EMATOLOGIA DI LABORATORIO:
percorsi diagnostici e obiettivi clinici.

Milano, 11-12 novembre 2010

Diagnosi differenziale anatomopatologica della trombocitemia essenziale

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The history of myeloproliferative disorders: before and after Dameshek

1845: description of chronic myelogenous leukemia

In October, 1845, John Hughes Bennett (1812–1875), an English pathologist at the Royal Infirmary in Edinburgh, Scotland, published the first report of CML entitled 'Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood'.[1]

1879: description of primary myelofibrosis

A German surgeon, Gustav Heuck (1854–1940) was the first to describe PMF, in 1879, under the title of 'Two cases of leukemia with peculiar blood and bone marrow findings'.[6] Heuck described two young patients with massive splenomegaly, circulating nucleated red blood cells, and increased number of morphologically abnormal leukocytes; he referred to the two

1892: description of polycythemia vera

Louis Henri Vaquez (1860–1936), a French physician, was the first to describe PV in 1892 in a 40-year-old man with chronic 'cyanosis' distended veins, vertigo, dyspnea, palpitations, hepatosplenomegaly and marked erythrocytosis.[4] It should be

1934: description of essential thrombocythemia

Essential thrombocythemia is the last of the classic MPDs to be formally described (as 'hemorrhagic thrombocythemia') in 1934 by Emil Epstein (1875–1951) and Alfred Goedel, both Austrian pathologists.[7] Their patient presented with extreme thrombocy-

W. Dameshek (1900 -1969)

EDITORIAL

Some Speculations on the Myeloproliferative Syndromes

WILLIAM DAMESHEK

blood 1951 6: 372-375

TABLE 1.—*The Myeloproliferative Disorders*

Myelostimulatory Factor's)

Syndromes	Bone marrow				Potential bone marrow
	Erythroblasts	Granulocytes	Megakaryocytes	Fibroblasts	Myeloid metaplasia of spleen and liver
Chronic Granulocytic Leukemia	±	+++	+ to +++	+	++
Polycythemia Vera	+++	++	++ to +++	+ to +++	+ to +++
Idiopathic or Agnogenic Myeloid Metaplasia of Spleen	±	±	+++	+ to +++	+++
Megakaryocytic Leukemia	±	±	+++	+	+ to +++
Erythroleukemia (including diGuglielmo syndrome)	+++	+	±	±	+ to +++

Degrees of Proliferation: + slight
++ moderate
+++ marked

1960: the discovery of the Philadelphia chromosome

Peter Nowell (1928–), an American tumor biologist, was working at the University of Pennsylvania, PA, USA, when he unexpectedly visualized individual metaphase chromosomes in leukemia cell culture slides that were tap water-rinsed before Giemsa staining.[83] This was not intentional, from his part,

1967: Fialkow and the stem cell origin of clonal myeloproliferation

onstrate the Fialkow and colleagues applied a similar technique to confirm the clonal nature of CML (1967),[94] PV (1976),[95] PMF (1978),[96] and ET (1981).[97] They also showed the presence of a single G-6-PD

1967: establishment of the Polycythemia Vera Study Group

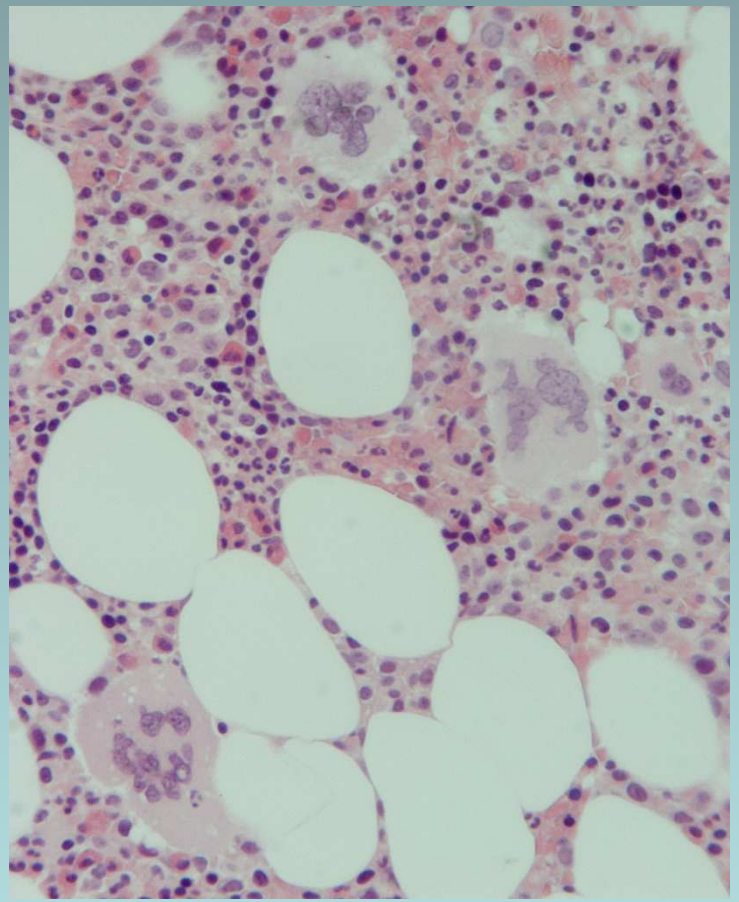
In 1967, Louis Wasserman (1912–1999), an American hematologist from the Mount Sinai Hospital in New York, assembled a multinational group of clinical investigators and founded the Polycythemia Vera study Group (PVSG), under the auspices and funding support of the US National Cancer Institute.[111] Their

Criteri diagnostici della TE (sec. PVSG)

1. Piastrine >600x109/L
2. Emoglobina <13 g/dL o valori normali di massa eritrocitaria
3. Depositi di ferro identificabili nel midollo osseo o assenza di risposta alla terapia marziale
4. Assenza del cromosoma Philadephia e assenza molecolare del gene ibrido bcr/abl
5. Fibrosi collagene nel midollo osseo:
   a) Assente
   b) Inferiore ad 1/3 della BOM in assenza di splenomegalia e di reazione leuco-eritroblastica
6. Assenza di dimostrabili cause di piastrinosi

La diagnosi di TE come diagnosi di esclusione !

Criteri diagnostici della TE (WHO, 2001)



Criteri positivi

1. Piastrinosi >600x109/L
2. Biopsia osteomidollare: proliferazione principalmente a carico della serie piastrinopoietica, con incremento del numero di megacariociti giganti, maturi.

