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Expression of CD4$^+$T Cells in Myeloproliferative Diseases and the Effect of Ruxolitinib Treatment on Prognosis

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Myeloproliferative disorders (MPDs) are rare diseases in which the bone marrow produces too many red, white, or platelets. Myeloproliferative disorders are neither acute nor leukaemia. To study ruxolitinib's effect on MPD therapy and CD4$^+$ T cell expression. In total, 66 JAK2V617F-positive MPD patients were admitted to our hospital. The patients were randomly assigned to control and research groups (each 33). Hydroxyurea pills were given to the control group and ruxolitinib to the observation group. The MPN-10 assesses 10 of the most clinically relevant symptoms, including fatigue and generates a Total Symptom Score (TSS). In addition, by comparing myelofibrosis (MF), spleen length, JAK2V617F gene expression, peripheral blood lymphocyte and T cell levels, and prognostic levels, analyze the shortcomings of each group. Post-treatment, MPN-10, MF, and spleen length diameter were reduced in both groups ($P < 0.05$), with the study group showing a higher reduction than the control group ($P < 0.05$). Compared to prior treatment, JAK2V617F gene expression was reduced in all groups after 6 months and a year of medication. The study category had a higher decrease in expression than the control group. After therapy, CD4 and CD4/CD8 levels rose, but CD8 and Treg levels decreased. The study group had increased CD4 and CD4/CD8 levels, whereas the control group had lower CD8 and Treg levels. The study group had a greater 1-year survival rate than the control group, but the control group had lower mortality and adverse event rates. In JAK2V617F-positive MPD patients, ruxolitinib reduces JAK2V617F gene expression, myelofibrosis, and therapeutic impact.

Keywords: CD4$^+$ T cells; myeloproliferative diseases; prognosis; ruxolitinib
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Introduction

Myeloproliferative disorders (MPD) are a kind of neoplastic disease characterised by the clonal proliferation of a multilineage myeloid cell with a relatively high level of differentiation. Clinically, MPD is characterised by the proliferation of numerous blood cells, as well as the enlargement of the spleen or lymph nodes (Braun and Zeiser 2020). Myeloproliferative disorders (MPDs) include a range of subgroups, such as primary myelofibrosis (PMF), essential thrombocythemia (ET), and polycythemia vera (PV), among others. Previous research (Guy and James 2019) has shown a significant association between mutations in the JAK2V617F gene and their profound influence on MPD. These mutations have been found to exhibit a high degree of specificity across various strains, therefore serving as reliable indicators for verifying the diagnosis of MPD, as outlined by the most recent diagnostic criteria.

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The primary clinical interventions for the condition include the administration of interferon, surgical excision of the spleen, and chemotherapy. However, patients often have intolerance and encounter a higher incidence of adverse effects, resulting in suboptimal therapeutic outcomes. In a research conducted by Al-Ali et al. (2020), it was shown that the use of Ruxolitinib as a monotherapy demonstrated efficacy in the treatment of MF. However, it was seen that this treatment did not lead to a reduction in the JAKV617F allele load. The potential impact of reducing the JAKV617F allele load on prognosis remains uncertain. However, it is worth noting that a higher allele load has been linked to increased clinical symptoms, a heightened risk of disease progression, and thrombosis. Consequently, the reduction of the JAKV617F allele load is regarded as a clinical treatment endpoint for MF and a valid indicator for evaluating treatment efficacy. The objective of this study was to assess the efficacy and prognosis of Ruxolitinib in the treatment of persons diagnosed with JAK2V617F-positive MPD.

Materials and Methods

Inclusion criteria

Patients were ≥ 18 years of age with palpable splenomegaly ≥ 5 cm below the left costal margin and a confirmed diagnosis of primary MF (WHO criteria) or post–polycythemia vera or post–essential thrombocythemia MF (International Working Group for Myelofibrosis Research and Treatment [IWG-MRT] criteria) (Fujii et al. 2020).

Exclusion criteria

Endocrine dysfunction; previous history of MPD-related treatment; major illness with severe cardiovascular, cerebrovascular, hepatic, or renal disease; haematological with immune system disorders; psychiatric disorders; incomplete data.

