XIX CONGRESSO NAZIONALE SIES 2026

Investigating Neutrophil Extracellular Traps as Thromboinflammation Markers in Myeloproliferative Neoplasms

Elena Torre

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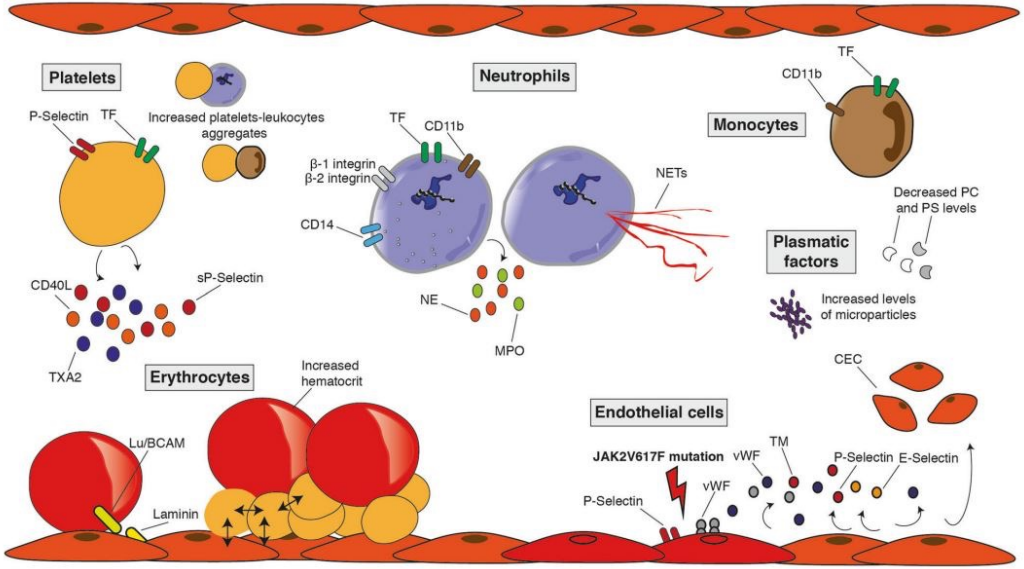
Palazzo degli Affari

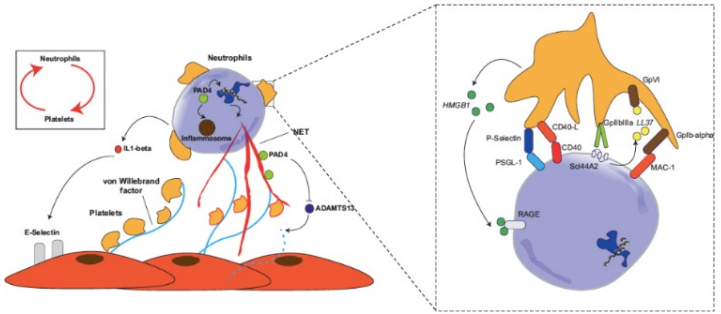
I have no conflict of interest to disclose

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Thrombosis is the main cause of morbidity and mortality in MPN patients

- Neutrophil Extracellular Traps (NETs) play a pivotal role in **immunothrombosis** in MPN.
- Recent studies show increased NETosis in JAK2V617F-mutated neutrophils and in mouse models, highlighting their role in thrombogenesis.





Guy, A. et al., Leukemia 35, 935–955 (2021)

Early Evidence: NETosis and Thrombosis in MPN

Study	Cohort and model	Key findings	Other findings
Oyarzun, 2016	66 MPN patients/52 HDNs + ex vivo	↑ NET formation vs HDNs; highest in MF	Lower NETs in CALR vs JAK2-mutated
Wolach, 2018	19 MPN patients/11 HDNs + JAK2 mice	NETosis enhanced; PAD4-dependent thrombosis	Ruxolitinib reduced NETs and thrombosis in mice
Guy, 2019	52 MPN patients with thrombosis/54 HDNs	↑ MPO-DNA complexes; higher in thrombotic patients	NETs linked to thrombosis history
Schmidt, 2021	102 MPN patients/28 HDNs	↑ NET levels in MPNs vs HDNs; association with JAK2 and CALR	No clear ruxolitinib effect on induced NETosis, no link with thrombosis

Early studies demonstrate ↑NETosis in MPN. Heterogeneous findings regarding its association with thrombosis.