Criteri di esclusione

1. Nessuna evidenza di Policitemia Vera

a) Normale massa eritrocitaria; Hb <18.5 g/dL (uomo) e 16.5 g /dL (donna)

b) Depositi di ferro identificabili nel midollo osseo, ferritina sierica normale, MCV normale

c) Se la precedente condizione non è rispettata, assenza di incremento della terapia marziale ad incrementare la massa eritrocitaria o l'Hb ai livelli della PV

2. Nessuna evidenza di Leucemia Mieloide Cronica: non evidenza di cromosoma Philadelphia; non evidenza di gene di fusione BCR-ABL
3. Nessuna evidenza di Mielofibrosi Idiopatica Cronica

a) Fibrosi collagene assente

b) Fibrosi reticolinica minima o assente

4. Nessuna evidenza di Sindrome Mielodisplastica

a) No del(5q), t(3;3)(q21;q26), inv(3)(q21;q26)

b) No significativa displasia dei granulociti, assenza o presenza di solo pochi micromegacariociti

5. Nessuna evidenza di piastrinosi secondaria a:

a) infiammazione o infezione

b) neoplasia

c) precedente splenectomia

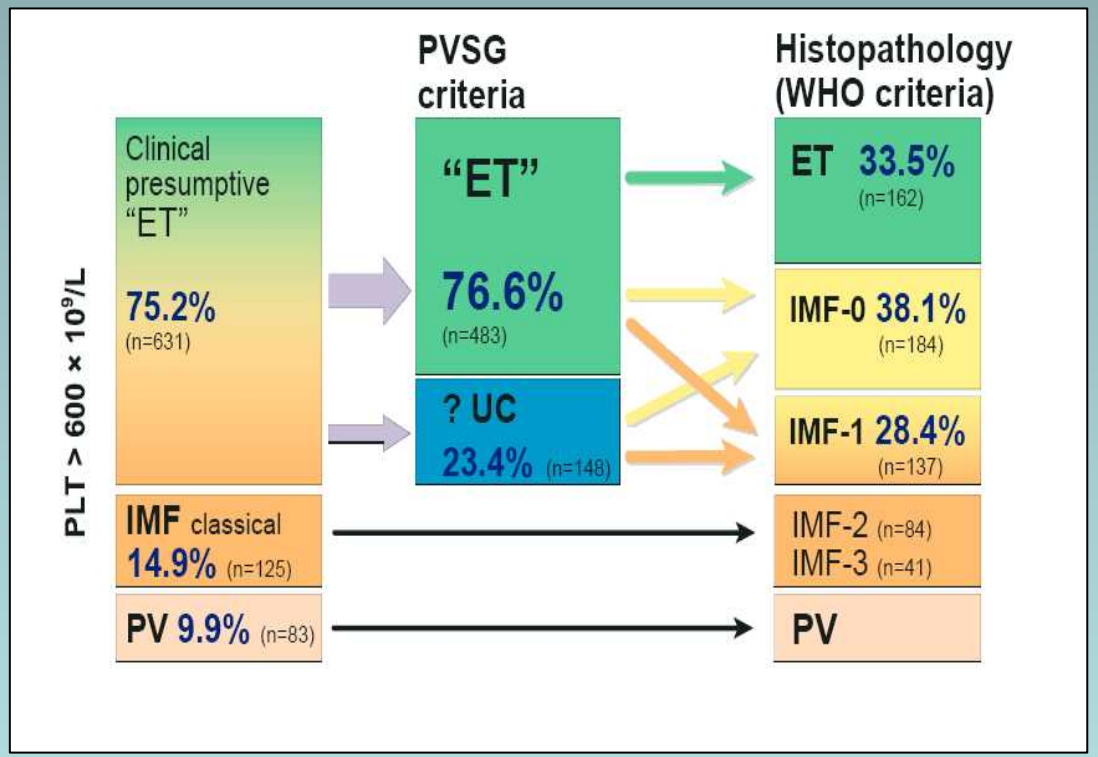
J. Thiele · H. M. Kvasnicka

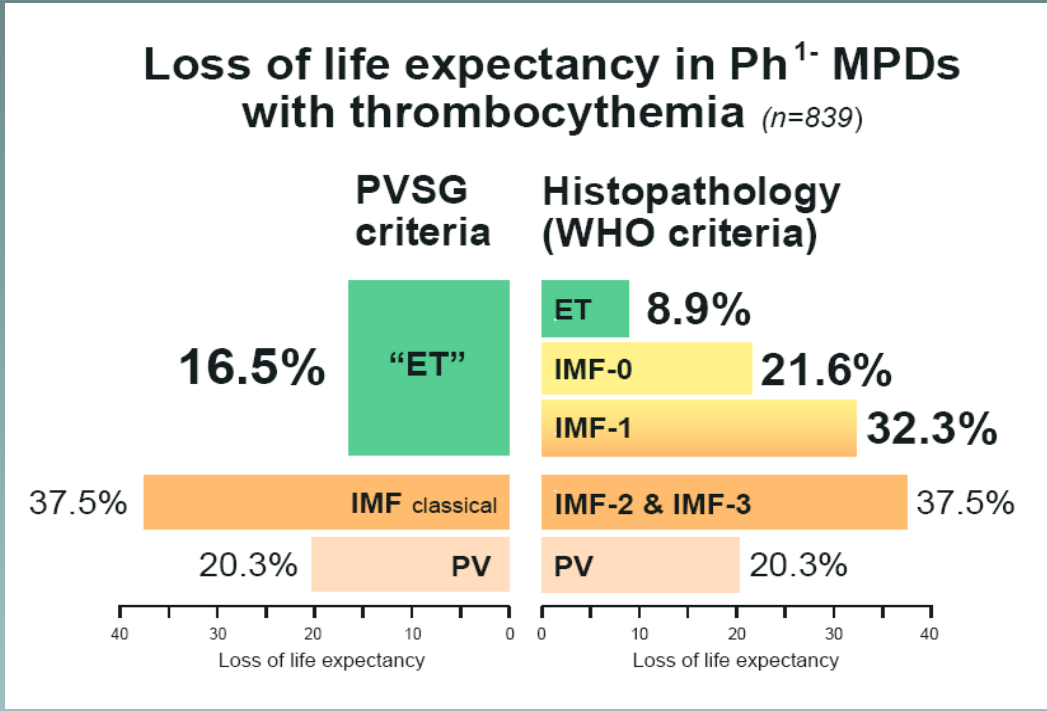
Chronic myeloproliferative disorders with thrombocythemia: a comparative study of two classification systems (PVSG, WHO) on 839 patients

