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Ruxolitinib mitigates oxidative stress and ROS-induced structural and functional fibrinogen alterations in primary myelofibrosis

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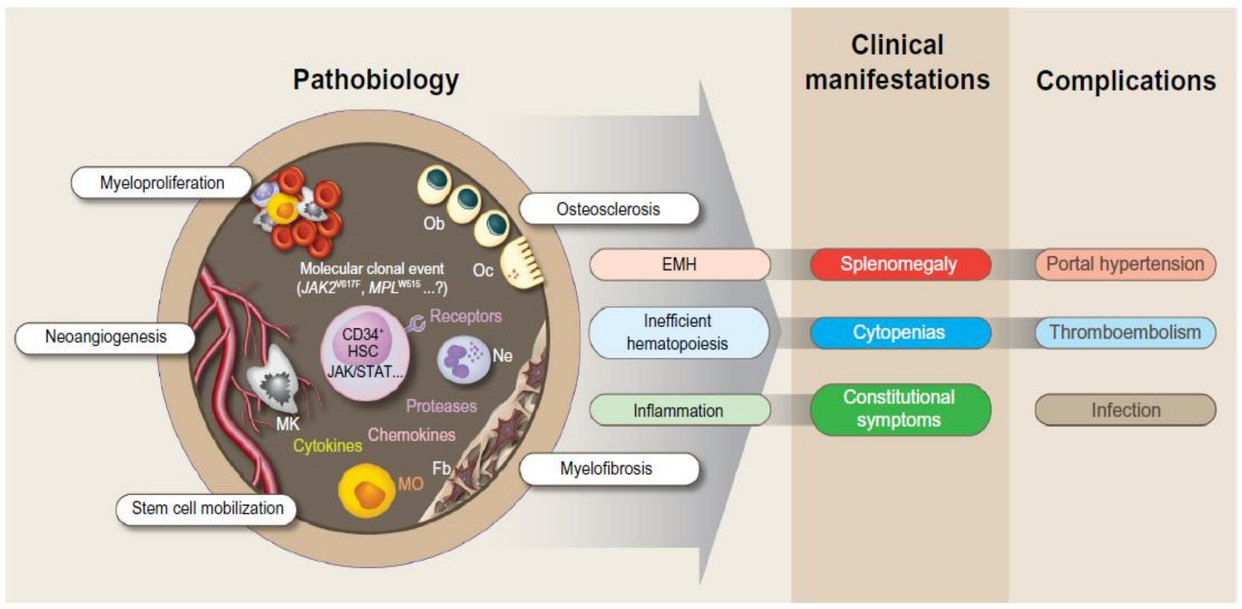
INTRODUCTION & AIM

Myelofibrosis (MF) is a myeloproliferative neoplasm, characterized by clonal myeloproliferation, bone marrow fibrosis, extramedullary hematopoiesis, and abnormal inflammation.

About 90% of MF patients carry mutations in the JAK2, CALR, or MPL genes. Among these, JAK2 mutations promote cytokine independence, constitutive activation of STAT proteins, and increased reactive oxygen species (ROS) production within hematopoietic stem cells (1).

Excessive ROS and inflammation are potential triggers for thrombotic events, a leading cause of morbidity and mortality in myeloproliferative neoplasm patients (1).



To explore mechanisms of inflammation-induced thrombosis in MF, we analyzed oxidation-induced fibrinogen alterations in MF patients compared to healthy controls and the effects of Ruxolitinib treatment, a first-in-class JAK inhibitor.

METHODS

We performed a single-center retrospective study in samples from MF at diagnosis (n=15) and during Ruxolitinib treatment in respect with of age-matched healthy control subjects (n=15).

Plasma redox status and the structural and functional fibrinogen alterations were assessed in both MF patients (before and after Ruxolitinib treatment) and controls.

Fibrinogen was purified using the previously described ethanol precipitation method (2). ROS-induced fibrinogen oxidation was assessed by measuring dityrosine content and intrinsic fibrinogen fluorescent spectra. The fibrinogen secondary structure was assessed via circular dichroism (CD) spectra (2).

Fibrinogen functional analysis included the assessment of thrombin- catalysed fibrin polymerization kinetics, and fibrin susceptibility to plasmin-induced lysis (3).