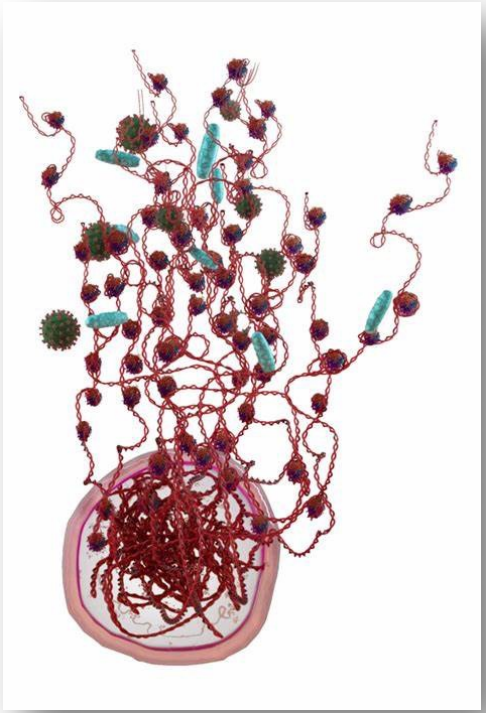
NETs as Biomarkers and Therapeutic Targets in MPN

Study	Cohort and model	Key findings	Other findings
Massarenti, 2023	128 HU vs IFN-treated PV patients	NETs correlate with JAK2V617F allele burden and NLR	IFN reduced NETs more than HU
Wolach, 2024	39 MPN (ET+PV) patients + HDNs	IFN-α suppresses NET formation in HDNs	Direct immunomodulatory effect
Guy, 2024	Murine	↑NETosis and spontaneous thrombosis in mice expressing JAK2V617F in all HSC lineages.	Aspirin reduced NETosis and thrombosis in the murine model
Guy, 2025	394 (ET+PV) therapy-naïve patients	NET markers associated with thromboinflammatory markers and conventional risk score	Aspirin associated with lower H3Cit

Recent studies suggest that NET levels reflect clonal burden and may be modulated by IFN and ASA.

How to detect NETs?

- NETs detection and quantification have not yet been standardised
- ISTH SCC project ongoing for comparing the currently available assays for measuring NETs in plasma samples
- **Nu.Q® NETs** is currently the only CE-marked automated chemiluminescence immunoassay for NET quantification available for clinical use in Europe.

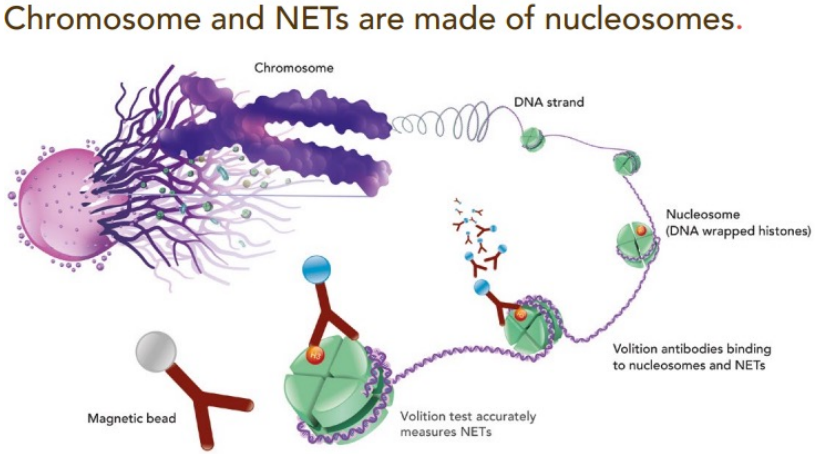


CE-IVDD, Belgian Volition SRL, Isnes, Belgium

Martinod K, ISTH, Vascular Biology SSC Subcommittee Project, March 2019
Bonifay A, et al. J Thromb Haemost. 2024 Oct;22(10):2910-2921