Ann Hematol 2003; 82: 148-152

- ✓ CMPD Ph-
- ✓ PTL >600 x 10^9/l per almeno due mesi
- ✓ Grading IMF (0 – 1 – 2 – 3)*

(*) Thiele et al. Ann Hematol 1999, 78: 495-506





Thiele et al. Ann Hematol 2003; 82: 148-152

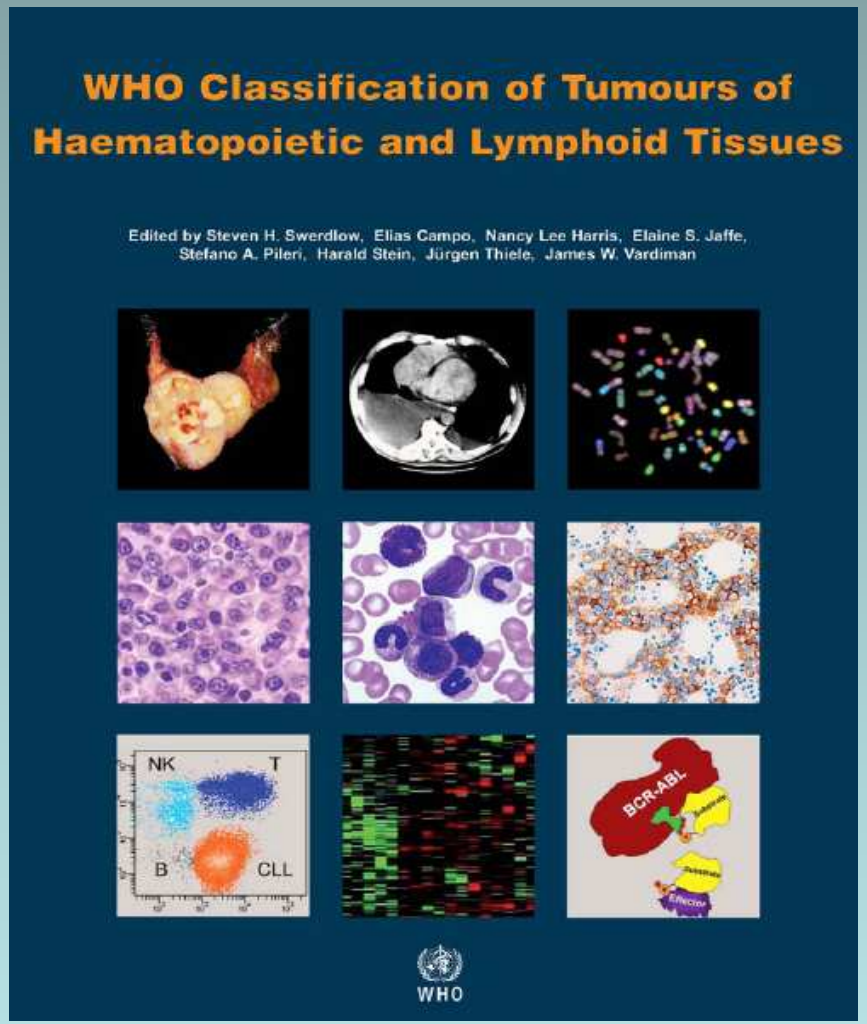
1) La diagnosi basata su criteri positivi (BOM) riduce la percentuale di ET ed aumenta quella di IMF

2) Viene introdotto il concetto di fase cellulare (prefibrotica) della IMF

3) La BOM è indispensabile per identificare le varie fasi (prefibrotica, fibrotica precoce, fibrotica avanzata) di IMF

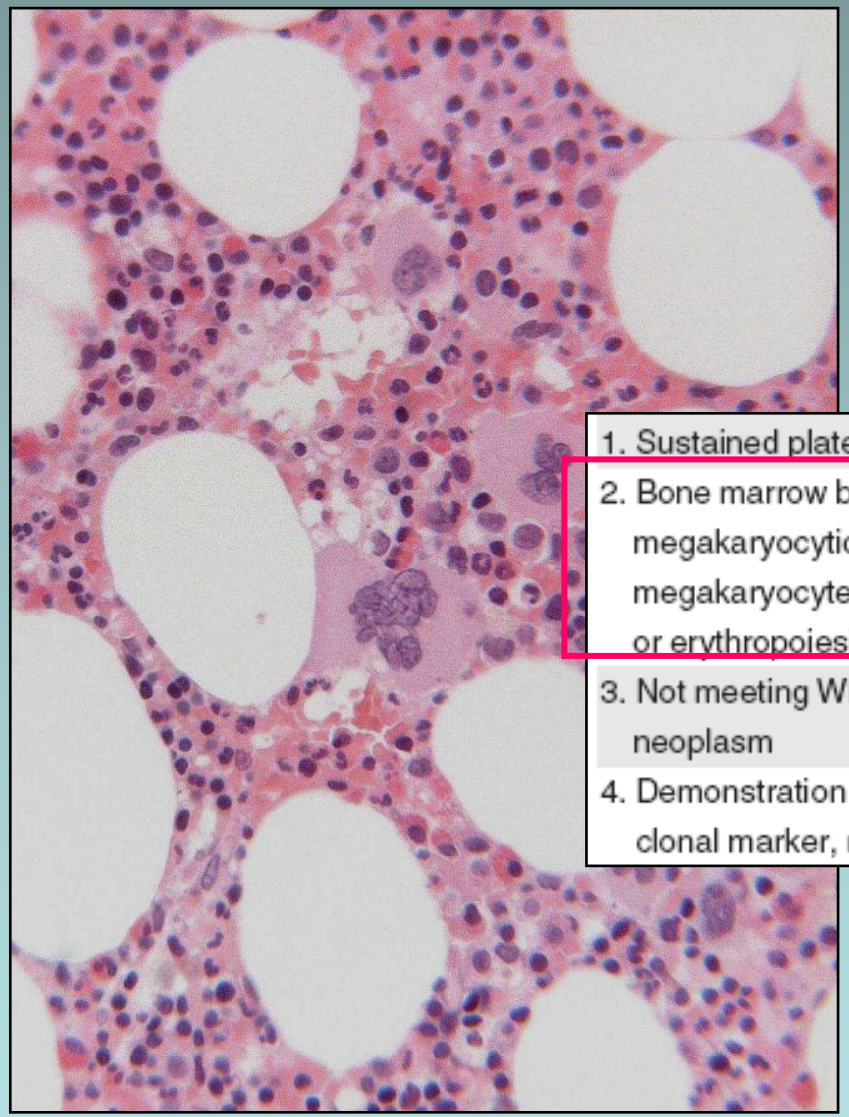
4) La prognosi dei pazienti ET (PVSG) riclassificati IMF /WHO) è peggiore

