

Original Research Article

Study of mutation profile in myelofibrosis and response to low dose ruxolitinib: a tertiary care experience

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ABSTRACT

Background: Myelofibrosis is a rare myeloid neoplasm characterized predominantly by anaemia and splenomegaly. Mutations in Janus kinase 2 (JAK2), calreticulin (CALR), and myeloproliferative leukaemia (MPL) play key roles. This study aimed to examine the demographic profile, transfusion dependency, and mutations (JAK2, CALR, and MPL) associated with myelofibrosis and to evaluate the response to Ruxolitinib treatment in these patients.

Methods: This retrospective study included 30 patients at Madras Medical College, Chennai, from January 2022 to August 2024. Patients aged between 20 and 70 years at the time of diagnosis, primary myelofibrosis (PMF) or secondary myelofibrosis confirmed by clinical findings, laboratory tests, bone marrow biopsy, and genetic mutation such as JAK2 V617F, CALR, MPL, or triple-negative were included.

Results: The mean age was 52.37 ± 13.08 years, with splenomegaly and anaemia being common. Of the 30 patients, 73.3% were on Ruxolitinib and 59.1% were transfusion-dependent. CALR-positive and triple-negative patients were entirely transfusion-dependent, whereas JAK2-positive patients were predominantly non-transfusion-dependent ($p < 0.0001$). Ruxolitinib treatment showed mild reduction on spleen size but significantly improved quality of life ($p = 0.031$). Non-transfusion-dependent patients had better quality of life scores ($p < 0.0001$).

Conclusions: Genetic testing, including both driver and non-driver mutations, plays a crucial role in the diagnosis, prognosis, and treatment of myelofibrosis. Transfusion dependency and anaemia severity are negative prognostic factors, while Ruxolitinib improves the quality of life.

Keywords: Myelofibrosis, Ruxolitinib, Splenomegaly, Anaemia, JAK2

INTRODUCTION

Myelofibrosis (MF) is a chronic rare myeloid neoplasm that is diagnosed in an estimated 1 per 100,000 people. MF is characterized by splenomegaly, constitutional symptoms, and cytopenia, and can be a primary or secondary myelofibrosis due to polycythemia (10-15%) and essential thrombocythemia (4.3%).¹

Two distinct clinical phenotypes, myeloproliferative and myelodepletive (cytopenic), have been recognized in MF. The biology of these two phenotypes differs, and their relationship with their molecular profiles is complex. The myelodepletive phenotype, often linked to primary MF (PMF) and cytopenias (anaemia, thrombocytopenia, or

severe pancytopenia), typically requires red blood cell and/or platelet transfusions and presents with circulating blasts and bone marrow fibrosis grade 2 or higher, resembling bone marrow failure. Conversely, the myeloproliferative phenotype usually evolves from polycythemia vera (PV) or essential thrombocythemia (ET) to secondary MF, showing myeloid lineage expansion and normal or high blood counts (leukocytosis or elevated platelet counts). Patients with this phenotype have normal to mildly decreased haemoglobin (Hb), minimal transfusion needs, hypercellular bone marrow, large splenomegaly, and constitutional symptoms.²

Perturbation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway

is a hallmark of MF. This finally leads to hematopoietic stem defects with abnormal proliferation of megakaryocytes, which fail to express the hematopoietic transcription factor GATA1. Consequently, the secretion of inflammatory cytokines results in myeloproliferation, increased bone marrow fibrosis (BMF), and extramedullary haematopoiesis.

A new prognostic model (DIPSS-plus) has been established and includes red blood cell transfusion as one of the eight risk factors.³ Anaemia is a key hallmark of MF, which increases in frequency and severity with disease progression.⁴ Anaemia is a powerful risk factor for primary myelofibrosis. The presence and severity of anaemia in primary myelofibrosis signify a clonally advanced and biologically aggressive disease. JAK2 V617F, calreticulin (CALR), myeloproliferative leukaemia (MPL) and triple-negative mutations are common in myelofibrosis.⁵ Ruxolitinib, a selective inhibitor of JAK 1 and 2, has clinically significant activity in myelofibrosis.⁶

As all agents of this class, the drug mainly inhibits dysregulated JAK-STAT signalling, present in all patients with MF irrespective of their JAK2 mutational status, but it is not selective for the mutated JAK2, which explains its efficacy in both JAK2-positive and JAK2-negative MF. Ruxolitinib is highly effective in reducing the spleen and controlling the symptoms of MF, with this resulting in a marked improvement in the patients' quality of life.⁷

Patients tested negative for the three driver mutations, in which an extended mutation panel including at least ASXL1, EZH2, IDH1/2, and SRSF2 is recommended.⁸

Aim

This study aimed to examine the demographic profile, transfusion dependency, and mutations (JAK2, CALR, and MPL) associated with myelofibrosis and to evaluate the response to Ruxolitinib treatment in these patients.

