaryocyte arrangement, distribution, cytoplasmic granularity in case groups according to megakaryocyte count

	MEG COUNT	N	Mean	Minimum	Maximum	p- value
MEGAKARYOCYTE CLUSTER	DECREASED	3	.00	0	0	
	NORMAL	10	.10	0	1	0.042
	INCREASED	18	.50	0	1	
	TOTAL	**31**	**.32**	**0**	**1**	
DIFFUSE	DECREASED	3	.00	0	0	
	NORMAL	10	.00	0	0	0.205
	INCREASED	18	.22	0	1	
	TOTAL	**31**	**.13**	**0**	**1**	
PARSINUSOIDAL/ PARATRABECULAR	DECREASED	3	.00	0	0	0.070
	NORMAL	10	.00	0	0	
	INCREASED	18	.33	0	1	
	TOTAL	**31**	**.19**	**0**	**1**	
PAR-SINUSOIDAL	DECREASED	3	1.00	1	1	
	NORMAL	10	1.00	1	1	0.324
	INCREASED	18	.83	0	1	
	TOTAL	**31**	**.90**	**0**	**1**	
GANULARITY	DECREASED	3	1.00	1	1	
	NORMAL	10	.90	0	1	0.148
	INCREASED	18	.61	0	1	
	TOTAL	**31**	**.74**	**0**	**1**	
HYPOGRANULAR	DECREASED	3	.00	0	0	
	NORMAL	10	.10	0	1	0.244
	INCREASED	18	.33	0	1	
	TOTAL	**31**	**.23**	**0**	**1**	

Table 3: Comparision of megakaryocyte nuclear features, % of micromegakaryocyte in case groups according to megakaryocyte count

	Meg Count	N	Mean	Minimum	Maximum	p- value
1-2 Nuclei	Decreased	3	30.000	20.0	40.0	
	Normal	10	41.500	15.0	75.0	
	Increased	18	52.778	20.0	85.0	0.096
	Total	**31**	**46.935**	**15.0**	**85.0**	
2-3 Nuclei	Decreased	3	38.333	30.0	50.0	
	Normal	10	35.000	10.0	50.0	0.708
	Increased	18	32.778	10.0	50.0	
	Total	**31**	**34.032**	**10.0**	**50.0**	
4-5 Nuclei	Decreased	3	26.667	10.0	40.0	
	Normal	10	18.500	10.0	30.0	
	Increased	18	12.500	.0	40.0	0.118
	Total	**31**	**15.806**	**.0**	**40.0**	
> 5 Nuclei	Decreased	3	5.000	.0	10.0	
	Normal	10	5.000	.0	15.0	
	Increased	18	1.944	.0	15.0	0.215
	Total	**31**	**3.226**	**.0**	**15.0**	
% of Micromegakaryocytes	Decreased	3	26.667	15.0	35.0	
	Normal	10	39.000	20.0	70.0	0.196
	Increased	18	48.611	15.0	80.0	
	Total	**31**	**43.387**	**15.0**	**80.0**	
Bare nuclei	Decreased	3	.00	0	0	
	Normal	10	.70	0	1	0.010
	Increased	18	.83	0	1	
	Total	**31**	**.71**	**0**	**1**	
Emperipolesis	Decreased	3	.00	0	0	
	Normal	10	.20	0	1	
	Increased	18	.39	0	1	0.312
	Total	**31**	**.29**	**0**	**1**	
Disjointed Nuclei	Decreased	3	.00	0	0	
	Normal	10	.50	0	1	0.218
	Increased	18	.56	0	1	
	Total	**31**	**.48**	**0**	**1**	