Project Proposal

1. Detect circulating H3.1 nucleosomes as a surrogate of NETs using the [ ] **NETs assay**
2. Evaluate the effect of cytoreductive therapy
3. Explore correlations with clinical and biological parameters
4. Assess potential implications for risk stratification.



The project aims to deepen our **understanding of NETs in MPN**

Study Design

Monocentric, prospective study

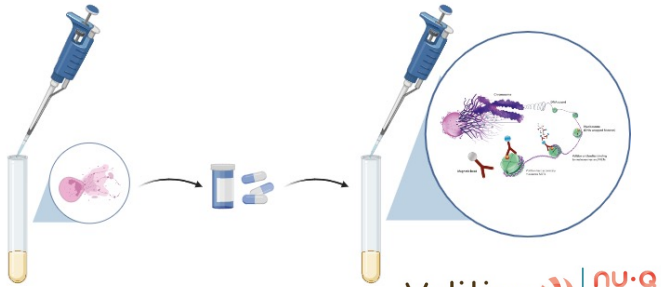
NHS
Guy's and St Thomas'
NHS Foundation Trust

KING'S HEALTH PARTNERS
CANCER BIOBANK
KING'S College LONDON
APPROVED

MPN Clinic

Pre-treatment

Post-treatment



Volition | nu·a nets

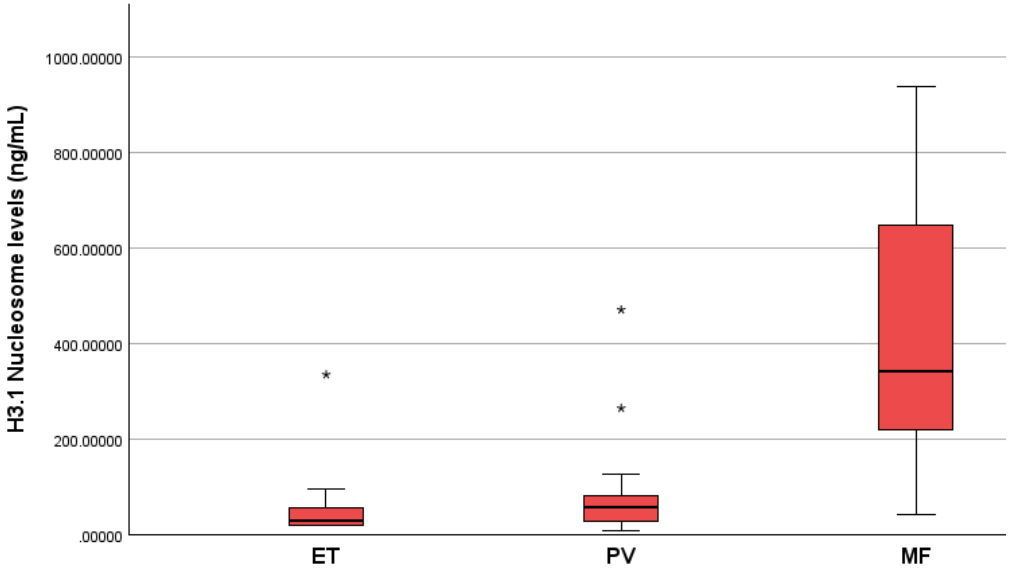
Patients' cohort

45 patients
(23 female, 22 male)

