Bologna, 21 Aprile 2026

Dott.ssa
Evita Massari

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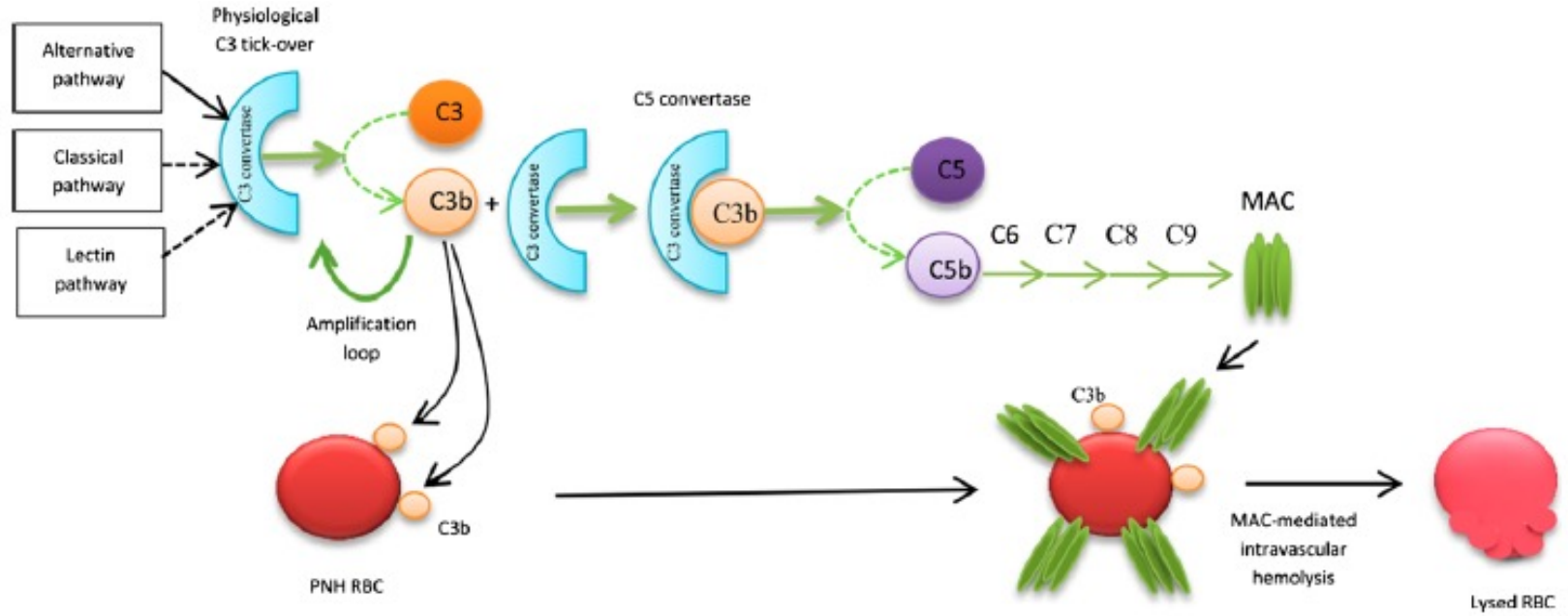
EPN
Diagnosi di laboratorio

NESSUN CONFLITTO D’INTERESSI

EPN

- disordine raro ed acquisito a carico della cellula staminale ematopoietica pluripotente
- mutazione somatica del gene PIG-A
- incapacità di sintetizzare l'àncora glicofosfatidilinositolo
- perdita di due importanti proteine regolatorie del complemento: DAF (CD55) e MIRL (CD59)
- RBC più vulnerabili all'azione del complemento
- emolisi intravascolare complemento mediata



Devalet et al. EJH 2015; 95: 190-198

EMOGLOBINURIA

DOLORE ADDOMINALE

DISTONIA

SPOSSATEZZA

TROMBOSI

INSUFFICIENZA MIDOLLARE

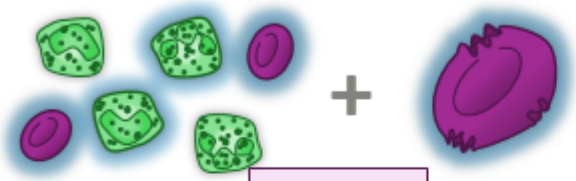
Meccanismi espansione cloni EPN

- Espansione clonale di cellule PIG-A mutate e sopravvivenza ad attacco T citotossico (AA)
- Ematopoiesi clonale (MDS)
- Mutazioni rare (deficit congenito CD59, mutazioni PIG-T)
- Altre mutazioni (es. JAK2 o CALR, più spesso associate a MPN)

- *Devalet B et al. EJH 2015; 95: 190-198*
- *Rotoli B, Luzzatto L. Semin Hematol 1989; 26(3):201-7*
- *Savage et al. Exp Hematol 2009 37:42-51.e1*
- *Richards SJ et al. Br J Haematol 2020, 189, 954-966*
- *Brodsky RA. Blood 2014; 124(18):2804-2811*
- *D'Addio et al. Ann Hematol 2025 Sep;104(9):4487-4494*

Classificazione IPIG PNH

Classic PNH



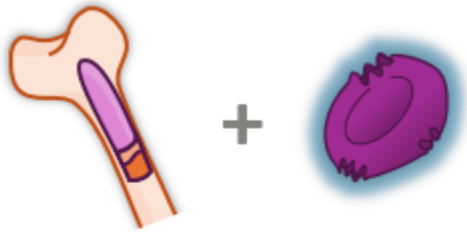
>50%

Characterised by intravascular haemolysis, with no evidence of another BMF syndrome[1]

