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SHORT COMMUNICATION

Summary and Review of the Abstracts on Philadelphia-Negative Myeloproliferative Neoplasms Presented at Haematocon 2017

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Abstract There are lot of grey zones in Philadelphia negative chronic myeloproliferative neoplasms (CMPNs) and that's the reason they are in hit list of researchers. Having a spectrum of disorders their diagnosis is very important and especially to differentiate from each other since they overlap with each other in many ways. Diagnosis doesn't start from lab but with clinical phenotype. Clinical phenotype not only able to provide us the diagnosis but also helps in management of the disease per se. When diagnosis comes, the old timer but an evergreen morphology plays an important role which along with the newer generation tool "molecular" helps in differentiating these disorders. Lot of studies have already come up from the world. Indian data has also started coming up. When we say about the Indian data nothing holds more important role than Indian Society of Haematology and Blood Transfusion, ISHBT. This small review will cover all papers with BCR-ABL negative CMPNs which were presented at the annual national conference of the ISHBT (Haematocon 2017) which was conducted at Guwahati. These abstract papers from various reputed institutes and centres will provide a short academic journey towards ongoing research activities at these places and will able to guide us regarding Philadelphia negative CMPNs and also stimulate our brain for some left or conflicted areas.

Keywords Chronic myeloproliferative neoplasm · Philadelphia negative · Thrombophilia in CMPN · Megakaryocyte morphology in CMPN · JAK 2 mutation in CMPN

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Introduction

Chronic myeloproliferative neoplasms (CMPNs) which include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) and other less well-characterized chronic myeloproliferative disorders are clonal diseases which originate in pluripotential haematopoietic stem cell. The clonal expansion results in an increased abnormal haematopoiesis thus producing group of interrelated syndromes which are then classified as per the predominant phenotypic expression of the clone. Numerous studies have been done recently in the field of CMPNs resulting in the incorporation of some major changes in WHO 2017 classification of hematolymphoid malignacies.

Methodology

A short review from abstracts comprising of research data pertaining to Philadelphia negative CMPNs from our country which were presented during Haematocon 2017 held from 2nd Nov 2017–05 Nov 2017 at Guwahati were being done. All abstracts presented as oral or in poster form were reviewed. All Philadelphia positive cases and mastocytosis cases were excluded.

Molecular Studies in CMPNs

Role of molecular studies in myeloproliferative neoplasms is progressing. While enough studies are available on JAK2 mutational analysis there are only few studies available for

MPL and CALR mutations which have shown an established role in CMPNs. Recent studies have shown that mutations mapping to the juxtamembrane region of the thrombopoietin receptor MPL (exon 10) are present in 5-10% of the patients with PMF and 1% of the patients with ET who were negative for both JAK2 and CALR mutations. MPL mutations are known to have gain of function mutations leading to receptor activation in the absence of thrombopoietin binding with constitutional activation of the JAK-STAT signaling. Presently there are no FDA approved tests available for detection of JAK2 V617F, MPL mutation. Till date traditional Sanger sequencing is being used which have proven to have relatively poor sensitivity. Other methods which have been commonly used to detect these mutations includes restriction fragment length polymorphism, denaturing high-performance liquid chromatography, high-resolution melting-curve analysis, pyrosequencing, and various allele-specific PCR systems with electrophoretic analysis of the products. Despite of these extensive lists, still these methods have never achieved the sensitivity of less than 5% of alleles which is shown in Table 1. However, few studies are available for MPL mutations. Direct sequencing and PCR are available for its detection. It has been seen that MPL mutations which are below 10–15% are difficult to detect by Sanger sequencing [17]. Therefore, now multiplexed allele-specific PCR assay is an upcoming tool which can not only detect the majority of MPL mutations with high sensitivity but also can be easily deployed in laboratory. One of the studies done by Hemamalini et al. from CMC Vellore was "Comparison of Direct Sequencing and Allele Specific PCR Assay for Detection of MPL Mutations in Essential Thrombocythemia and Primary Myelofibrosis" in which they have compared the sensitivities of allele specific PCR and Sanger sequencing for detection of MPL mutations in essential thrombocythemia and primary myelofibrosis patients. They did retrospective study in which they found 80 patients suspected with ET or PMF between 2012 and 2016 (who were negative for JAK2 V617F, CALR and JAK2 exon 12 mutations. They extracted DNA from peripheral blood leukocytes using a commercial kit (GentraPuregene Blood Kit, Qiagen, Hilden, Germany) followed by Sanger sequencing and allele specific PCR (targeting W515L, W515K, W515A and S505N) which were done as per the established protocols. They found MPL mutation in 12 out of 80 patients (15%) using either of the two methods. Sanger sequencing identified mutations in 10 while allele specific PCR identified the mutations in 12 patients. Out of those mutations seven patients had W515L while five patients had W515K. Other rare mutations like S505N, W515A were not identified. It was found that direct sequencing failed to detect the nucleotide change in 2 patients with W515L mutation. While no additional mutation other than four common mutations were identified by allele specific PCR it enabled to pick up two additional positive cases in their study, thus making it to have better sensitivity. This study is similar to other study [17] in which all MPL mutation positive cases were detected by allele-specific PCR, while 17 out of 58 (29%) low-level mutations were either difficult to identify or were completely missed by Sanger sequencing. Therefore, this study reflects that triple negative (JAK-2, MPL and CALR) needs a concern to be confirmed by allele specific PCR which is more sensitive and reliable for their detection especially if previously it has been done by direct sequencing. This study provides an important information regarding a simple and robust assay capable to detect the majority (approximately 98%) of MPL mutations