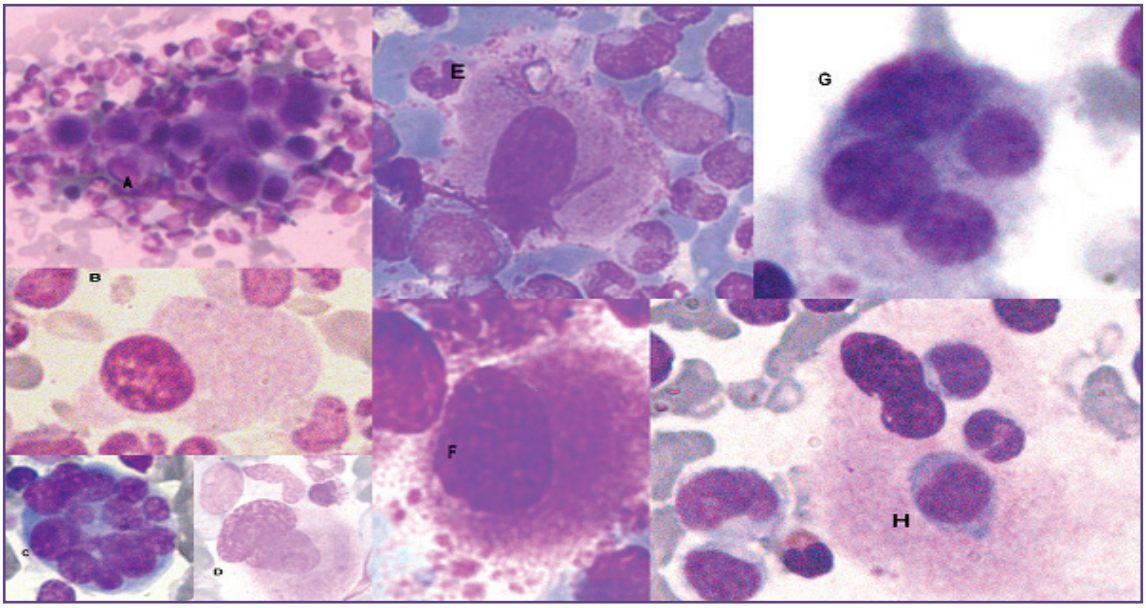

Fig. 1: Bone marrow aspirate (Leishman stain)
A: Megakaryocyte cluster (100X)
B: Hypogranular megakaryocyte (400X)
C: Multilobated megakaryocyte (400X)
D: Bilobed megakaryocyte (400X)
E: Monolobated megakaryocyte (400X)
F: Micromegakaryocyte (400X)
G: Disjointed nuclei in megakaryocyte (400X)
H: Emperipolesis (400)

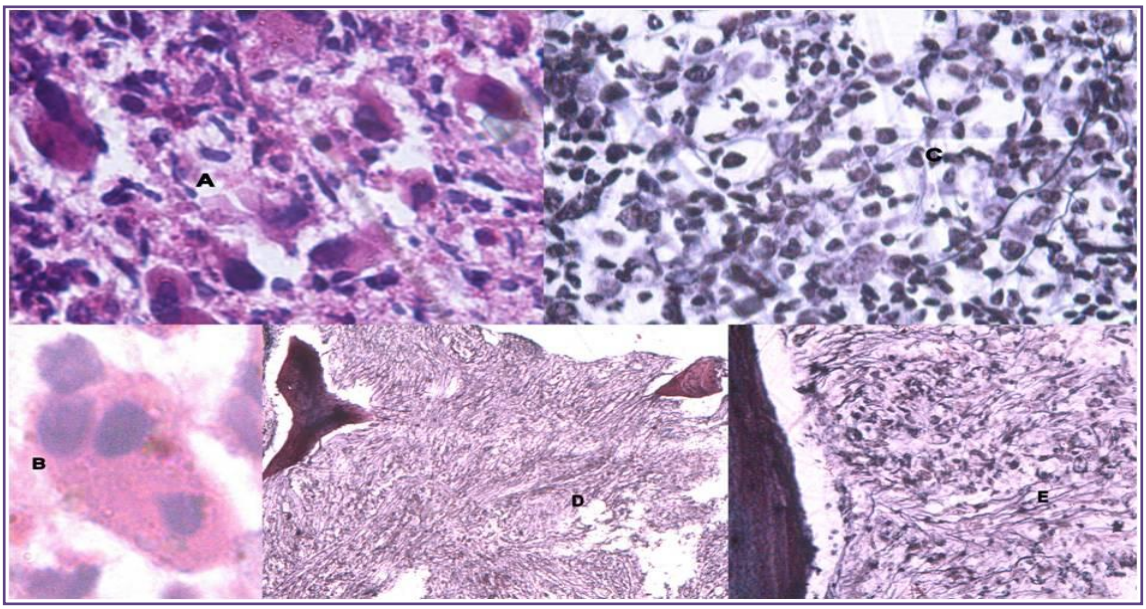

Fig. 2: Trephine biopsy section
A: Increased megakaryocyte with micromegakaryocytes (Hematoxylin & eosin 400X)
B: Disjointed nuclei in megakaryocyte (Hematoxylin & eosin 400X)
C: Grade I fibrosis (Reticulin stain 400X)
D: Grade III fibrosis in CML-BP (Reticulin stain 40X)
E: Grade II fibrosis (Reticulin stain 40X)