Patients characteristics evaluated at baseline

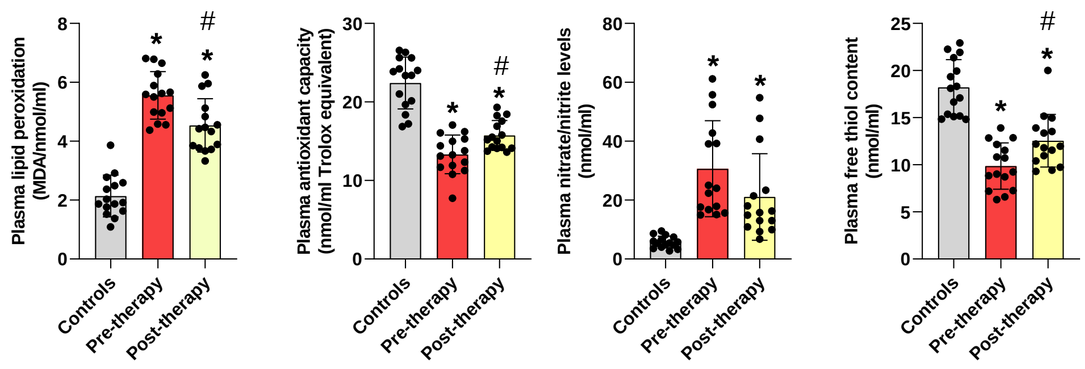
Age (years)	
Median [range]	67 [51-83]
Sex, n (%)	
Female	6 (40)
Male	9 (60)
Type of MF, n (%)	
Primary MF	11 (73.4)
PPV-MF	2 (13.3)
PET-MF	2 (13.3)
Constitutional symptoms, n (%)	
No	2 (13.3)
Yes	13 (86.7)
Splenomegaly, n (%)	
No	3 (20)
Yes	12 (80)
IPSS, n (%)	
Intermediate-1	5 (33.4)
Intermediate-2	5 (33.3)
High	5 (33.3)
Fibrosis grade, n (%)	
1	9 (60)
2	5 (33.3)
3	1 (7.7)
Driver mutations, n (%)	
JAK2	12 (80)
MPL	0 (0)
CALR	2 (13.3)
Triple negative	1 (6.7)
Blood count, median (range)	
Hb, g/dL	10.2 [7-13.6]
WBC, 10^3/mmc	12.1 [6.5-149]
PLT, 10^3/mmc	249 [102-848]

PPV: post-polycythemia vera; PET: post-essential thrombocythemia; IPSS: international prognostic system score.

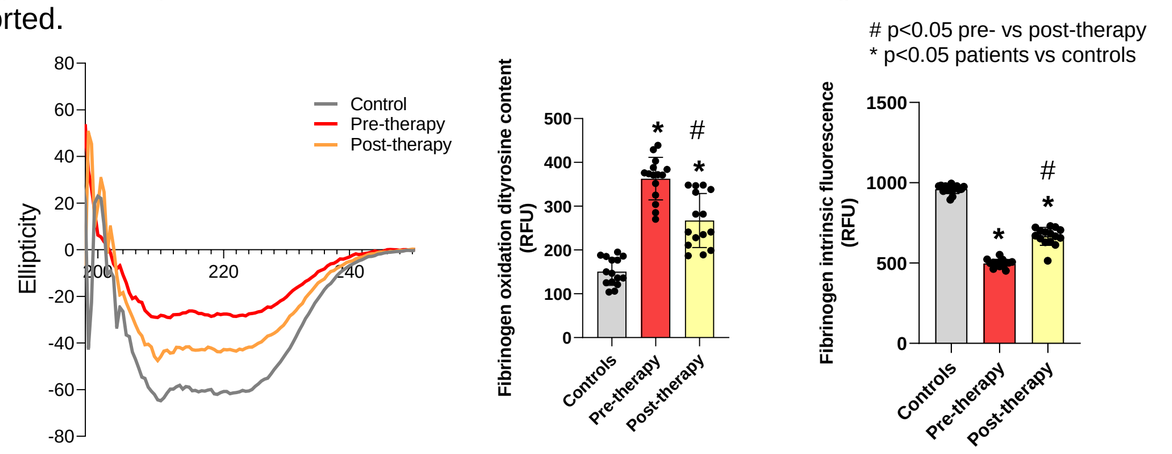
RESULTS & DISCUSSION

MF patients displayed a significant increase in plasma lipid peroxidation associated with a reduced total antioxidant capacity and free thiol plasma content.