Age at pre-treatment sampling (years), median (range)	59 (21-79)
MPN subtype, n (%)	
- PV	20 (44)
- MF	16 (36)
- ET	9 (20)
Driver gene mutations, n (%)	
- *JAK2V617F*	36 (80)
- *JAK2V617F* allele burden (%), median (range)	26 (7.4-87)
- *CALR*	6 (13)
- *MPLW515L*	2 (5)
- triple-negative	1 (2)
Palpable splenomegaly, n (%)	17 (38)
Prior history of thrombosis*, n (%)	10 (22)
Hb (g/dL), median (range)	13.1 (7.9-17.7)
WBC ($x10^9$/L), median (range)	9.7 (5-43)
PLT ($x10^9$/L), median (range)	444 (23-1378)
ANC ($x10^9$/L), median (range)	7 (3-33)
NLR ratio, median (range)	3.8 (1.3-46)
LDH (IU/L), median (range)	307 (142-194)

*Before a median time of 3 years before the baseline sample

Baseline H3.1 nucleosome levels (NETs) varied across MPN subtypes (p<0.001)



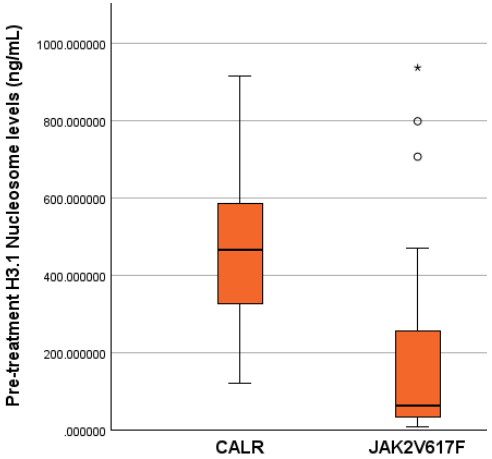
	Pre-treatment
H3.1 Nucleosome levels median (ng/mL), (IQR)	67 (35-320)

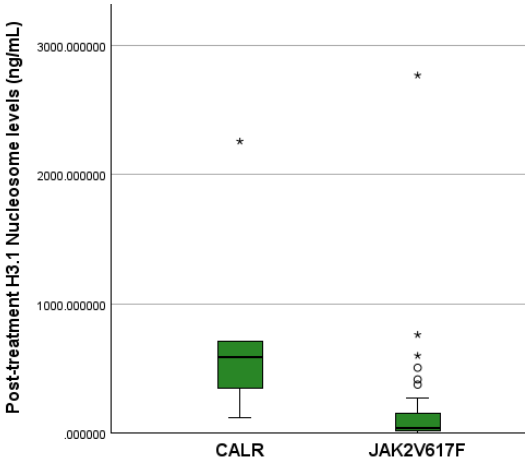
MF patients had a higher level of H3.1 nucleosomes compared to ET (p<0.001) and to PV (p<0.001).

	N of patients	Pre-treatment NETs (ng/mL) median (IQR)
MPN Subtype		
PV	20	58 (23-88)
MF	16	341 (202-676)
ET	9	30 (19-76)
p-value		p<0.001

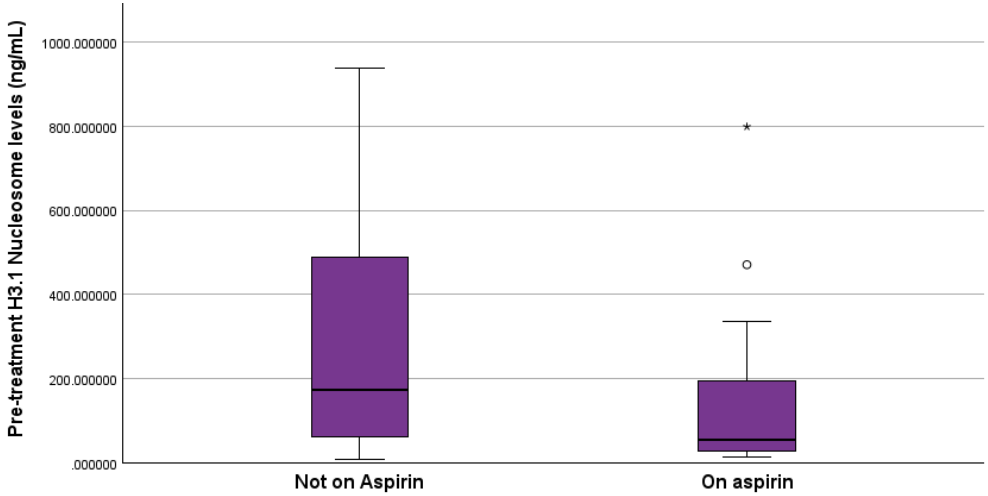
Differences in NET levels in pre- and post-treatment samples