Principali modifiche nella classificazione delle neoplasie mieloproliferative (WHO 2008)



- Gli algoritmi diagnostici per la diagnosi di ET, PV, MFP sono stati modificati includendo:
  - le informazioni relative a JAK2
  - le caratteristiche istologiche come criteri diagnostici
- Il livello di piastrinosi è stato ridotto a 450 x 10^9/L

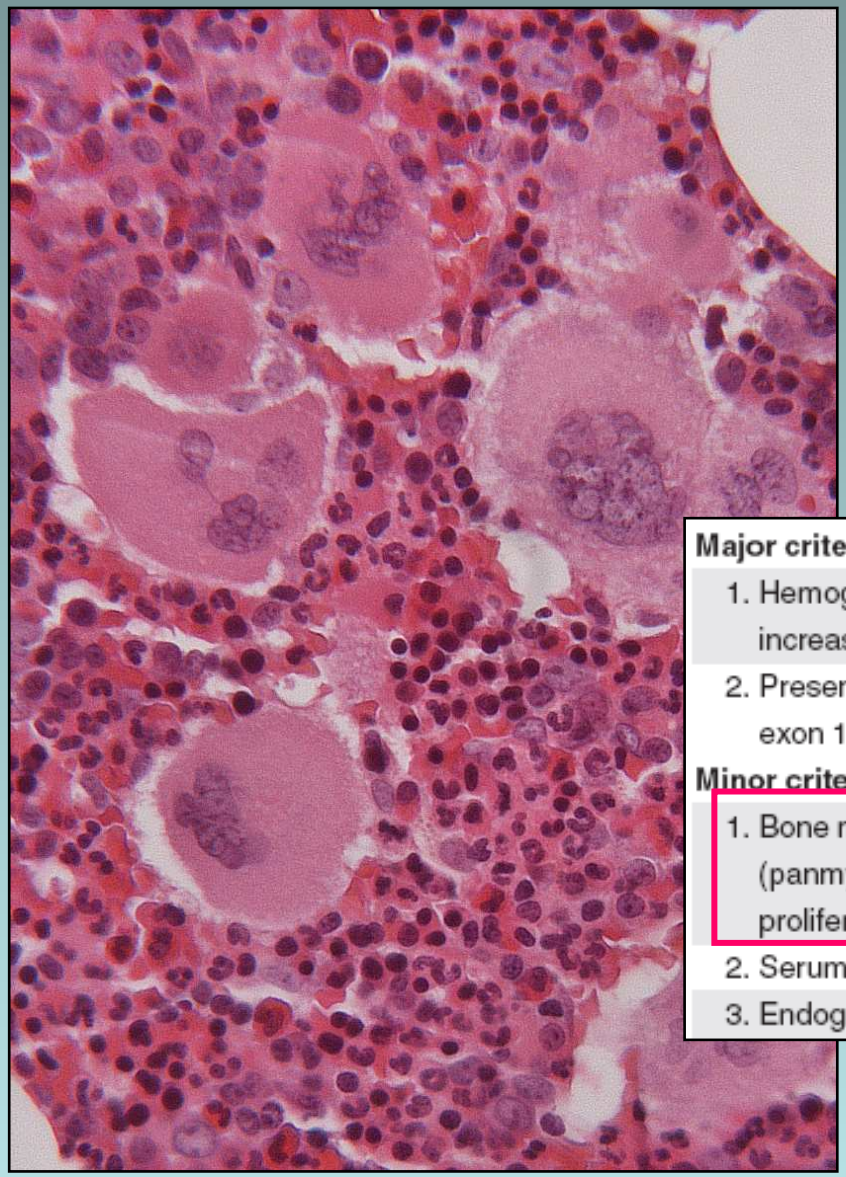
WHO criteria for Essential Thrombocythemia



1. Sustained platelet count ≥ 450 × 10^9/L*
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes; no significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
3. Not meeting WHO criteria for PV,† PMF,‡ CML,§ MDS,¶ or other myeloid neoplasm
4. Demonstration of *JAK2*617V>F or other clonal marker, or in the absence of a clonal marker, no evidence for reactive thrombocytosis‖

(2008)