Table 1 Comparison of methodolgies for JAK-2 detection

Method	Sensitivity (%)	Advantage	Disadvantage
qPCR (AS, LNA) [1–4]	0.1–0.01	High sensitivity; quantitative	Detects only target mutations
PCR (AS) [5–7]	1	High sensitivity; simple to perform	Detects only target mutations; not quantitative
Melting curve Analysis [6, 8–10]	5–10	Simple to perform; semiquantitative; low cost	Detects target mutation only; moderate to low sensitivity; poor reproducibility in low samples
Pyrosequencing [11]	5–10	Simple to perform; quantitative; low cost	Detects target mutation only; relatively low sensitivity
RFLP [8, 12]	1–10	Low cost	Relatively low sensitivity; requires post-PCR manipulation; unreliable in low þ samples; not quantitative
Sanger sequencing [13–16]	20	Detects all mutations; bidirectional confirmation	Low sensitivity; time-consuming; not quantitative

AS allele-specific, *LNA* locked nucleic acid, *RFLP*, restriction fragment length polymorphism

associated with PMF and ET with high analytical sensitivity (approximately 2.5%). Other methods described for the detection of MPL mutations are high-resolution melting, quantitative PCR, bead-based assay with locked nucleic acid modified probes, amplification refractory mutation, pyrosequencing and single allele-specific PCR and their sensitivity are either similar or lower than allele-specific PCR (approx 3–0.1%). Another major disadvantage among all these tests is that they failed to detect S505N mutation, which is present in a significant proportion (10.3%) of MPL mutation positive cases [18].

JAK 2 and Its Association with Clinical Phenotype in MPNs

MPNs have been associated with thrombophilia which has been extensively reviewed by Pati et al. in their editorial [19]. These vascular events present in approx of 12–39% of PV and ET patients [20]. International Prognostic Score of Thrombosis in ET (IPSET) in 2012 introduced markers of thrombotic risk (age, cardiovascular risk factors, prior history of thrombosis) [21]. JAK2 mutation also now is considered a well-established risk factor for thrombosis in ET [21, 22]. Besides V617F allele burden has also been associated with acquired activated protein C resistance, low free PS and increased levels of soluble markers of endothelial and platelet activation including elevated tissue factor [23–25]. In continuation with these studies, a study from AIIMS [26] was presented with a paper titled "Impact of Combined JAK2V617F Mutation and the Inherited Thrombophilic Factors on Thrombotic Risk in CMPNs" to study the combined role of JAK2 V617F and inherited thrombophilia in hypercoagulative state in CMPN patients. A total of 131 CMPN patients (PV, PMF and ET) positive for JAK2 V617F with thrombosis and 85 controls (JAK2 V617F negative CMPN patients without thrombosis) were studied. The DNA samples were screened for inherited thrombophilia which included FV Leiden, MTHFR C677T polymorphism, PT 20210 mutation and deficiencies of Protein C, Protein S and AT III. There were 131 patients out of which 74 were males and 57 were females (M:F—2.3:1) with median age 52 years (range 16–85 years). In 37 (28.2%) patients combined JAK2 V617F and inherited thrombophilic factors (predominantly FV Leiden) were present as compared to 7/85 (8.2%) in controls. Among inherited thrombophilic factors FV Leiden mutation was exclusively present in JAK2 V617F positive patients and was negative in controls ($p = 0.004$), the frequency of MTHFR C677T polymorphism was higher in JAK2 V617F positive patients as compared to controls but the difference was not statistically significant ($p < 0.07$) in the latter. Thus, the study emphasized that JAK2 V617F mutation in combination with inherited thrombophiliamay increase the risk of thrombosis in CMPN patients and thus this finding could further assist in risk stratification, setting up the investigation and treatment protocols in CMPN patients.