METHODS

This retrospective study included 30 patients at the Department of Clinical Haematology, Madras Medical College, Chennai, from January 2022 to August 2024.

Inclusion criteria

Patients aged between 20 and 70 years at the time of diagnosis, PMF, or secondary MF confirmed by clinical findings, laboratory tests, and bone marrow biopsy, and confirmed by genetic mutations such as JAK2 V617F, CALR, MPL, or triple-negative were included.

Exclusion criteria

Patients aged <20 and >70 years, those with other haematological disorders that did not satisfy the diagnostic

criteria, and those who did not adhere to treatment were excluded.

Demographic, clinical, laboratory, and treatment data were collected from medical records. Key parameters included genetic mutation status (JAK2, CALR, MPL, or triple-negative), splenomegaly measurements obtained through ultrasound, transfusion dependency, and treatment regimens such as Ruxolitinib, prednisolone, and thalidomide. Quality of life (QoL) was assessed using the myelofibrosis symptom assessment form (MF-SAF). The primary outcomes focused on analyzing genetic mutation profiles and their correlation with transfusion dependency, evaluating the impact of Ruxolitinib on splenomegaly, QoL, and prognostic scores based on the dynamic international prognostic scoring system-plus (DIPSS-plus), and comparing the influence of different treatment regimens on QoL and transfusion dependency.

Statistical analysis

Data are presented as mean, standard deviation, frequency, and percentage. Continuous variables were compared using the independent sample t-test, while the Mann-Whitney U test was used to compare non-normally distributed continuous data between independent groups. Categorical variables were compared using Pearson's Chi-square test. Significance was defined as p values less than 0.05 using a two-tailed test. Data analysis was performed using IBM-statistical package for the social sciences (SPSS) version 25.0 (IBM-SPSS Corp., Armonk, NY, USA).

RESULTS

The mean age was 52.37 ± 13.08 , with a wide age range. The TLC had a mean of 11636.67 ± 6571.08 cells/ μ l, indicating considerable variability in the immune response. Hemoglobin levels and anemia were low, with a mean of 8.34 ± 3.90 g/dl. The mean PLT count was 181240 ± 133873.11 cells/ μ l. The initial spleen size has a mean of 20.31 ± 3.84 cm, indicating splenomegaly, which decreases to 16.88 ± 2.14 cm after six months, showing improvement. The DIPSS plus score averages 2.23 ± 1.14 , indicating mild to moderate risk. The overall quality of life SAF had a mean score of 5.43 ± 1.59 , while the MF-SAF score was higher at 7.43 ± 0.86 a better quality of life (Table 1).

Among the patients with myelofibrosis, 11 (36.7%) were females and 19 (63.3%) were males. Regarding the bone marrow biopsy fibrosis grading, 14 (46.7%) patients had grade II fibrosis, whereas 16 (53.3%) patients had grade III fibrosis. Karyotyping revealed that 10 (33.3%) patients had a 46 XX karyotype and 17 (56.7%) patients had a 46 XY karyotype. Genetic mutation distribution showed that three (10%) patients were CALR positive, six (20%) patients were JAK 2 negative, 15 (50%) patients were JAK 2 positive, and six (20%) patients were triple-negative. Eight (26.7%) patients were not treated with Ruxolitinib

and 22 (73.3%) patients were treated with Ruxolitinib. Only seven (23.3%) patients did not receive Tab. Prednisolone + Cap. Thalidomide and 13 (43.3%) patients were transfusion-dependent (Table 2).

Table 1: Clinical and quality of life outcomes in myelofibrosis patients.

Variables	Mean±SD
Age (in years)	52.37±13.08
Total leukocyte count (TLC)	11636.67±6571.08
Hemoglobin (HB)	8.34±3.90
Platelet count (PLT)	181240±133873.11
Initial spleen size	20.31±3.84
6 months spleen size	16.88±2.14
DIPSS plus score	2.23±1.14
Overall quality of life (SAF)	5.43±1.59
Overall quality of life (MF-SAF)	7.43±0.86

Table 2: Clinical and genetic characteristics.