Study subjects

The JAK2V617F gene was found to be positive in 66 individuals suffering from myeloproliferative diseases who were admitted to Tangshan People's Hospital from March 2019 to March 2020. These individuals satisfied the requirements for inclusion and exclusion, 33 individuals from each of the control and study categories were randomly assigned to each category. In the control category, there were 15 men and 18 women with an average age of 62.43 ± 3.23 years, a range of 50 to 70 years. There were 12 PV cases, 9 PMF cases, and 12 ET cases. The trial category consisted of 15 PV, 10 PMF, and 8 ET patients, with a gender split of 17 men and 16 women, ages ranging from 55 to 75. There was no statistical significance between the two categories' overall statistics ($P > 0.05$). The Experimental Ethics Committee of Tangshan People's Hospital approved all the experiments conducted (No. 2019/4764/066). The informed consent form, which was authorized by the hospital's ethics committee, was signed by all participants in the study.

Experiment

This study had a 24-week double-blind double-dummy treatment phase. Patients were randomly assigned to the control group in a 1:1 ratio through a web-based interactive response system and given hydroxyurea tablets (national drug quantification number H42022293; manufacturer: Wuhan Zhonglian Group Siyao Pharmaceutical Co., Ltd.). Specification: 0.5 g/tablet, oral, 20-60 mg twice daily. Patients in the observation group were given Ruxolitinib (national drug quantification number H20040317; Specification: 5 mg/tablet, oral, 25 mg twice daily (or modified according to the label). Treatment assignment was stratified by transfusion dependence (yes or no; defined as ≥ 4 units of RBCs or Hb level < 8 g/dL in the 8 weeks before random assignment, excluding patients associated with clinically overt bleeding) and by platelet count (< 100 × 109/L, ≥ 100 × 109/L and ≤ 200 × 109/L, or > 200 × 109/L). A record of all transfusions received during screening and throughout the study was kept in the patients' diaries.

Indicator observation

(1) MPN-10 evaluated using MPN Total Symptom Rating Scale (Guaraná et al. 2022) each symptom has a score of 0-10 for assessment, with 0 being no symptoms and 10 being the most severe, with higher scores indicating more severe symptoms. (2) MF scores range from 0 to 3 points (Krečak et al. 2021). MF-0 represents basic normal bone marrow tissue, while MF-3 represents a complete loss of bone marrow tissue, completely replaced by proliferative fibres and the presence of osteophytes. (3) Spleen length diameter was measured using color ultrasound. The splenic length diameter was measured using color ultrasound. (4) JAK2V617F gene expression assay: Before therapy, six months, and a year following therapy, patients' fasting venous blood was drawn; it was centrifuged, mononuclear cells were separated; total genomic RNA was extracted using the Trizol technique; and cDNA was synthesised using reverse transcription. Table 1 lists the primers and probes. GAPDH served as an internal standard, and $2^{-\Delta\Delta}$ct was utilized to determine the relative gene expression values. A fully automated biochemical analyzer was used to measure the peripheral blood lymphocyte subsets and T-cell counts. (5) Patients were followed up until March 2021, and the 1-year survival rate and mortality rate of the 2 groups were counted. (6) The adverse effects such as fever, malaise, rash, abdominal distension and muscle pain were counted and contrasted among the two categories of patients.

Statistical analysis

Data processing and analysis were done using the SPSS 22.0 licensed statistical tool. The measurement results were reported by mean ± standard deviation, repeated measurements and a t-test for independent samples

Table 1. Gene primer sequences.

Gene primer	Sequence
JAK2 upstream primer	5'-CAGCAAGTATGATGAGCAAGCTTT-3'
JAK2 downstream primer	5'-TGAACCAGAATATTCTCGTCTCCAC-3'
Probe sequence	5'-FAM-TCACAAGCATTTGGTTTT-MGB-3'
JAK2 V617F downstream primer	5'-CCAGAATATTCTCGTCTCCACTGAA-3'

Table 2. Comparison of MPN-10 score, MF score and splenic length diameter before and after treatment in two categories ($\bar{x} \pm s$).

Group	MPN-10 score (points)		MF score (points)		Spleenlength diameter (cm)	
	Treatment		Treatment		Treatment	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Control (33)	39.23 ± 9.88	26.34 ± 6.21*	1.98 ± 0.23	1.03 ± 0.21*	13.34 ± 3.23	10.34 ± 3.21*
Study (33)	39.21 ± 9.56	14.22 ± 4.21*	1.99 ± 0.34	0.46 ± 0.01*	13.45 ± 3.65	8.11 ± 2.12*
t	0.008	9.280	0.140	15.570	0.130	3.330
P	0.993	0.001	0.889	0.001	0.897	0.001

*$P < 0.05$ compared with pre-treatment.