WHO criteria for Polycythemia Vera



Major criteria

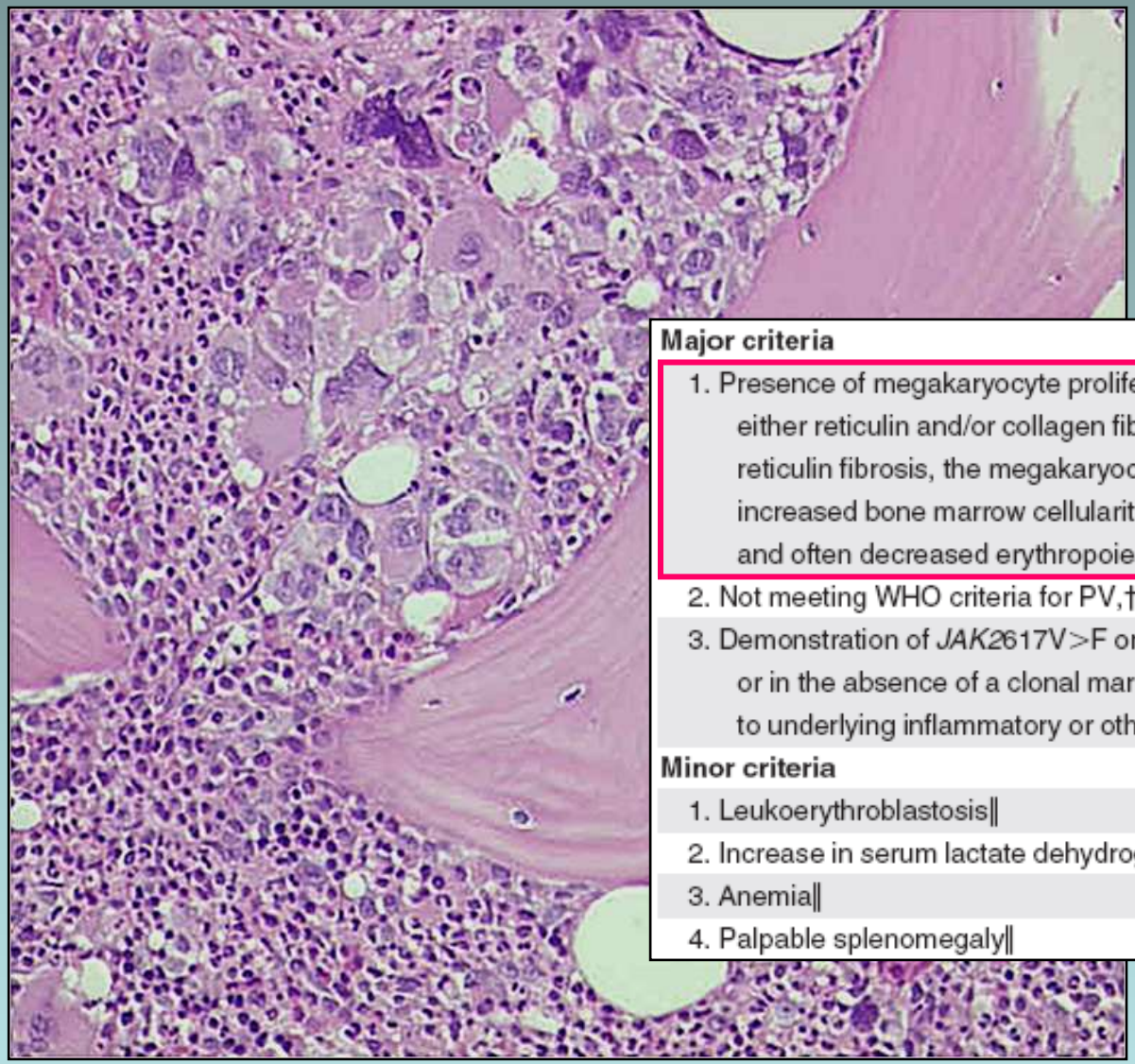
1. Hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume*
2. Presence of *JAK2*617V>F or other functionally similar mutation such as *JAK2* exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro

(2008)

WHO criteria for Primary Myelofibrosis



Major criteria

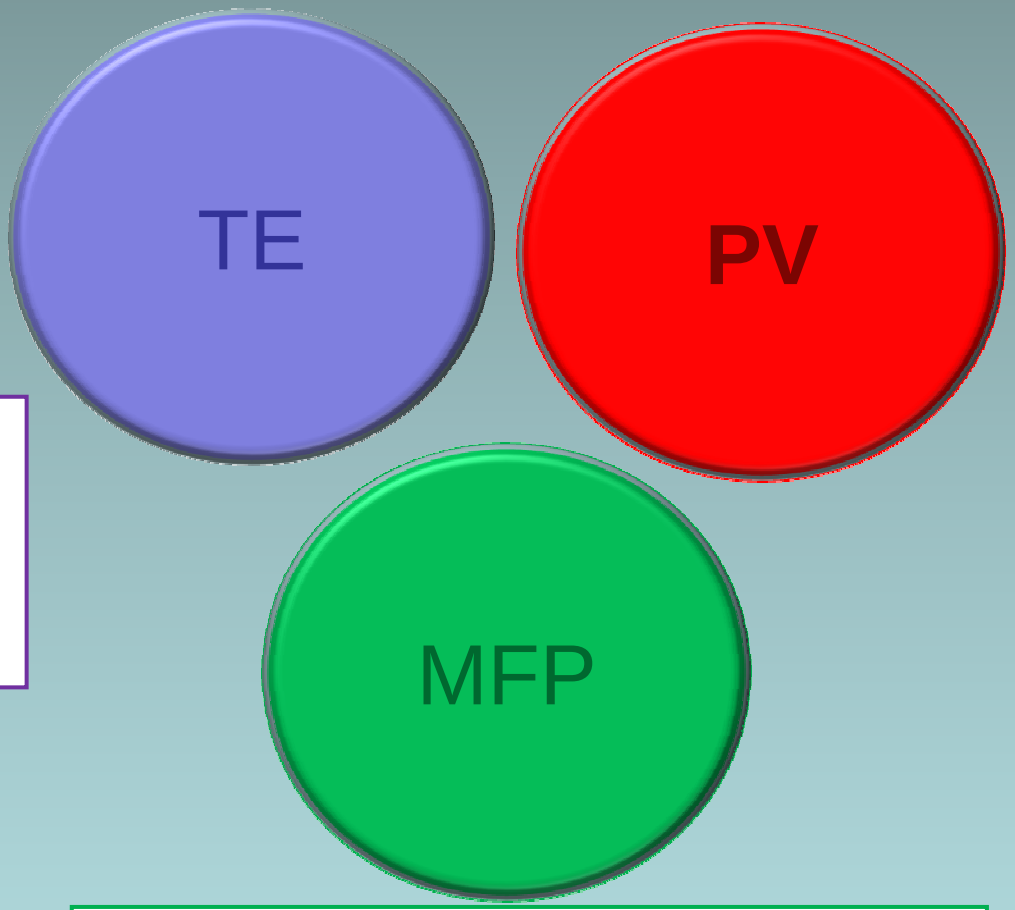
1. Presence of megakaryocyte proliferation and atypia,* usually accompanied by either reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (ie, prefibrotic cellular-phase disease)
2. Not meeting WHO criteria for PV,† CML,‡ MDS,§ or other myeloid neoplasm
3. Demonstration of *JAK2*617V>F or other clonal marker (eg, *MPL*515W>L/K), or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases¶

Minor criteria

1. Leukoerythroblastosis‖
2. Increase in serum lactate dehydrogenase level‖
3. Anemia‖
4. Palpable splenomegaly‖

(2008)

Le NMP Ph-negative (sec. WHO, 2008)