Characteristics	Frequency (%)
Sex	
Female	11 (36.7)
Male	19 (63.3)
BMB-fibrosis grading	
Grade II	14 (46.7)
Grade III	16 (53.3)
Karyotyping	
46 XX	10 (33.3)
46 XY	17 (56.7)
Metaphases couldn't be analysed	2 (6.7)
Not done	1 (3.3)
Genetic mutation	
CALR positive	3 (10)
JAK 2 negative	6 (20)
JAK 2 positive	15 (50)
Triple-negative	6 (20)
Tab. Ruxolitinib (5 mg) BD	
No	8 (26.7)
Yes	22 (73.3)
Tab. Prednisolone (0.5 mg/kg/day) + Cap. Thalidomide (100 mg) OD	
No	7 (23.3)
Yes	23 (76.7)
Transfusion dependency	
No	17 (56.7)
Yes	13 (43.3)

All CALR-positive patients 3 (100%) were transfusion-dependent ($p<0.0001$), while JAK2 positive patients showed a predominance of non-transfusion dependency 14 (93.3%), with only one (6.7%) transfusion-dependent. All triple-negative patients 6 (100%) were transfusion-dependent (Table 3).

Table 3: Correlation of genetic mutations and Ruxolitinib use with transfusion dependency.

Variables	Transfusion dependency (%)		P value
	No	Yes	
Genetic mutation			
CALR positive	0	3 (100)	<0.0001
JAK 2 positive	14 (93.3)	1 (6.7)	
JAK 2 negative	3 (50)	3 (50)	
Triple negative	0	6 (100)	
Tab. Ruxolitinib 5 mg BD			
Yes	9 (40.9)	13 (59.1)	0.004
No	8 (100)	0	

There was reduction in spleen size with no significant differences in spleen size between the 25th quartile, median, and 75th quartile groups for both those who received the treatment (1, 3, and 5.95 cm, respectively) and those who did not (0.38, 2.23, and 3.85 cm, respectively; $p=0.629$). There was a significant improvement in the overall quality of life post-treatment for those receiving Ruxolitinib, with a median change of -1.75 compared to -2 for non-treated patients ($p=0.031$).

Regarding spleen size, no significant difference was found between patients treated with Ruxolitinib and those who did not, both at baseline ($p=0.393$) and at six months ($p=0.448$). However, the MF-SAF score is a significantly higher in non-transfusion dependent patients (8.13 ± 0.35 versus 7.18 ± 0.85 , $p=0.006$). The DIPSS plus score showed no significant difference between the two groups ($p=0.076$) (Table 4).

Table 4: Comparison of Ruxolitinib use on spleen size, quality of life, and DIPSS plus score.

Variables	Tab. Ruxolitinib		P value
	Yes	No	
Difference in spleen size (initial-post)			
25th quartile	1	0.38	0.629
Median	3	2.23	
75th quartile	5.95	3.85	
Difference in overall quality of life (pre-post)			
25th quartile	-3	-2	0.031
Median	-2	-1.5	
75th quartile	-1.75	0	
Spleen size			
Initial	20.68±3.81	19.29±4	0.393
6 months	17.06±2.13	16.38±2.22	0.448
Overall quality of life (SAF)	4.86±1.39	7±0.93	<0.0001
Overall quality of life (MF-SAF)	7.18±0.85	8.13±0.35	0.006
DIPSS plus score	2.45±1.22	1.63±0.52	0.076

Among patients receiving Ruxolitinib (5 mg) along with prednisolone and thalidomide, five (31.3%) were not transfusion-dependent, while 68.8% were transfusion-dependent ($p=0.132$). In the group receiving Ruxolitinib without prednisolone and thalidomide, four (66.7%) were not transfusion-dependent, and two (33.3%) were transfusion-dependent.

For patients not receiving Ruxolitinib, all seven (100%) of those on prednisolone and thalidomide were transfusion-dependent. There was no significant association between Ruxolitinib treatment and transfusion dependency ($p=0.132$) (Table 5).

Table 5: Impact of Ruxolitinib and prednisolone + thalidomide on transfusion dependency in patients.