Besides the association of JAK2 617F allelic burden with thrombosis; another fascinating aspect among the clinicians is an association of former with clinical phenotype. Recently a study by Shixiang et al. has studied an impact of JAK2 617F mutation burden on disease phenotype [27] in which they have found some conclusive results like, JAK2 V617F mutation burden in PV to be higher than ET; strong correlation of JAK2 V617F mutation allelic burden with WBC counts, haemoglobin level, splenomegaly, and thrombosis. There is paucity of data showing the association between JAK2V617F allele burden and clinical phenotype in Indian polycythemia vera (PV) patients. In continuation with it a study from AIIMS was done by Sudha et al. showing the Influence of JAK2 V617F Allele Burden on Clinical Phenotype of Polycythemia Vera Patients [28] wherein blood samples were obtained from a total of 90 PV patients with JAK2 V617F positivity and allele burden was quantified by real-time polymerase chain reaction (RQ-PCR). Out of the 90 patients, 74 (82.22%) were males and 16 (17.78%) were females (median 45 years, range 35–78). Patients aged more than 50 years had significantly higher JAK2 V617F allele burden (median 40.15%, range 0.49–91.62%) as compared to patients with age < 50 years (median 48.59%, range 0.56–86.74%; $p = 0.0032$). Patients with splenomegaly had significantly higher JAK2 V617F allele burden (mean 50.24%, range 6.91–84.17%) as compared to patients without splenomegaly (mean 33.82%, range 0.49–71.83%; $p < 0.017$). Patients with higher allele burden (median 57.20, range 43.4–72.03%) had significantly increased thrombotic events as compared to patients with lower allele burden (median 37.38, range 0.49–84.17%; $p < 0.043$). Out of 90 patients, 49 (54%) were homozygous and 41 (46%) were heterozygous by ASO-PCR. 32/49 (66%) homozygous patients had allele burden > 50% by RQ-PCR, however 17/49 (34%) patients had allele burden < 50% by RQ-PCR. Thus, the study showed that in PV patients higher JAK2 V617F allele burden was associated with increasing age, splenomegaly and thrombotic events. Therefore, authors have recommended that inclusion of JAK2 V617F allele burden by RQ-PCR may be considered for prognostication and setting up the treatment protocol in these patients. This study from India was similar to one International study by Alessandro et al. [29].

Clincopathological Spectrum of MPNs

Another study by Tahlan et al. [30] which focussed on Clinico-Pathological Spectrum of Myeloproliferative Neoplasm was presented at the national haematology

conference in 2017 in which 120 cases of myeloproliferative neoplasms diagnosed over a period of 12 years were reviewed. The clinical features including hepatosplenomegaly were noted. The haemogram findings and bone marrow aspiration and trephine findings were analyzed. They found that out of total 120 MPN cases, 97 were diagnosed as chronic myeloid leukaemia (CML), 6 as polycythemia vera (PV), 5 as essential thrombocythemia (ET), 11 as primary myelofibrosis (PMF) and 1 as chronic neutrophilic leukaemia (CNL). 53.3% of the patients in the study were male and 46.7% were female. The overall male–female ratio was 1.1:1. CML was the most common type of MPN followed by PMF. Fever was the most common clinical presentation (53.3%) followed by abdominal symptoms (34.2%). Haematological parameters and bone marrow aspiration and trephine biopsy findings were analysed for the various MPNs and were compared with previously published studies.