Table 3. Comparison of JAK2V617F gene expression before, 6 months and 1 year of therapy in two categories ($\bar{x} \pm s$).

Group	Cases (n)	JAK2V617F		
		Pre-treatment	6 months post-treatment	1year post-treatment
Control group	33	0.56 ± 0.04	0.45 ± 0.07*	0.22 ± 0.01*,#
Study group	33	0.55 ± 0.06	0.28 ± 0.01*	0.15 ± 0.01*,#
t		0.800	13.810	28.430
P		0.429	0.001	0.001

*$P < 0.05$ compared with pre-treatment, #$P < 0.05$ compared with 6 months post-treatment.

were used to compare the categories. Before and after therapy comparisons were made using ANOVA; count data were reported as frequencies and percentages; comparisons among categories were made using the 2 test; a discrepancy of $P < 0.05$ was deemed statistically meaningful.

Results

Correlation of MPN-10 score, MF score, and splenic length diameter prior and post treatment between 2 categories of patients

There was no variation in the MPN-10 score (Table 2). MF score and splenic length diameter before treatment ($P > 0.05$); MPN-10 score, MF score, and splenic length diameter decreased after treatment ($P < 0.05$) and MPN-10 score, MF score, and splenic length diameter were inferior in the study category after treatment ($P < 0.05$).

Correlation of JAK2V617F gene expression in 2 categories of patient's prior therapy, six months and 1 year post therapy

As seen in Table 3, there was no distinction in the expression of the JAK2V617F gene prior to therapy ($P > 0.05$); the expression of the JAK2V617F gene dropped after six months as well as one year of therapy ($P < 0.05$); as well as the expression of the JAK2V617F gene was less in the study category after six months as well as one year of therapy ($P < 0.05$).

Comparison of peripheral blood lymphocyte subsets and T-cell levels prior to and post-therapy in 2 categories

The levels of T cells and peripheral blood lymphocyte subsets were the same in each group prior to therapy, as indicated in Table 4 ($P > 0.05$); After therapy, CD4, CD4/CD8 levels were greater, CD8 and Treg levels were decreased, both of which were statistically significant ($P < 0.05$). In the study category, these results were also statistically significant.

Correlation of the prognosis of patients in the 2 categories

According to Table 5, the study category's 1-year survival rate was 75.75% (25/33), which was greater than the control category's [36.36% (12/33)], ($P < 0.05$). When compared to the control category's death rate, which was 21.21% (7/33), the study category's mortality rate was

Table 4. Correlation of T-cell counts and lymphocyte subsets in peripheral blood in the two categories prior to and following treatment [($\overline{x} \pm s$), %].

Group	$CD4^+$ treatment		$CD8^+$ treatment		CD4/CD8 treatment		Treg treatment	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
Control group (33)	31.23 ± 9.8	34.23 ± 9.12*	37.23 ± 8.23	35.34 ± 7.12*	0.88 ± 0.12	1.14 ± 0.11*	13.34 ± 3.21	7.45 ± 1.11*
Study group (33)	32.23 ± 9.66	41.22 ± 9.11*	36.87 ± 8.86	29.45 ± 6.54*	0.86 ± 0.16	1.33 ± 0.12*	13.45 ± 3.44	3.45 ± 0.21*
t	0.416	3.115	0.171	3.500	0.575	6.705	0.134	20.340
P	0.680	0.003	0.865	0.001	0.568	0.001	0.894	0.001

*$P < 0.05$ compared with pre-treatment.

Table 5. Correlation of prognosis of patients in 2 groups (cases, %).

Group	Cases (n)	1-year survival	Mortality rate
Control group	33	12 (36.36)	7 (21.21)
Study group	33	25 (75.75)	1 (3.03)
χ^2		10.395	4.924
P		0.001	0.026

Table 6. Comparison of adverse rates in two categories (cases, %).