Discussion

A spectrum of megakaryocyte alterations was seen in our study group. The mean megakaryocyte count per 10 hpf was higher in our cases in comparison to the normal controls. On classifying cases as CML with granulocytic proliferation (CML-G) and CML cases with both granulocytic and megakaryocytic proliferation (CML-GM) on the basis of the criteria set by Yookarin Khonglah et al., the distribution in each group was 42% and 58% respectively. [8] These values were intermediate to the distribution of cases reported in the studies by Yookarin Khonglah et al. (67% & 33%) and Bartl R et al. (45% & 55%). [8, 9] The increased megakaryocyte count was reflected in peripheral blood smear as raised platelet count. A fair number of cases (n=13) however had a normal or low megakaryocyte count in coherence with the previous reports. [10] The variability in megakaryocyte count may be due to underlying molecular triggers or marrow microenviorment. The cases with increased megakaryocyte count (CML-GM subgroup) also showed prominent clustering of megakaryocytes as reported in earlier studies. [11] No megakaryocyte clustering was observed in CML-G. This difference is probably due to the higher megakaryocyte count in the CML-GM group when compared to the other two groups. The distribution pattern was predominantly parasinusoidal. In cases with increased megakaryocyte count occasional paratrabecular or diffusely distributed megakaryocytes could be appreciated however predominant paratrabecular distribution characteristic for Myelodysplastic Syndrome (MDS) [12] was not seen. The paratrabecular area in CML is usually obliterated with granulocytic proliferation.

Dysplastic features namely hypolobation and micromegakaryocyte were constantly seen in all patients consistent with the previous studies. [11, 13] These hypolobated and dwarf megakaryocytes are significant in differentiating cases of CMLCP with raised platelet counts from other MPNs such as ET (hyperlobated megakaryocytes), PMF (enlarged megakaryocytes with cloudy nuclear chromatin). The nuclear lobulation was in general shifted towards left with decreased number of mature forms. Disjointed nuclei characteristically seen in PMF and MDS were seen in half of the cases however the percentage of megakaryocytes was low (<10%). Cytoplasm hypo granularity is predominantly a feature of megakaryocytes in MDS. [14] Majority of the cases had normal granular megakarocytes, however 6 cases with increased megakaryocyte count had few dysplastic hypo granular megakaryocytes again a characterstic feature of MDS. [14] The presence of these dysplastic changes may be explained by proneness to dysplasia due to increased proliferation.

Miscellaneous parameters such as bare nuclei (probably representing final stage of megakaryopoiesis after platelet shedding) were seen in varying proportions in all cases. However they are not of much significance in differentiation among the MPN as it is increased in all CMPD. [11] Emperipolesis was observed in 29% of cases, a value comparable to that reported by Bobik Ret al. [15] as 25% and Cashell AW et al. [16] as 17%. The parameter however is not much significant in differentiating among the various diagnoses of MPDs as emperipolesis has been consistently reported in all classes of MPN. [15, 16]

A positive correlation was seen between the megakaryocyte count and grade of fibrosis suggesting a pathogenetic link between the two. [17, 18] Growth factors like platelet derived growth factor (PDGF) and LOX protein have been suggested as factors inducing marrow fibrosis. [19]

Conclusion

A spectrum of morphological changes is seen in megakaryocytes in cases of CML -CP. An increase in megakaryocyte count in over half of the cases indicate towards a stem cell abnormality however since not all cases show an increase in the megakaryocyte count other factors at the molecular level do come into play. Probability of clustering increases with increase in megakaryocyte count. The distribution was predominantly parasinusoidal. Hypolobated nuclei and micromegakaryocytes were a consistent finding in all cases. Additional dysplastic features of hypogranular cytoplasm and nuclear fragmentation were seen in cases with increased megakaryocyte count. The megakaryocyte count showed positive correlation with reticulin fibrosis grade.