Patients often have a **large PNH clone (>50%)** leading to **haemolysis**[2]

Treatment with anti-complement therapies[1,3-5]

PNH in the context of another bone marrow failure (BMF) syndrome



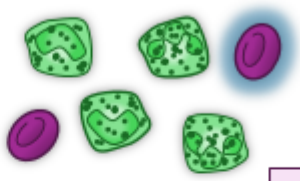
Characterised by evidence of haemolysis and presence of a current or previous BMF syndrome[1]

The **BMF syndrome is the primary factor** contributing to the symptoms of the disease[3]

Treatment is focused on **addressing the underlying BMF syndrome**[1,4,5]

Patients with **large PNH clones** may benefit from **anti-complement therapies**[4,5]

Subclinical PNH



<10%

Characterised by the presence of a **few PNH clones (<10%)**, and **no evidence of haemolysis or thrombosis**[1-3]

Yearly monitoring is recommended[1,4,5]

BMF, bone marrow failure; IPIG, International PNH Interest Group; PNH, paroxysmal nocturnal haemoglobinuria.

1. Parker et al. *Blood* 2005 **2.** Hill et al. *Nat Rev Dis Primers* 2017 **3.** Brodsky *Blood* 2014 **4.** Parker *Hematology Am Soc Hematol Educ Program* 2016 **5.** Sahin et al. *Am J Blood Res* 2015.

Monitoraggio clone EPN nelle citopenie

PAZIENTI CON AA SENZA EVIDENZA DI CLONE EPN
Ogni 6 mesi per i primi 2 anni, poi annualmente se non emergono segni o sintomi

PAZIENTI CON AA CON EVIDENZA DI CLONE EPN
Ogni 3 mesi fino a stabilizzazione per i primi 2 anni, poi meno frequentemente

PAZIENTI CON MDS
Generalmente non presentano un clone in evoluzione

- *Dezern AE, Borowitz MJ Cytometry B Clin Cytom. 2017 Dec 13*
- *Parker C et al. International PNH Interest Group. Blood 2005: 106(12):369*
- *Killick et al. British Journal of Hematology 2016; 172(2):187-207*

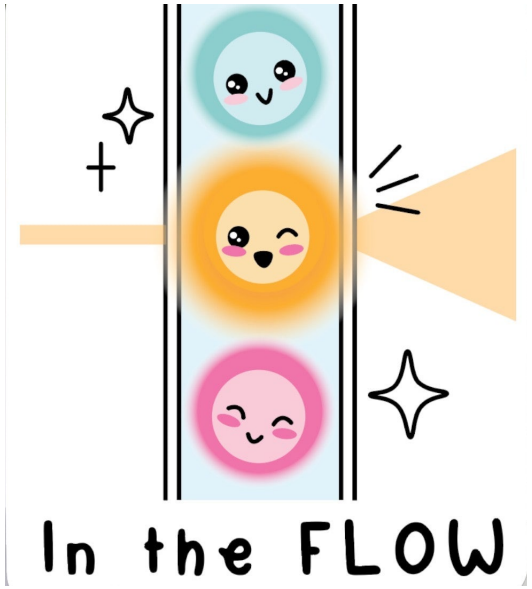
Citofluorimetria: il gold standard diagnostico

- Only **peripheral blood should be analyzed for PNH clones**

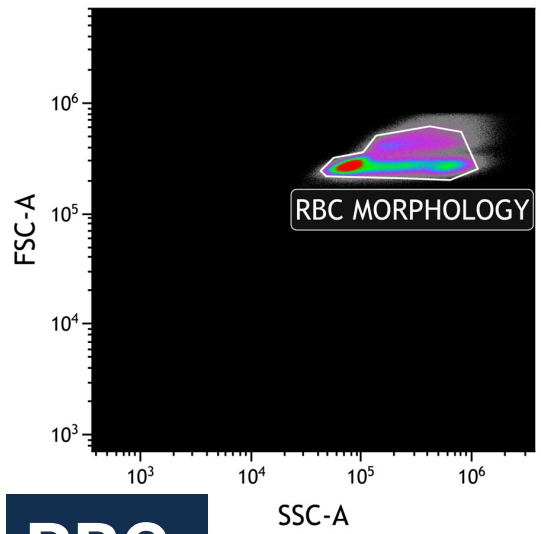
- **A screening test can be made according to the following 'Reasons for Testing':**
  - **DAT-, Hemolytic Anemia, with ↑LDH, ↓Aptoglobin, ↑ Reticulocytes**
  - Unilinear, bilinear or trilinear refractory cytopenias
  - Low-grade Myelodysplastic Syndromes, Aplastic Anemia
  - Unusual thromboses, especially if associated with hemolysis or cytopenias

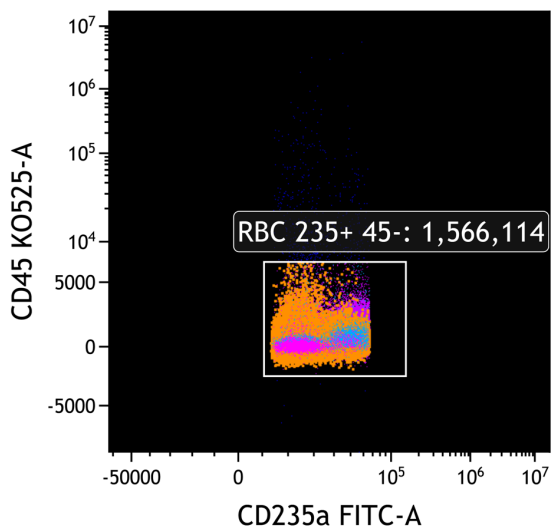
- **DIAGNOSIS:** 6-color assay including Granulocytes, Monocytes and Red Cells
  - deficiency of at least **TWO different GPI-Linked molecules on (possibly) two different cell populations**
  - Lymphocytes have no role