-Proliferation of enlarged, mature mega

- No increase or left-shift of granulopoiesis or erythropoiesis

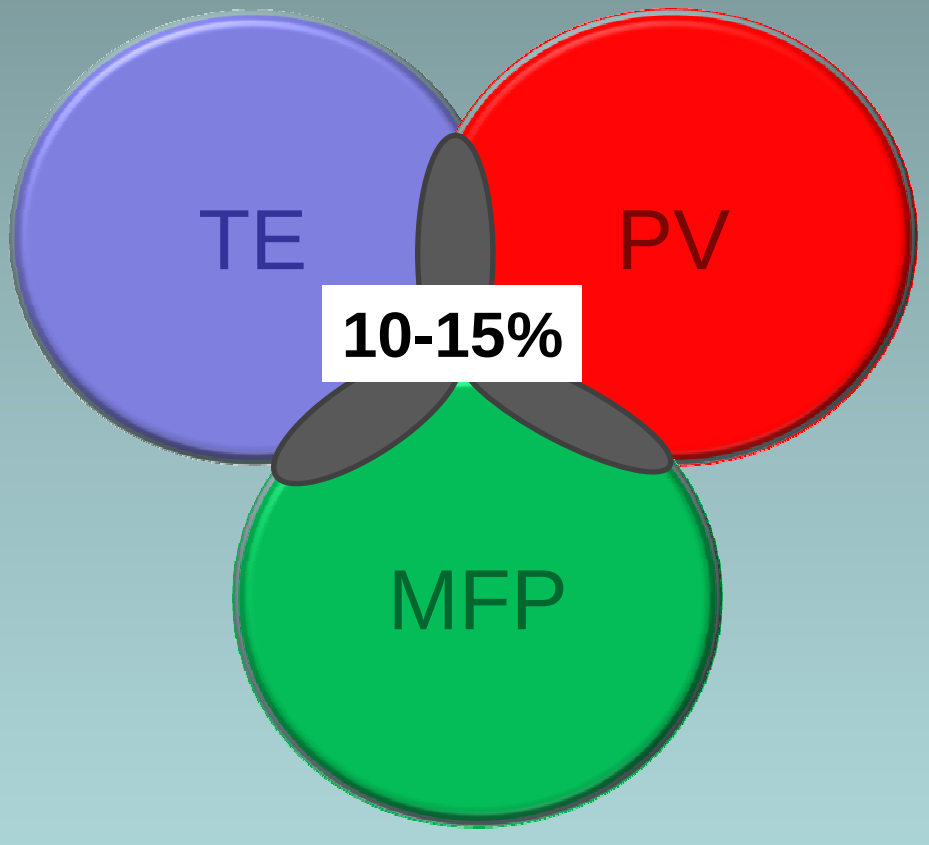
- Hypercellular bone marrow

- (panmyelosis)

- Megakaryocyte proliferation and atypia, with reticulin and/or collagen fibrosis

- in the absence of fibrosis, the megas changes must be accompanied by an increased bone marrow cellularity with granulocytic proliferation and decreased erythropoiesis

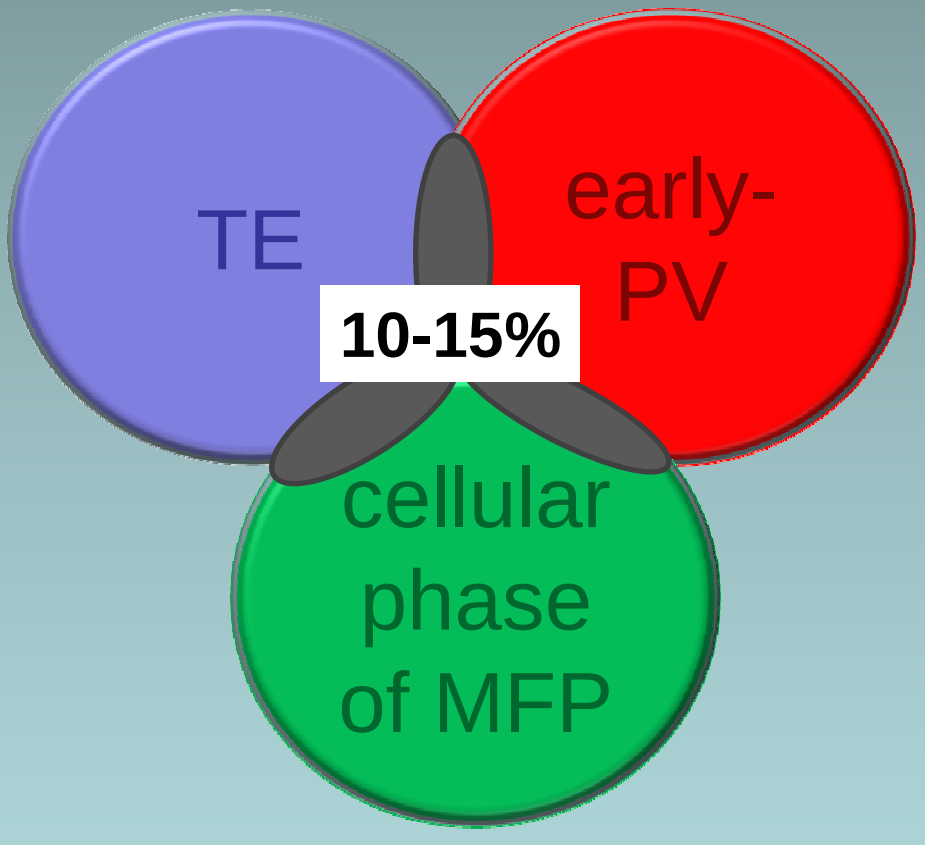
La categoria delle “NMP-U”



1) **Casi con caratteristiche cliniche e/o morfologiche intermedie tra due entità**

2) **Casi in fase iniziali (prodromica)**

La categoria delle “NMP-U”



La diagnosi differenziale riguarda soprattutto le fasi iniziali delle NMP !