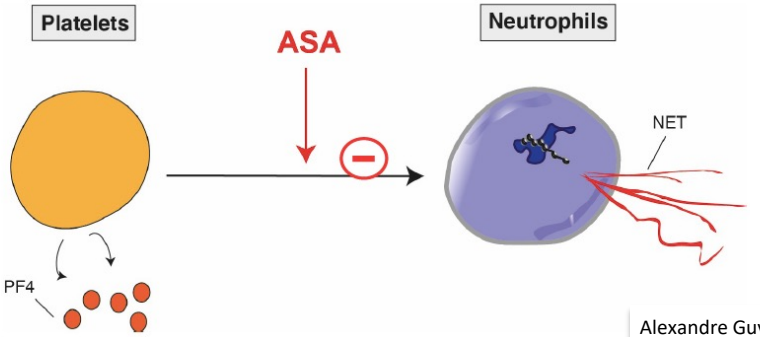
	N of patients	Pre-treatment NETs (ng/mL) median (IQR)	Post-treatment NETs (ng/mL) median (IQR)
Gender			
Male	22	121 (35-357)	122 (20-480)
Female	23	61 (35-317)	43 (21-119)
p-value		p=0.496	p=0.454
MPN Subtype			
PV	20	58 (23-88)	22 (18-52)
MF	16	341 (202-676)	447 (198-710)
ET	9	30 (19-76)	32 (20-51)
p-value		**p<0.001**	**p<0.001**
Driver mutation			
JAK2V617F	36	62 (31-261)	39 (20-153)
CALR	6	465 (275-669)	590 (291-1100)
MPL	2	89 (18-89)	85 (70-85)
p-value		**p=0.036**	**p=0.015**
Palpable splenomegaly			
Yes	17	292 (53-475)	268 (104-708)
No	38	46 (21-114)	38 (19-66)
p-value		**p<0.001**	**p<0.001**
Previous thrombosis			
Yes	10	64 (59-353)	54 (20-384)
No	35	94 (26-326)	53 (21-268)
p-value		p=0.429	p=0.913





Impact of antiaggregant therapy on NETs in pre-treatment samples





Alexandre Guy

	N of patients	Pre-treatment NETs (ng/mL) median (IQR)	Post-treatment NETs (ng/mL) median (IQR)
Aspirin treatment			
Yes	27	56 (26-264)	32 (19-75)
No	18	173 (60-513)	153 (52-627)
p-value		p=0.035	p=0.004

- 27 patients on aspirin had significantly lower baseline H3.1 nucleosome levels than the other patients (p=0.035).

No significant differences in H3.1 nucleosomes were observed between those with and without a history of thrombosis (p=0.429).