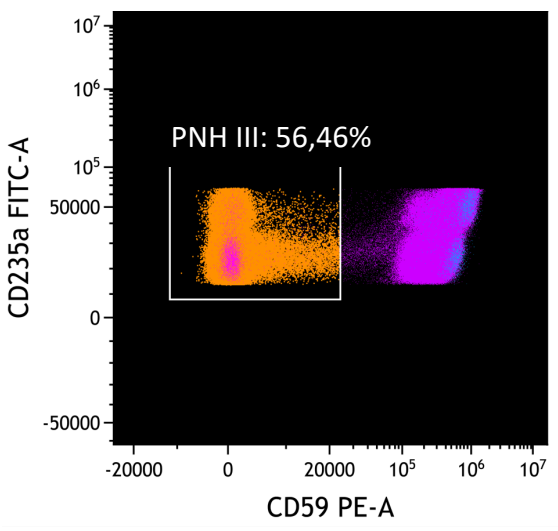
- **ARTIFACTS:** platelets, blasts, basophils, dendritic cells, NK, dead cells



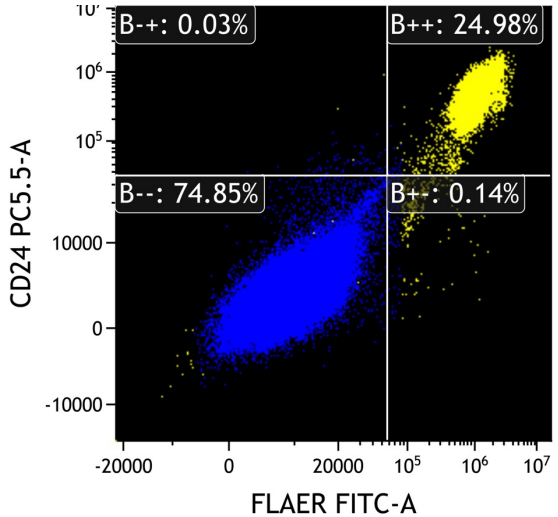
ICCS/ESCCA Consensus Guidelines. Cytometry Part B (Clinical Cytometry) 94B:16-81 (2018)