Essential thrombocythemia or chronic idiopathic myelofibrosis? A single-center study based on hematopoietic bone marrow histology

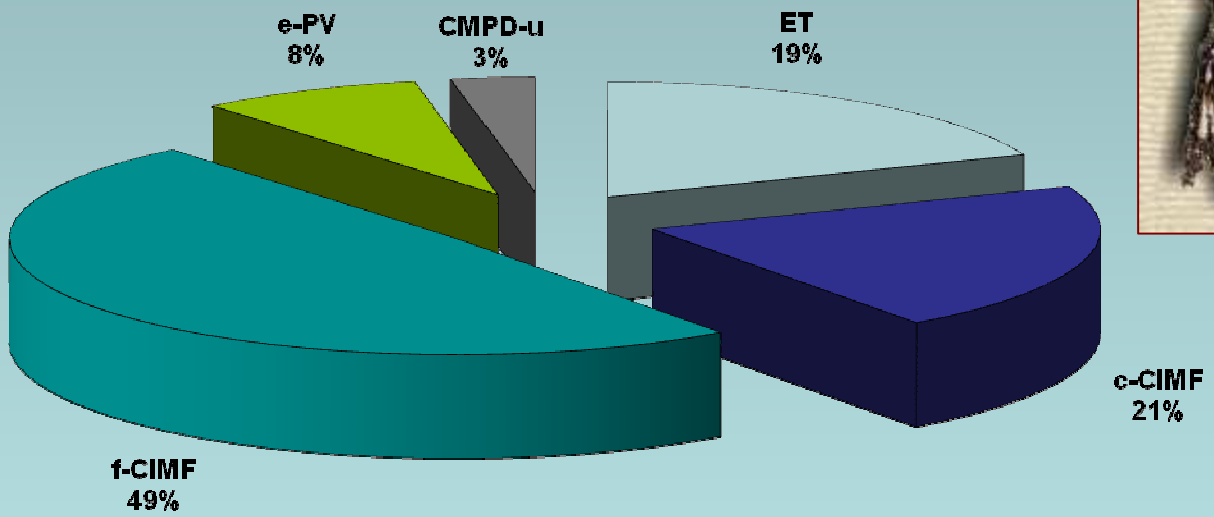
Gianelli *et al.* Leukemia & Lymphoma 2006; 47: 1774-81

Patients = 116 ET pts. (PVSG)

Gender: 44 M 72 F

Age: median 55 yrs. (range:19-83)

Follow-up: medium 121 months

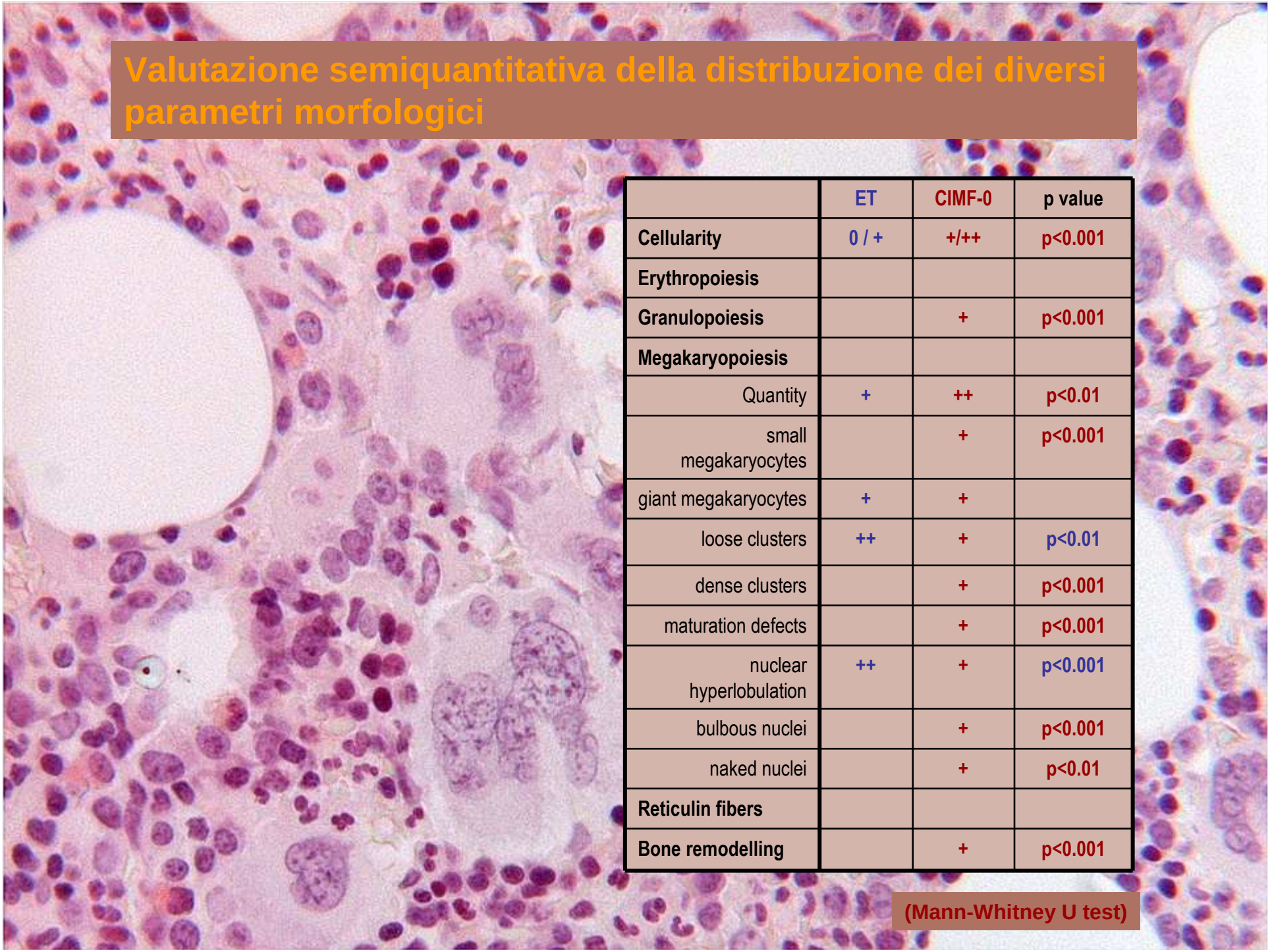


Classification according to the WHO (2001)

Clinical characteristics of the patients

WHO classification	ET	CIMF-0 (*)	CIMF-1(*)	CIMF-2(*)	e-PV
Patients	22	24	44	13	9
Age: median (range)	54 (19-73)	52 (31-79)	59 (24-82)	66 (36-83)	60 (22-70)
M / F (ratio)	8/14 (0.6)	8/16 (0.5)	17/27 (0.6)	6/7 (0.9)	5/4 (1.3)
Hb (g/dL) median (range)	13.5 (12.1-16)	13.7 (10.1-17.3)	13.7 (11.1-18.9)	12.2 (10.9-12.9)	14.9 (12.4-15.2)
WBC (109/L) median (range)	7,7 (3,9-11,3)	7,1 (3,9-14,1)	8,4 (4,5-12,4)	6,6 (6,5-7,4)	7,1 (5,7-9,3)
PLT (109/L) median (range)	780 (636-1.413)	859 (600-1.870)	840 (605-1.690)	990 (768-2.120)	907 (639-1.215)
Splenomegaly No. (%)	0 (0%)	4 (17%)	8 (18%)	5 (38%)	2 (22%)