NET levels reflect disease burden

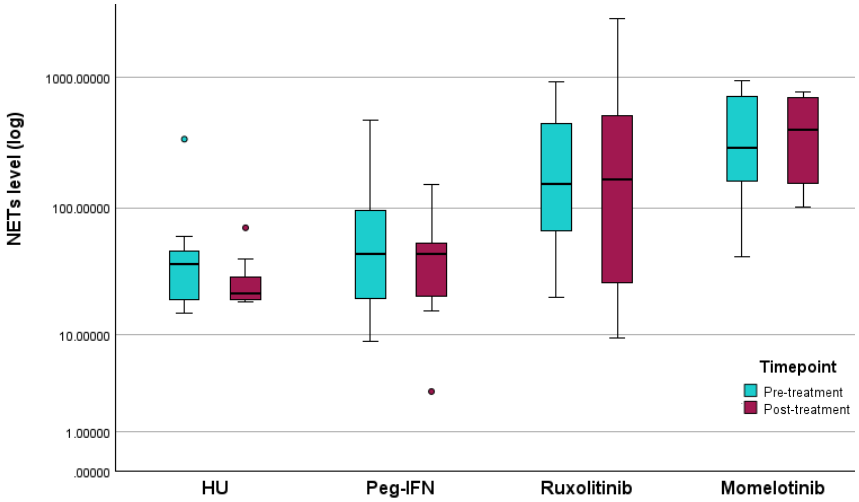
- Higher in leucocytosis (WBC ≥11×10^9/L)
- Higher in elevated NLR (≥4.5)
- No association with platelet count

	N of patients	Post-treatment NETs (ng/mL) median (IQR)
WBC (x10^9/L)		
<11	35	36.1 (20-75)
≥11	10	492.3 (347-705)
p-value		**p<0.001**
NLR		
<4.5	30	37 (20-106)
≥4.5	12	211 (56-676)
p-value		**p=0.013**
PLT (x10^9/L)		
<450	34	39.7 (20-384)
≥450	11	70 (48-150)
p-value		p=0.731

Baseline	r-value	p-value
RBC	−0.493	<.001
WBC	0.421	0.004
ANC	0.363	0.016
JAK2VAF	0.511	0.026
LDH	0.725	<.001
Post-treatment		
RBC	−0.636	<.001
WBC	0.649	<.001
ANC	0.571	<.001
JAK2VAF	0.723	0.003
LDH	0.712	<.001

No significant linear correlation was observed between NETs and continuous NLR values.

Impact of Cytoreduction on NET Levels



	Pre-treatment	Post-treatment	p-value
H3.1 Nucleosome levels median (ng/mL), (IQR)	67 (35-320)	53 (20-308)	0.141

- No difference across treatment types (p=0.562).
- However, 60% showed a reduction in NET levels following cytoreductive therapy.

	HU	Peg-IFN	Ruxolitinib	Momelotinib	Total
N of pts	11	10	18	6	45
Reduction, n (%)	6 (55%)	6 (60%)	11 (61%)	4 (67%)	27 (60%)
Median % reduction (IQR) *	−53 (−69 to −42)	−46 (−65 to −11)	−44 (−64 to −38)	−38 (−43 to −28)	

*Calculated among responders

Limitations

- Small sample size
- Biologically heterogeneous MPN population
- Use of frozen samples may have affected assay sensitivity and specificity
- Follow-up samples not collected at standardised timepoints
- Limited follow-up to evaluate incident clinical events, including thrombosis.

Conclusions and Future Perspectives

- ✓ H3.1 nucleosome levels differ across MPN subtypes
- ✓ Highest levels observed in MF
- ✓ NET levels correlate with markers of disease burden (leucocytosis, LDH, splenomegaly, JAK2V617F allele burden)
- ✓ Cytoreductive therapy did not significantly reduce NET levels in this cohort
- ✓ Prior antiplatelet therapy associated with lower NET levels
- ✓ First study evaluating the impact of JAK inhibitors on NET levels in paired pre- and post-treatment samples.
- ➢ NETs represent a promising biomarker of thromboinflammation in MPN and warrant further longitudinal investigation, with a potential role in risk stratification.

Acknowledgements

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ASSOCIAZIONE ITALIANA CONTRO LEUCEMIE LINFOMI E MIELOMA

Società Italiana Ematologia Sperimentale SIES

MPN Team
Claire Harrison
Shahram Kordasti
Natalia Curto-Garcia
Jennifer O'Sullivan
Priya Sriskandarajah
Maria Georgiou
Patrick Harrington
Hugues de Lavallade
Benissa Narciso
Susan Asirvatham
Claire Woodley

Elena Torre
e.torre@nhs.net