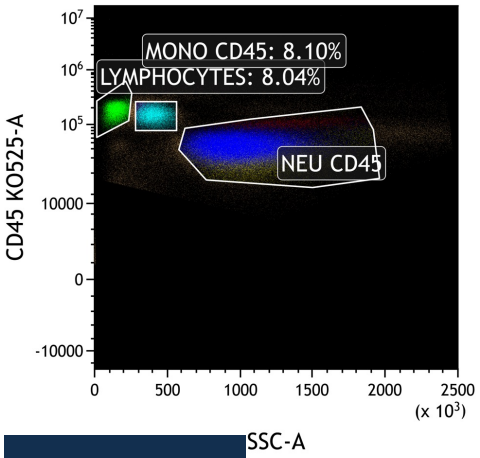

RBC MORPHOLOGY
FSC-A
SSC-A

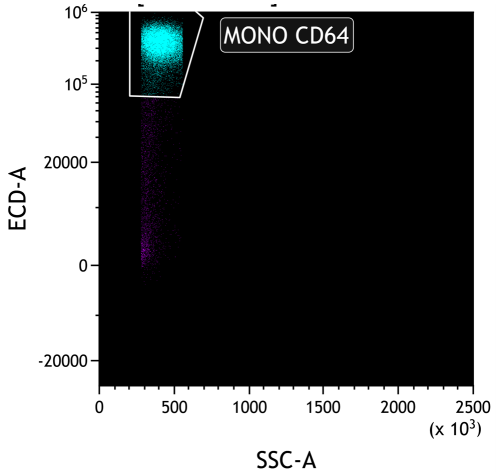

RBC 235+ 45-: 1,566,114
CD45 KO525-A
CD235a FITC-A

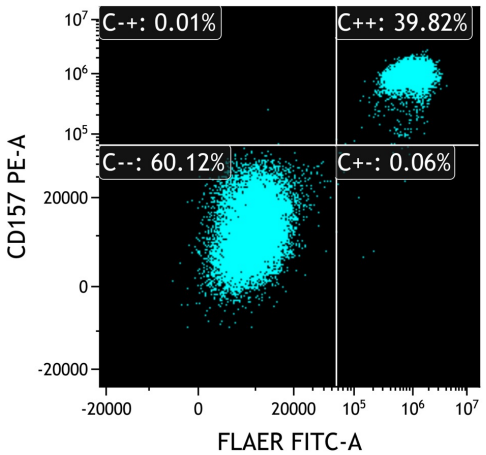

PNH III: 56,46%
CD235a FITC-A
CD59 PE-A

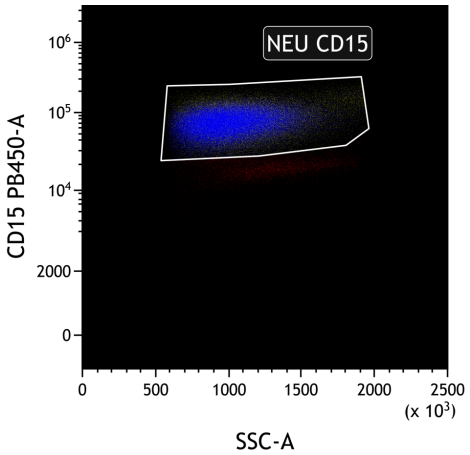
RBC

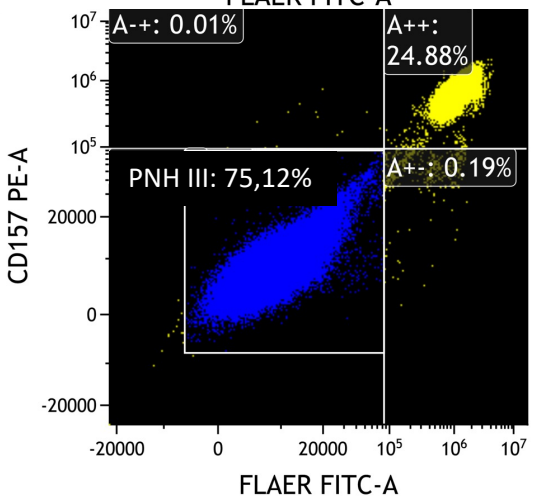

B-+: 0.03%
B++: 24.98%
B--: 74.85%
B+-: 0.14%
CD24 PC5.5-A
FLAER FITC-A


MONO CD45: 8.10%
LYMPHOCYTES: 8.04%
NEU CD45
CD45 KO525-A
SSC-A


MONO CD64
ECD-A
SSC-A


C-+: 0.01%
C++: 39.82%
C--: 60.12%
C+-: 0.06%
CD157 PE-A
FLAER FITC-A


NEU CD15
CD15 PB450-A
SSC-A

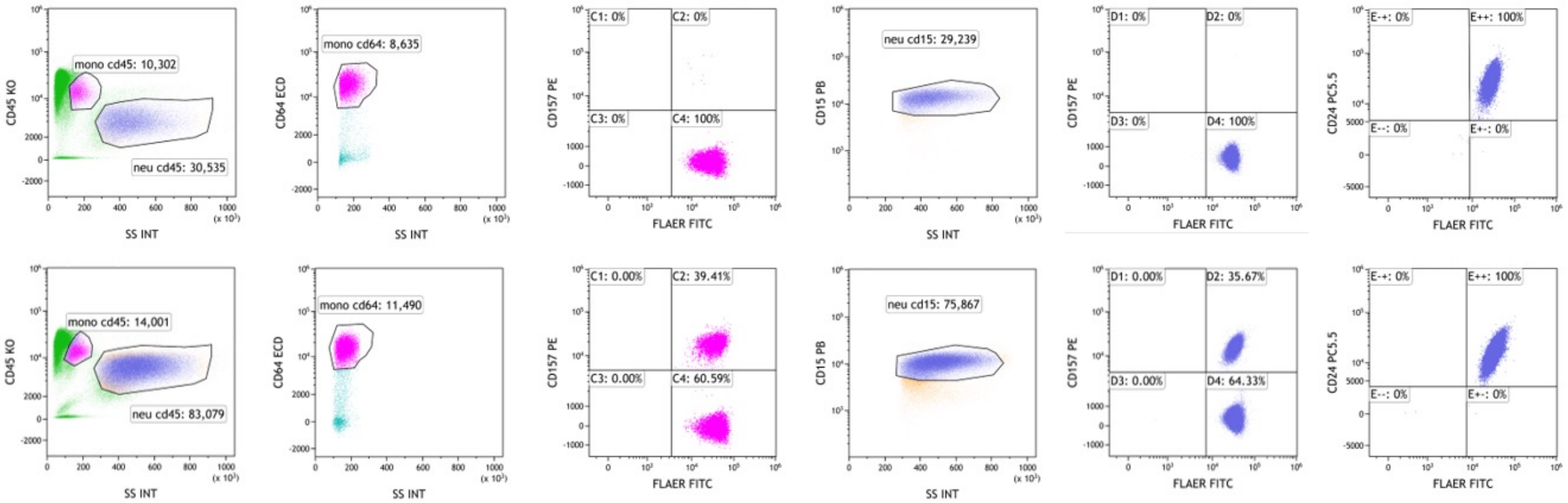

A-+: 0.01%
A++: 24.88%
PNH III: 75,12%
A+-: 0.19%
CD157 PE-A
FLAER FITC-A

WBC

Don’t always trust CD157 expression: the importance of combination with FLAER for diagnosis of PNH

E. Massari[1], M. Monti[1], G. Poletti[1], M. Rosetti[1], M. Monterosso[1], C. Casadei[1], A. Clementoni[1], V. Polli[1], M. Olivieri[1], V. Libri[1], T. Fasano[1]

1 Clinical Pathology Unit, Hub Laboratory, AUSL della Romagna, Cesena, Italy



C3d: determinazione e strategia di gating

- frammenti complemento C3d, prodotti di splicing del C3b si legano a RBC riconosciuti dai recettori del complemento sui fagociti -> fagocitosi