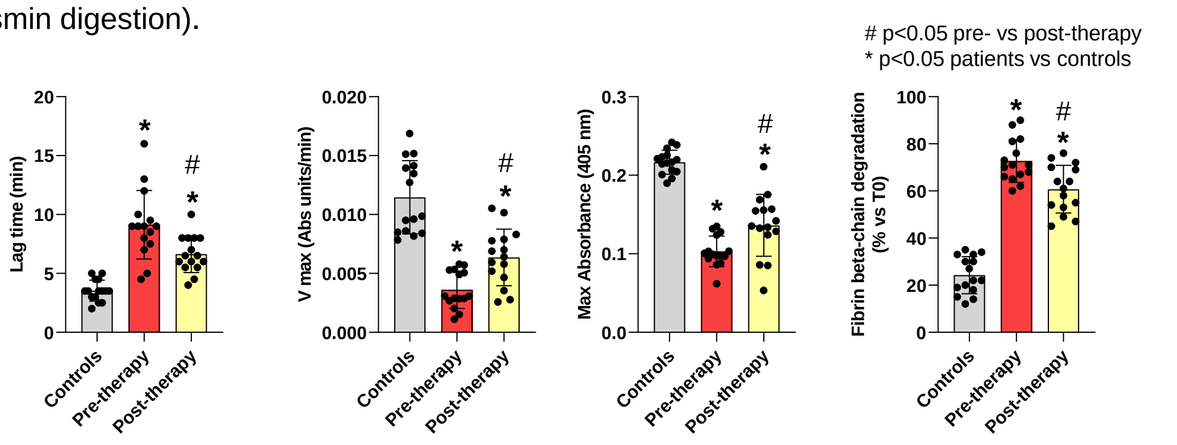
# p<0.05 pre- vs post-therapy
* p<0.05 patients vs controls



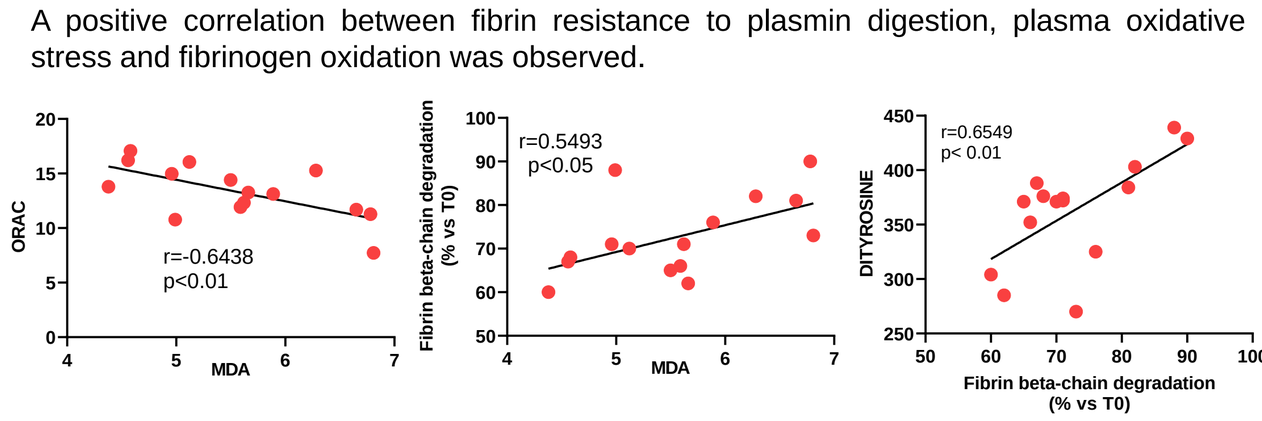
Plasma oxidative imbalance was associated to fibrinogen oxidation, resulting in structural changes. Representative circular dichroism spectra of fibrinogen and fibrinogen oxidation analysis via dityrosine content assessment and intrinsic fibrinogen fluorescence were reported.



Structural changes impaired fibrinogen polymerization into fibrin (Lag time, Vmax and Max Abs of thrombin-catalysed fibrin polymerization curves were reported) and reduced fibrin susceptibility to plasmin-induced lysis (quantification of residual fibrin β chain after 6 h of plasmin digestion).



A positive correlation between fibrin resistance to plasmin digestion, plasma oxidative stress and fibrinogen oxidation was observed.



CONCLUSIONS

- MF patients exhibit a prothrombotic profile sustained by oxidative modifications of fibrinogen.
- Ruxolitinib treatment is able to restore redox homeostasis, with a potential effect in cardiovascular prevention in MF patients.

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