Does Morphology have a Role to Play Now in CMPNs?

A hallmark of the histopathological diagnosis of MPNs is the number, morphology and topography of megakaryocytes which can help in differentiating among each other. Keeping this in mind a study was done by Tanya et al. [31] in JIPMER and was presented as "Megakaryocyte Morphology and Morphometry in Philadelphia Negative Myeloproliferative Neoplasms". There is significant overlap in the clinical, haematological as well as morphological features of the various subtypes of Ph negative MPN. The importance of bone marrow trephine biopsy plays a major role in distinguishing various subgroups of Ph negative MPN. Study from JIPMER aimed to assess and compare the megakaryocytic morphology and morphometry in Ph negative MPN wherein all newly diagnosed (based on WHO 2008 criteria) cases of Ph negative MPNs (n = 22) between January 2014 and June 2017 were included, out of which 7 cases were of ET, 7 were of PMF, 8 were of PV. They studied the following parameters in their bone marrow trephine biopsy:

1. Morphology—Location, type, number of megakaryocyte/mm^2, megakaryocytes in clusters, attached to trabeculae, intrasinusoidal megakaryocytes, megakaryocytes with naked nuclei, and showing emperipolesis along with megakaryocyte atypia and degree of marrow fibrosis.
2. Morphometric analysis was done on a minimum of 10 megakaryocytesin each case by Olympus Microscope using Progress(R)Capture Pro software at 409 and these parameters were assessed: (a) cytoplasmic major diameter (CMD), (b) nuclear major diameter (NMD), (c) nuclear to cytoplasmic area ratio, (d) nuclear circularity. They found that the number of megakaryocytes/mm^2 was found to be significantly high in ET as compared to PMF (8.6 vs 4.13; $p < 0.05$).Megakaryocytes in both dense and loose clusters were seen more in ET as compared to PV and PMF. Paratrabecular location of megakaryocytes were more in PMF as compared to ET and PV. One of the case of PMF showed a very high number of intrasinusoidal megakaryocytes (45.3 megs/mm^2). Megakaryocytes with naked nuclei and with emperipolesis were commonly seen in all the cases. Atypia was seen in 4 out of 7 cases of PMF and none in ET and PV. The degree of fibrosis (using WHO 2008 criteria) was significantly high for PMF (7 out of 8 cases showed grade 3 fibrosis). 6 out of 8 cases of PV and 5 out of 7 cases of ET showed grade 1 fibrosis. The mean CMD for PV was 13.02 which was more than both ET and PMF. Hence, the cytoplasmic area was more in PV as compared to ET and PMF. PV showed more of cloud-like nuclei and the NMD for PV was more as compared to ET and PMF. ET showed more of staghorn type of nuclei. The nuclear to cytoplasmic area was more in PV as compared to ET and PMF. Summarising the study, the number of megakaryocytes/mm^2 and clustering was more in ET, large size of megakaryocytes was seen in PV, atypia and fibrosis was more in PMF. Thus, the studied showed despite of overlapping of MPNs the morphology and morphometry of megakaryocytes can lead to clue to reach the diagnosis in various Ph negative MPN. The similar study by Nataliya et al. [32] showed that in ET, megakaryocytes were the largest of all MPNs, nuclei were more polymorphic and were characterized by a less regular cytoplasmic outline compared with normal forms. Thiele et al. [33, 34] also observed the similar pattern. Nataliya et al. also showed that morphologic findings concerning megakaryocytes didn't support a close relationship between ET and PV which was also observed by Gianelli et al. [35].

Conclusion

Abstracts from Haematocon 2017 covering broad spectrum of CMPNs have given in-depth knowledge as well as the update on this entity. Various massive Indian data has been coming from various established centres and this will enlightens mort ho the world literature in future. Importance of these data gains more importance because of different challenges and clinical scenarios in Indian population thus leading to difficulty in diagnosis. Forums

are required to raise these concerns and as far as Indian setting is concerned Indian Society of Haematology and Blood Transfusion has an important role to play while conducting annual conferences. This mini review from abstract from an annual conference in 2017 has focused upon updates and knowledge on CMPNs.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval Not sought/required as this review article does not contain any studies with human participants performed by any of the authors.

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