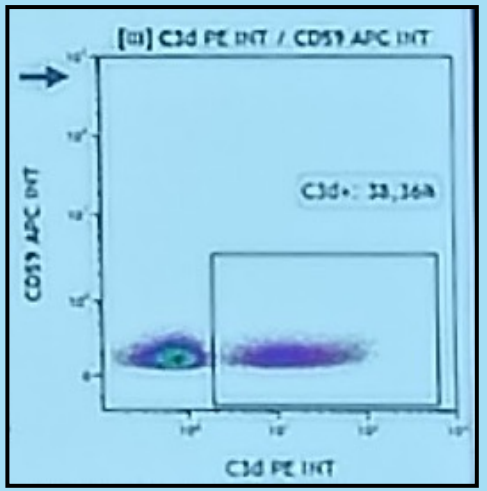
- determinazione C3d sugli RBC tramite FCM: valutazione emolisi extravascolare

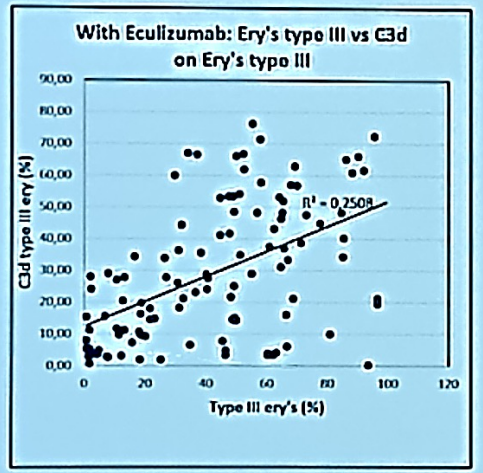
- scopo: follow-up emolisi extravascolare in pz EPN resistenti al trattamento

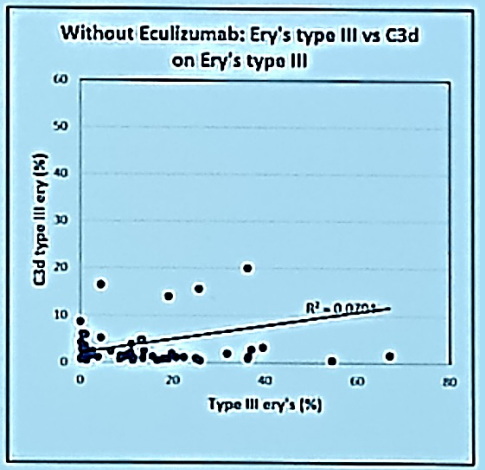
- I pazienti con campioni di espressione C3d >30% hanno mostrato un'emolisi extravascolare da moderata a grave. Le analisi di regressione tra C3d% e la conta assoluta dei reticolociti hanno mostrato un R2 di 0,56

espressione di C3d su RBC PNH, corrispondeva alla gravità della malattia ed era più elevata nei pz resistenti al trattamento e con emolisi extravascolare

Preijers F. ESCCA 2023







Conclusion: Untreated (without Eculizumab) C3d expression is very low.

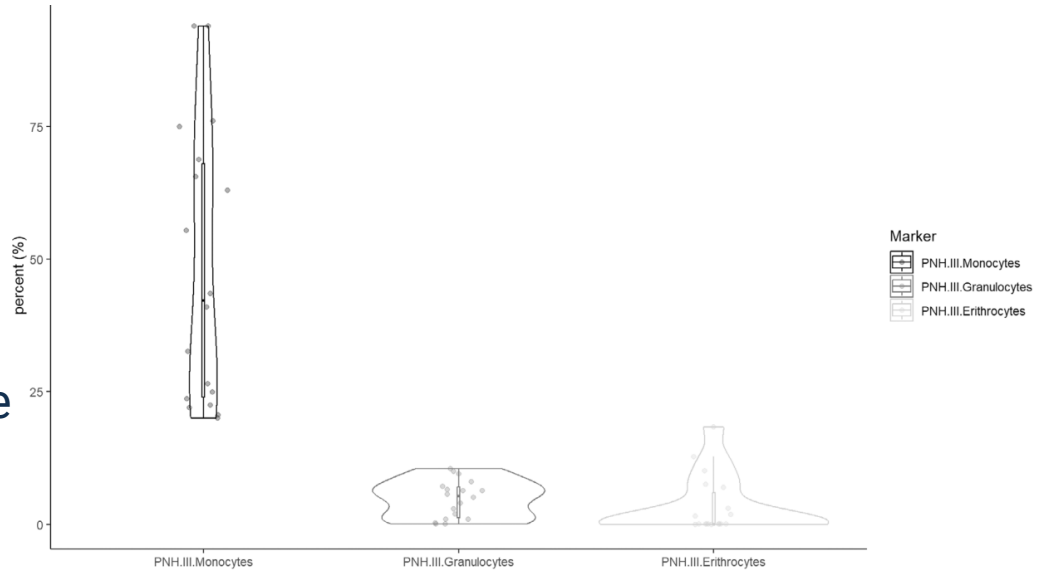
LETTER TO THE EDITOR

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

Description of 18 PNH patients with prevalent GPI-deficient monocytes. An Italian survey

F. Lanza[1]
E. Massari[2]
G. Marconi[1]
G. Poletti[2]
M. Krampera[3]
C. Lazzari[4]
G. Di Martino[4]
M. Raia[5]
G. Scalia[5]

- **18 patients** with **prevalent deficiency** of **GPI-linked molecules** on **monocytes**
- 720 Italian PNH patients
- both neutrophil granulocytes and erythrocytes almost normal
- **follow-up: no changes** in the expression of GPI deficient molecules in the three cell subpopulations selected
- first description of the so-called **'pure monocyte PNH'**
- **deficiency of GPI anchored proteins** in the examined patients occurred at a later stage of progenitor cell maturation, namely at the **monocyte differentiation phase**
- **NGS**: in addition to *PIG-A* mutations, several **additional genetic mutations** were detected, possibly suggesting a **stepwise clonal evolution** derived from a singular stem cell clone



Br J Haematol. 2025;206:1863–1867.