Group	Cases (n)	Feverish	Fatigue	Rash	Abdominal distension	Muscle soreness	Adverse reaction rate
Control group	33	1	2	1	28	2	8 (24.24)
Study group	33	0	1	0	0	0	1 (3.03)
χ^2							6.073
P							0.014

3.03% (1/33) fewer ($P < 0.05$). In comparison to the control category, it revealed that the study group had a superior prognosis.

Correlation of the two patient categories' unfavourable response rates

Common adverse effects after treatment in both groups were abdominal distension, fever, malaise, rash, and muscle aches. The rate of adverse reactions was 3.03% (1/33) in the study category, which ($P < 0.05$) was less than the figure of 24.24 (8/33) in the control group. The study group was safer than the control category (Table 6). Our results demonstrate the effect of rosolitinib on MPD treatment and $CD4^+$T cell expression.

Discussion

MPD is a common disorder of the haematological system, the pathogenesis of which is related to JAK1 and JAK2. MPN is clinically a clonal haematopoietic stem cell disease, the species of which is commonly dominated by malignant proliferative stem cells of a lineage of bone marrow, accompanied by symptoms of varying degrees of other cellular haematopoietic cells when used, this disease can co-exist or in transformation, eventually evolving into bone marrow failure (Fujii et al. 2020; Rampal et al. 2021). In 2008 the World Health Organization classified the disease such as true red blood cell symptoms, essential platelet symptoms, chronic leukemia cells, and primary bone marrow symptoms and injected JAK2V617F (Rampal et al. 2021) positivity into the diagnostic criteria for CD4/CD8 gene negative Treg (Patnaik and Lasho 2020).

The MPN-10 score, MF score, and spleen length diameter were decreased in the study category following therapy. This finding suggests that ruxolitinib was successful in reducing individuals' clinical complaints and spleen enlargement. According to the findings of a similar study (Yuan et al. 2020), JAK2V617F gene mutations are currently regarded as the gold standard for the management of PV, ET, and PMF since they occur in more than 80% of patients with PV as well as more than 65% of ET and PMF individuals in clinical practice. Rucotinib is a very effective JAK1 and JAK2 kinase inhibitor that specifically blocks their signaling (Liang et al. 2018; Prause et al. 2020). In this paper, JAK2V617F gene expression was lower in the study group after 6 months and 1 year of treatment. The current result indicates that ruxolitinib is effective in reducing JAK2V617F (Bose and Verstovsek 2017) gene expression and is worthy of clinical promotion. JAK2 inhibitors are tyrosine protein kinases that mediate a variety of cytokines and are important for haematopoietic and

immune functions. This study concluded that alectinib can successfully control peripheral blood lymphocyte and T cell levels in individuals who have notable effects because CD4, CD4/CD8 levels were increased, and CD8, Treg levels were reduced in the study category after therapy. Ruxolitinib was able to reduce the levels of the JAK2V617F mutation, PD-1, PD-L1, and Treg, which in turn slowed the course of MPN (Meyer 2017). Sørensen et al. (2019) research demonstrated that PD-1, PD-L1, and Treg were implicated in the pathogenesis of MPN in line with the conclusions of this article.

It has been demonstrated that JAK2 inhibitors can prevent downstream cells from proliferating by inhibiting the activation of the JAK-STAT signaling pathway (Simon et al. 2021). Rukotinib inhibits JAK2's aberrant signaling impact on cytokines, which prevents the JAK-STAT signaling pathway from being activated, thus preventing the abnormal proliferation of myeloid cells, thus achieving the therapeutic goal (Zeiser et al. 2021). findings were expressed that ruxolitinib was both safe and effective for treating myelofibrosis patients (Mesa et al. 2017). This was demonstrated by the outcomes of the 1-year follow-up. According to the study of Qu et al. (2022), ruxolitinib's unfavourable haematological effects were minimized when used in the management of MF individuals along with prednisone, thalidomide, danazol, increased haemoglobin and platelet counts, improved clinical efficacy, and had a good safety profile. All previous outcomes were consistent with the study's findings.

Conclusion

In brief, the administration of ruxolitinib has shown a notable capacity to effectively diminish the expression of the JAK2V617F gene in patients diagnosed with myeloproliferative neoplasms (MPNs) who tested positive for the JAK2V617F mutation. Furthermore, this intervention resulted in a reduction in the extent of myelofibrosis and yielded favourable treatment outcomes.

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Conflict of Interest

The authors declare no conflict of interest.

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