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ELEVENTH ANNUAL MEETING

Interferon Treatment Suppresses in vitro NETosis in Neutrophils Derived from Patients with Myeloproliferative Neoplasms

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INTRODUCTION

Interferon (IFN) & Myeloproliferative neoplasm (MPN):

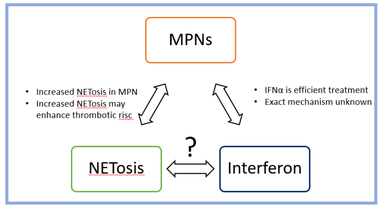
- IFN is an efficient cytoreductive agent for treating patient with polycythemia vera (PV) and essential thrombocytosis (ET).
- It reduces erythrocytosis, thrombocytosis, spleen size and the rate of thrombotic and hemorrhagic complications.

NETosis & MPN:

- Several studies have demonstrated increased neutrophil extracellular traps (NETs) formation among patients with MPN.
- NETs formation (NETosis) might contribute to the thrombotic tendency in MPN.

AIM

To evaluate the influence of IFN on NETosis of neutrophils derived from patients with MPN.

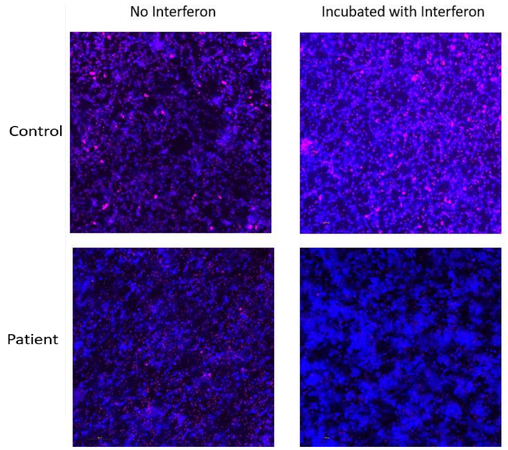


METHODS

- **Study group**: patients with MPN at a tertiary medical center who were not currently treated with cytoreductive therapy.
- **Control group**: Age and sex-matched controls.
- **Laboratory methods**: Neutrophils were harvested from blood samples and were divided to plates that were incubated with IFN and to plates that were not exposed to IFN.
  NETosis was assessed by measurement of neutrophil elastase activity in all samples and by immunofluorescence staining.
- Our results are being validated by cell death ELISA kit (Roche)

RESULTS

- 12 patients (7 men and 5 women) with MPN were included in our cohort. MPN was PV in 6 patients, ET in 5 patients and unclassified in 1 patient.
- Among all 12 samples, the level of NET-bound neutrophil elastase activity was significantly lower in samples that were incubated with interferon in comparison to samples that were not incubated with interferon *(16.2mU/mL (±16.53) vs 19.8 mU/mL (±17.3), respectively (p=0.002))* .
- Among neutrophils derived from controls, the level of NET-bound neutrophil elastase activity did not differ in samples that were incubated with interferon in comparison to samples that were not incubated with interferon.



Representative immunofluorescence images of human neutrophils derived from controls or MPN patients, unexposed or exposed to interferon. DAPI stain (4',6-diamidino-2-phenylindole) is shown in blue and MPO stain (anti myeloperoxidase antibodies) in red. Scale bar=100 µm.

Age (years), Sex (M/F)	MPN type	JAK2 v617f status	NE activity, without IFN (mU/mL)	NE activity with IFN (mU/mL)
55, M	PV	mutant	8.26	5.44
44, M	PV	mutant	4.26	2.45
43, F	uncMPN	mutant	8.71	7.29
62, F	ET	WT	3.59	2.61
42, M	ET	mutant	5.57	5.47
56, F	PV	mutant	3.85	2.59
35, M	PV	mutant	35.85	30.76
60, M	PV	mutant	35.21	31.68
43, M	ET	mutant	54.09	51.91

'Δ in NE activity with IFN vs no IFN' was calculated by dividing the difference between NE activity with and without IFN by the NE activity without IFN.

Table abbreviations: ET=essential thrombocytosis; F=female; IFN=interferon; M=male; NE=neutrophil elastase; PV= Polycythemia Vera; WT=Wild type

CONCLUSIONS

In-vitro **exposure to interferon inhibits NETosis** in neutrophils derived from patients with MPN. This inhibitory effect might be **disease specific**, as it was not demonstrated in neutrophils derived from controls. The influence of interferon on NETosis might **play a role in the therapeutic mechanism of interferon** in these diseases.

REFERENCES

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