PNH clones prevalence study in ph-negative myeloproliferative neoplasms: a multicenter Italian study

Alessandra D'Addio[1] · Michela Rondoni[1] · Marzia Salvucci[1] · Giovanni Marconi[1] · Massimiliano Bonifacio[2] · Ilaria Tanasi[2] · Omar Perbellini[3] · Giuseppe Carli[3] · Patrizia Tosi[4] · Simona Tomassetti[4] · Giovanni Poletti[5] · Evita Massari[5] · Marco Rosetti[5] · Elisabetta Fabbri[6] · Chiara Zingaretti[6] · Alessandro Lucchesi[7] · Maria Teresa Bochicchio[8] · Giorgia Simonetti[8] · Mauro Krampera[2] · Francesco Lanza[1]

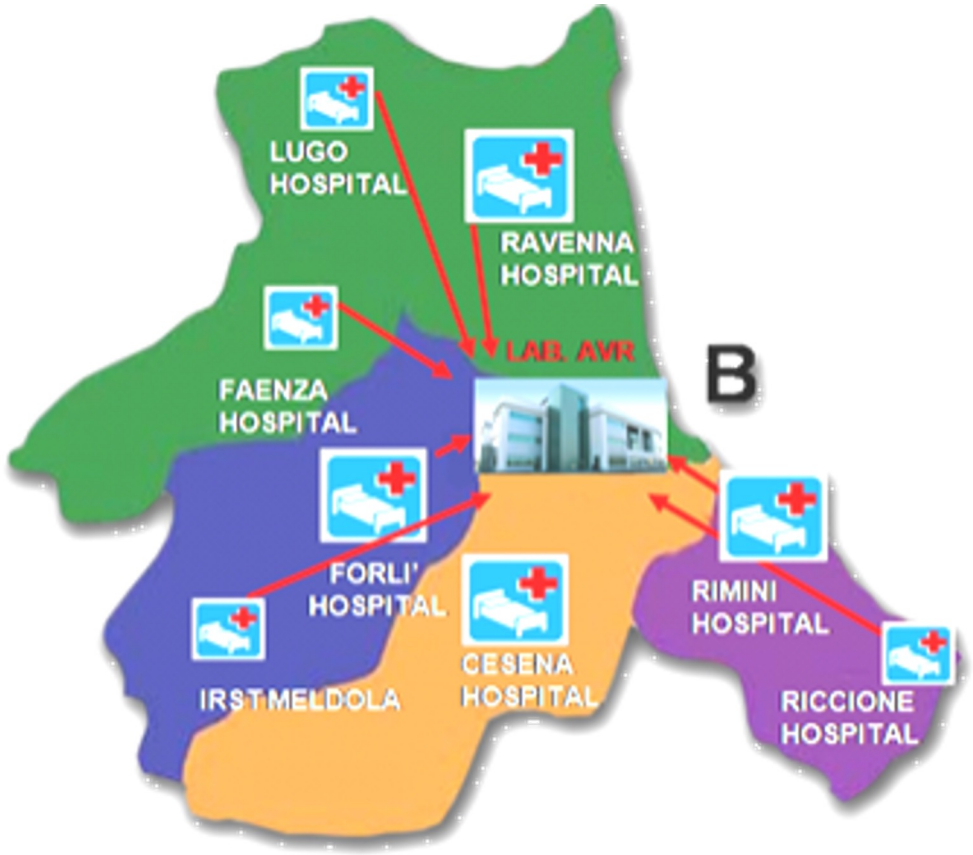
Ann. Hematol. 2025 Sep;104(9):4487-4494.

- 119 Ph- negative MPN patients having **anemia**, **LDH elevation**, **asthenia** and **history of thrombosis**
- standardized diagnostic test by using a **single lyophilized template**
- NGS was performed in 2 PNH-positive MPN cases and 13 PNH-negative MPN
- **Prevalence** of PNH positive clones was **3.23%** (n. 3 patients).
- all three patients had **splenomegaly**; none of them had thrombosis.
- 1 patient: ***CALR* mutated essential thrombocytopenia**, had a small clone (0.52%), clinically irrelevant
- 1 patient: ***JAK2*V617F primary myelofibrosis (PMF)** showed a PNH clone of 89.8%, severe anemia and hemoglobinuria and started eculizumab therapy
- 1 patient: ***CALR* mutated PMF** showed a PNH clone of 92.6% but without severe anemia and breakthrough hemolysis and eculizumab therapy was not undertaken
- ***PIGA* deletion** was detected in **PNH-positive cases** along with **mutations of myeloid-related genes**

Association of *CALR* mutation and *JAK2*V617F mutation with PNH positive clones?