Tab. ruxolit-inib 5 mg	Tab. prednisolone + cap. thalidomide	Transfusion dependency (%)		P value
		No	Yes	
Yes	Yes	5 (31.3)	11 (68.8)	0.132
	No	4 (66.7)	2 (33.3)	
No	Yes	7 (100)	0	-
	No	1 (100)	0	

A significant difference in the overall quality of life between patients who received Ruxolitinib along with prednisolone and thalidomide and those who did not were noted. However, no statistically significant difference was observed in the quality of life in patients not receiving Ruxolitinib with or without tab prednisolone and thalidomide (8.00 versus 8.14 ± 0.38 , $p=0.736$) (Table 6).

Table 6: Effect of prednisolone and thalidomide along with Ruxolitinib on overall quality of life in patients.

Tab. Ruxolitinib 5 mg	Tab. prednisolone + cap. thalidomide	Overall quality of life (MF-SAF) (%)	P value
Yes	Yes	6.94 ± 0.68	0.024
	No	7.83 ± 0.98	
No	Yes	8.14 ± 0.38	0.736
	No	8	

DISCUSSION

In our study, the mean age was 52.37 ± 13.08 , with a wide age range. Included 30 patients with both primary and secondary MF who were followed for 24 months which was similar to the study by Gerds et al where the median age was 52 years.⁹ In our cohort JAK 2 positive MF was seen in 50% of our patients. But it was contrary to the results of Gerds et al.¹⁰

In our study, all CALR-positive patients (100%) were transfusion-dependent, whereas most JAK 2-positive patients (93.3%) were non-transfusion-dependent. All

triple-negative patients (100%) were transfusion dependent. In our cohort no patients presented with culture positive sepsis. According to Chen et al, a case involving a post ET MF patient who was taking Ruxolitinib at a dosage of 20 mg twice a day presented twice with group B Streptococci infection.¹¹

In Drofenik et al study, the JAK2 allele burden exhibited a decrease in 19 patients, remained stable in five patients, and showed increase in four patients following Ruxolitinib therapy. No significant relationship was observed between the JAK2 allele burden and the calcium accumulation in coronary arteries. In post ET patients who demonstrated an increase in JAK2 mutation burden also showed a corresponding rise in their coronary calcium score.¹²

In our study, spleen size was reduced but not significantly different between patients treated with Ruxolitinib and those who did not at both baseline ($p=0.393$) and six months ($p=0.448$). However, non-transfusion-dependent patients had significantly higher overall quality of life (SAF) scores (7.00 ± 0.93 versus 4.86 ± 1.39 , $p<0.0001$). No significant difference was found in the DIPSS plus scores between groups ($p=0.076$). The research conducted by Polverelli et al revealed that patients with MF who had spleen enlargement of 5 cm or greater, especially when it extended beyond 15 cm below the left costal margin or was accompanied by symptoms related to splenomegaly, could benefit from treatment to reduce the spleen size before stem cell transplant. In our study with initial mean spleen size which was 20.31 cm, reduced to 16.88 cm by the end of 6 months follow-up.¹³ Our study is contrary to the study by Mauro et al, where there was a 35% reduction in spleen volume at the end of 6 months.¹⁴

In our study, most patients had more than grade II fibrosis which is similar to Passamonti et al. Some molecules have demonstrated early impacts on bone marrow fibrosis and myeloid gene variant allele frequencies in translational research. Although stem cell transplantation remains the sole curative option, it comes with significant obstacles.

In our study, CALR-positive and triple-negative patients were entirely transfusion-dependent, whereas JAK2-positive patients were predominantly non-transfusion-dependent ($p<0.0001$). Patel et al reported that JAK2-positive MF patients required fewer transfusions and found that the presence of >3 mutations showed an inverse relationship with both spleen response and overall survival.¹⁵ While Ruxolitinib treatment led to a reduction in spleen size; in our study. But this change was not statistically significant. However, QoL improved notably after six months of Ruxolitinib therapy.^{16,17} In the study by Gerds et al, CALR-positive and triple-negative patients demonstrated significant transfusion dependency.⁴

CONCLUSION

Genetic correlations could be a useful tool for clinical decision-making. The molecular genetics of MF has

opened new horizons in the diagnosis, prognosis, treatment decision-making, and monitoring of this disorder. Molecular testing for both driver and non-driver genetic events is recommended. The results demonstrate that dependency on transfusions, increasing transfusion requirements, and greater anaemia severity are negative prognostic factors for overall survival. Ruxolitinib which is a JAK2 inhibitor found to improve the QoL. However, the study's sample size was limited. Therapies that reduce transfusion dependency and anaemia severity may help to improve health outcomes.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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