A study of megakaryocyte parameters in other phases of CML may bring out the differences in various phases of CML. Also the megakaryocytic parameters helpful in prediction of the evolution of the disease may be identified.

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Competing Interests

None declared

Reference

1. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009 Jul 30;114(5):937-51
2. Georgii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. Baillieres Clin Haematol Dec 1998; 11(4):721-49.

3. BatesI, Burthem J. Bone Marrow Biopsy. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors Dacie and Lewis Practical Hematology. 11th ed. London:Churchill Livingstone Elsevier; 2012.123-137
4. Bain BJ, Lewis SM. Preparation and Staining Methods for Blood and Bone Marrow Films. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors Dacie and Lewis Practical Hematology. 11th ed. London:Churchill Livingstone Elsevier; 2012.57-68
5. Jones LM, Bancroft JD, Gamble M. Connective Tissues and Stains. In: Bancroft JD, Gamble M, editors Bancroft's Theory and Practice of Histological Techniques. 6th ed. Churchill Livingstone Elsevier; 2007.135-160.
6. Muhury M, Mathai AM, Rai S, Naik R, Pai MR, Sinha R. Megakaryocytic alterations in thrombocytopenia: A bone marrow aspiration study. Indian J Pathol Microbiol 2009; 52(4):490-4.
7. Thiele J, Kvasnicka HM, Faccheti F, Franco V, Walt JV, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 2005; 90(8):1128-32.
8. Khonglah Y, Basu D, Dutta TK. Bone marrow trephine biopsy findings in chronic myeloid leukemia. Malays J Pathol 2002; 24(1):37 - 43.
9. Bartl R, Frisch B, Wilmanns W. Potential of bone marrow biopsy in chronic myeloproliferative disorders. Eur J Haematol 1993; 50(1):41-52.
10. Kaloutsi V, Fritsch RS, Buhr T, Restrepo-Specht I, Widjaja W, Georgii A. Megakaryocytes in chronic myeloproliferative disorders: numerical density correlated between different entities. Virchows Arch A Pathol Anat Histopathol 1991; 418(6):493-7.
11. Nafe R, Holgado de Colombo S, Choritz H, Georgii A. Morphometry of megakaryocytes for supporting the histologic diagnosis of chronic myeloproliferative diseases. The Pathologist 1995;16(1):34-40.
12. Thiele J, Quitmann H, Wagner S, Fischer R. Dysmegakaryopoiesis in myelodysplastic syndromes (MDS): An immunomorphometric study of bone marrow trephine biopsy specimens. J Clin Pathol 1991; 44:300-305.
13. Michiels JJ. Diagnostic criteria of the myeloproliferative disorders (MPD): essential thrombocythaemia, polycythaemia vera and chronic megakaryocytic granulocytic metaplasia. Neth J Med 1997 Aug; 51(2):57-64.
14. Wong KF, Chan JK. Are dysplastic and hypogranular megakaryocytes specific markers for myelodysplastic syndrome? Br J haematol 1991; 77:509-514.
15. Bobik R, Podolak-Dawidziak M, Kiełbiński M, Jeleń M, Wróbel T.Emperipolesis in megakaryocytes in patients with thrombocytosis in the course of myeloproliferative disorders. Acta Haematol Pol 1995;26(2):179-83.
16. Cashell AW, Buss DH. The frequency and significance of megakaryocytic emperipolesis in myeloproliferative and reactive states. Ann Hematol Jun 1992; 64(6):273-6.
17. Lazzarino M, Morra E, Castello A, et al. Myelofibrosis in chronic granulocytic leukaemia: clinicopathologic correlations and prognostic significance. Br J Haematol 1986;64:227-40.
18. Buhr T, Georgii A, H. Myelofibrosis in chronic myeloproliferative disorders. Incidence among subtypes according to the Hannover Classification. Choritz Pathol Res Pract 1993; 189:121-32.
19. Papadantonakis N, Matsuura S, Ravid K. Megakaryocyte pathology and bone marrow fibrosis: lysyl oxidase connection. Blood 2012;120(9):1774-8.