Studio AVR-FLAER

“Progetto di studio di Area Vasta Romagna per lo screening di cloni EPN in pazienti con citopenia di diversa natura (idiopatica, in corso di anemia ipo-plastica o di mielodisplasia a basso grado IPSS), o associata a trombosi venosa idiopatica atipica o citopenia con segni di emolisi intravascolare: studio di validazione di un test rapido in citometria a flusso 3 colori”



Approvazione Comitato Etico 15/02/2017

Studio AVR-FLAER: criteri di inclusione

Diagnosi di:

- Anemia Aplastica oppure

- Mielodisplasia a basso rischio secondo IPSS oppure

- Citopenia Persistente da almeno 6 mesi valutata con biopsia ossea o aspirato midollare, definita come Hb < 12.5 g/L-uomo o < 11.5 g/L –donna **oppure** WBC < 4000/uL con PMN< 1000/uL oppure

- **Segni di emolisi**, definita come LDH> 1.5 x ULN **e/o** aptoglobina ridotta **e/o** reticolocitosi **E** test di COOMBS negativo, in assenza di altre patologie (congenite o acquisite) oppure

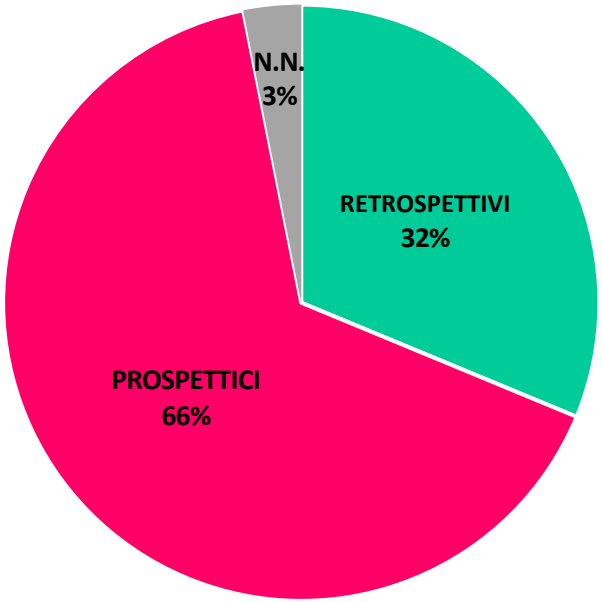
- Trombosi Venosa Idiopatica (confermata da uno dei seguenti esami strumentali: angio-TC, angio-RMN, angiografia), se insorta in una sede atipica (vene profonde addominali, vene cerebrali, vene superficiali del derma) o durante terapia anticoagulante primaria, o durante profilassi anticoagulante secondaria o concomitante a citopenia idiopatica secondo i criteri sopra definiti

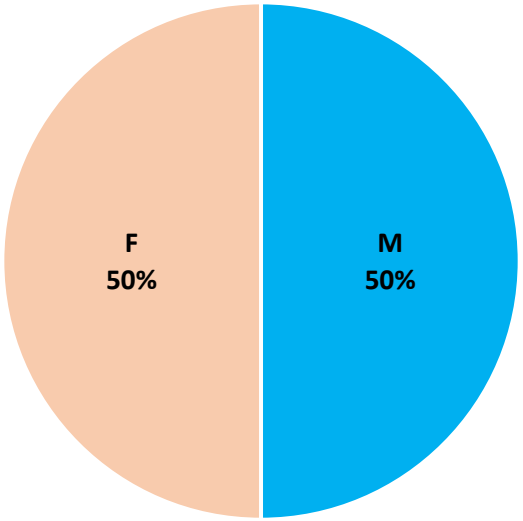
Studio AVR-FLAER: aggiornamento marzo 2026

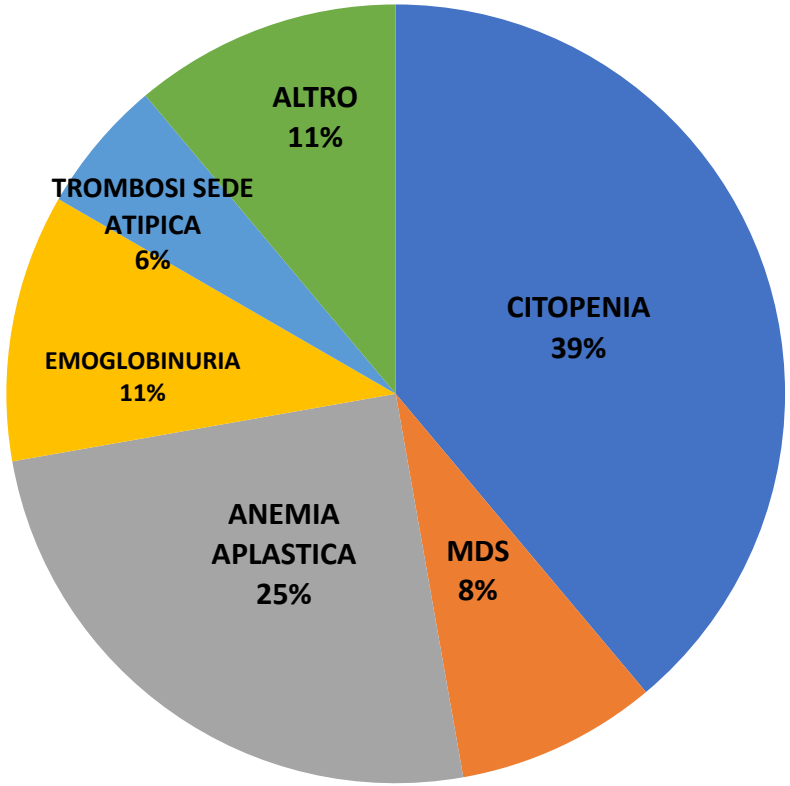
PAZIENTI ARRUOLATI: 174

PAZIENTI POSITIVI: 32 (19%)

ETÀ MEDIANA: 69 anni







Studio AVR-FLAER: pazienti positivi con citopenia

Pazienti positivi AVR-FLAER	clone RBC %	clone RET %	clone NEU %	clone MON %
MEDIANA	0,11	0,51	0,85	0,83

Pazienti positivi con citopenia AVR-FLAER	clone RBC %	clone RET %	clone NEU %	clone MON %
MEDIANA	0,23	0,51	1,63	1,33

Suddivisione dimensione clone per patologia	numero	mediana clone RBC	mediana clone RET	mediana clone NEU	mediana clone MON
CITOPENIA	14	0,19	2,51	1,69	1,28
MDS	3	8,56	35,9	45	41,30
AA	9	0,23	0	0,8	0,7

Valori emocromo	RBC (106/uL) I ANALISI	NEU (103/uL) I ANALISI	MONO (103/uL) I ANALISI	Hb (g/dL) I ANALISI
MEDIANA CITOPENICI	2,86	1,98	0,33	9,75
MIN	2,46	0,09	0,02	7
MAX	5,23	13,76	1,24	13,4

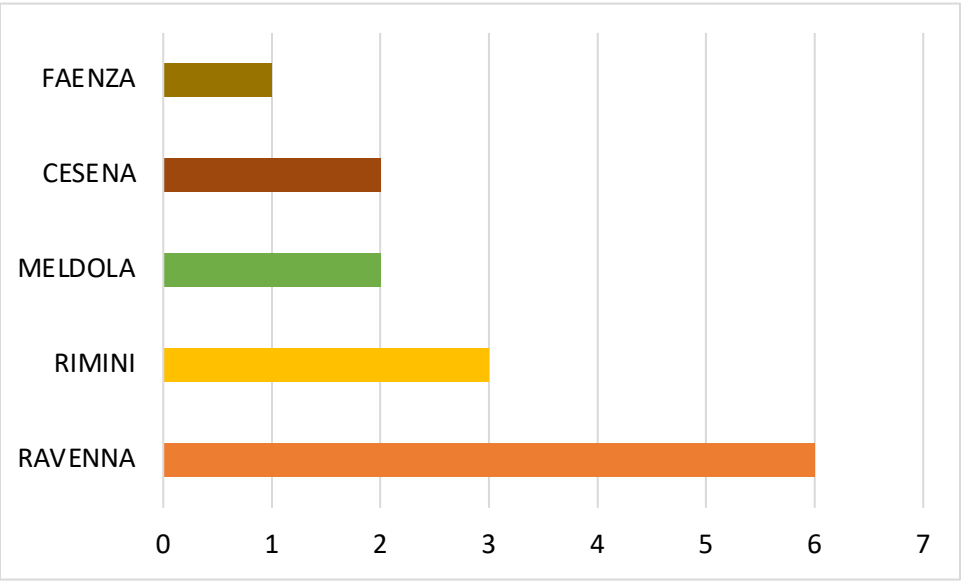
Pazienti positivi fuori da AVR-FLAER (2009-2026)

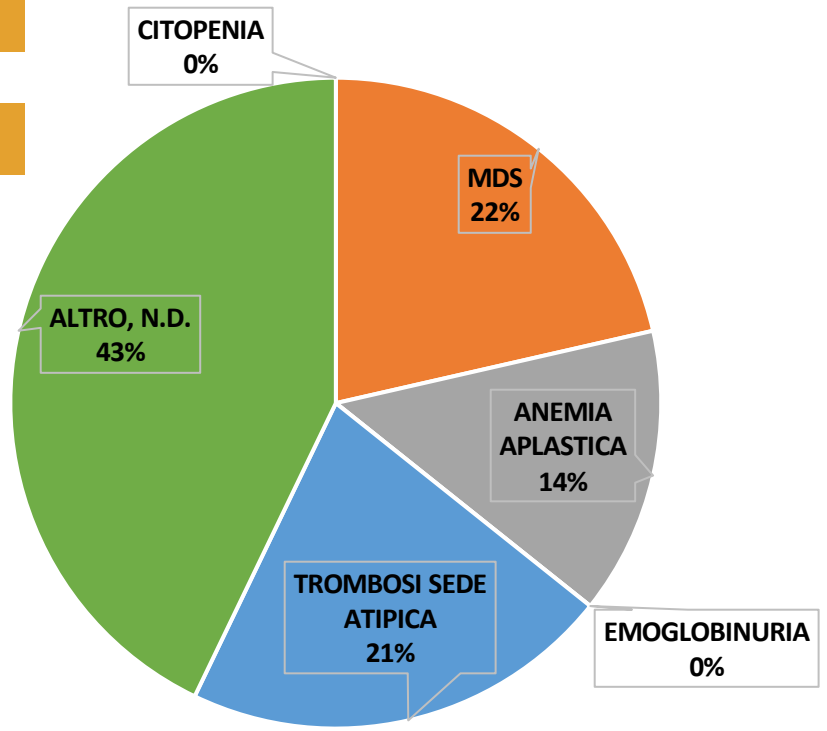
14 POSITIVI SU 304 TOTALI: 4,6%

NON ARRUOLABILI PER ETÀ: 2

ARRUOLATI IN MYELOPNH: 2

MICROCLONI





Take-home messages

Microcloni: ruolo ed implicazioni cliniche

Monitoraggio cloni su pazienti stratificati

Cloni PNH e MPN

Ruolo del laboratorio nelle terapie inibitorie del complemento

Aggiornamento linee guida (2018)

Grazie!

U.O. Patologia Clinica. Lab Ematologia

Dott.ssa V. Polli

Dott.ssa M. Zauli

Dott.ssa A. Clementoni

Dott.ssa M. Olivieri

Dott.ssa F. Germanò

Dott.ssa M.E. Fagioli

Dott. G. Poletti

Dott. M. Rosetti

Prof. F. Lanza

gli Ematologi della AUSL Romagna