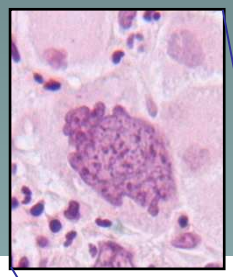
Valutazione semiquantitativa della distribuzione dei diversi parametri morfologici

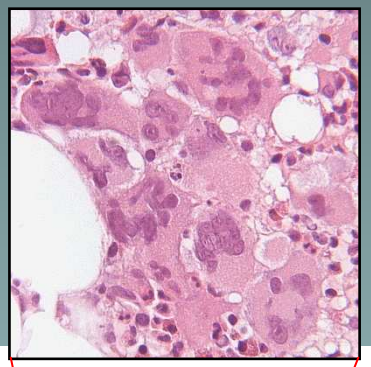


	ET	CIMF-0	p value
Cellularity	0 / +	+/++	p<0.001
Erythropoiesis			
Granulopoiesis		+	p<0.001
Megakaryopoiesis			
Quantity	+	++	p<0.01
small megakaryocytes		+	p<0.001
giant megakaryocytes	+	+	
loose clusters	++	+	p<0.01
dense clusters		+	p<0.001
maturation defects		+	p<0.001
nuclear hyperlobulation	++	+	p<0.001
bulbous nuclei		+	p<0.001
naked nuclei		+	p<0.01
Reticulin fibers			
Bone remodelling		+	p<0.001

(Mann-Whitney U test)

bulbous nuclei





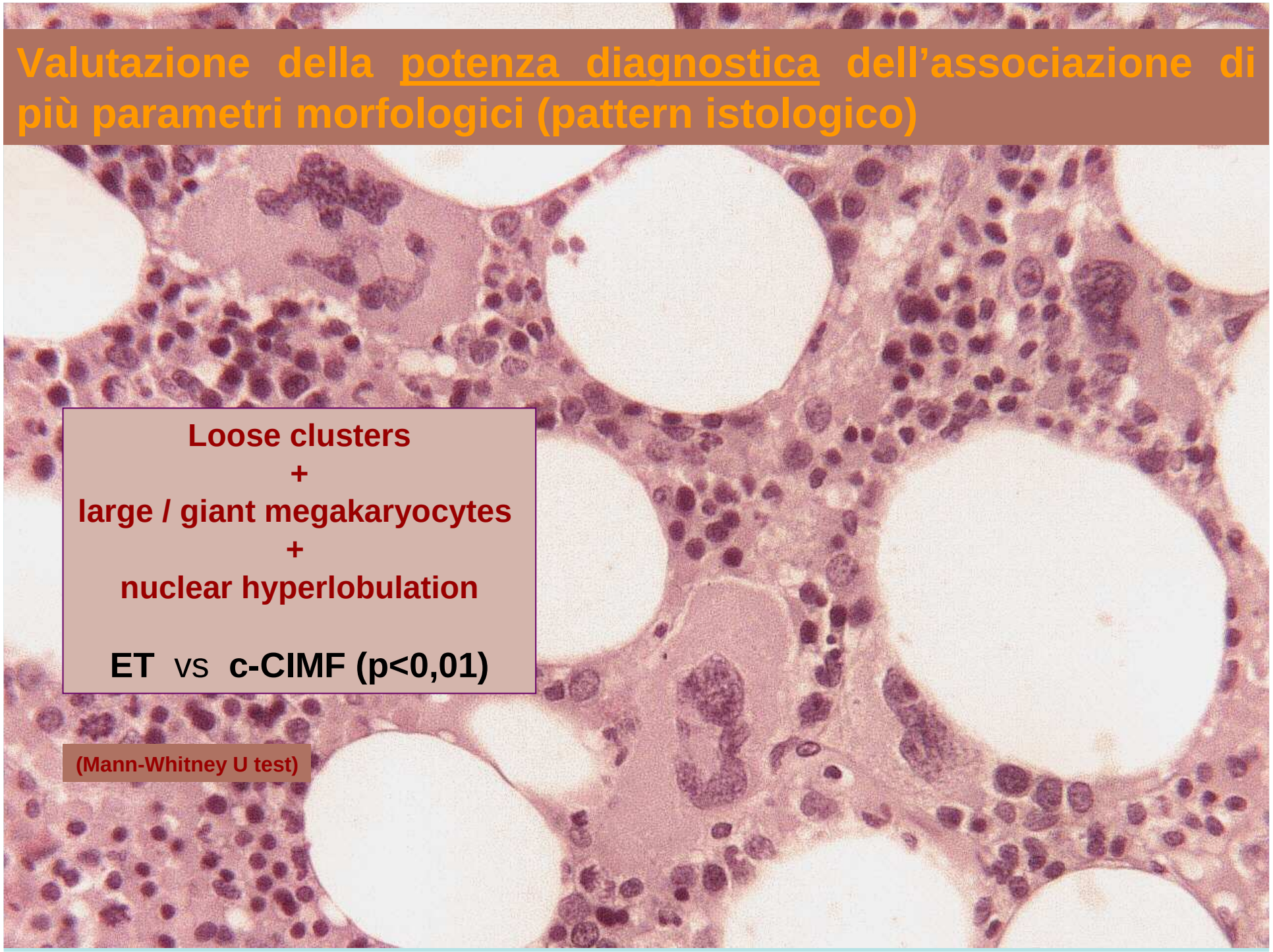
nuclear hyperlobulation

Table III. Comparison of the morphologic characteristics of ET and prefibrotic CIMF (CIMF-0) according to the WHO classification.

	ET				CIMF-0				
	0	1+	2+	3+	0	1+	2+	3+	Significance
Cellularity	18 (82%)	4 (18%)	0 (0%)	0 (0%)	0 (0%)	10 (42%)	13 (54%)	1 (4%)	$P < 0.001$
Erythropoiesis	21 (95%)	1 (5%)	0 (0%)	0 (0%)	19 (79%)	3 (13%)	2 (8%)	0 (0%)	$P = 0.098$
Granulopoiesis	20 (91%)	1 (5%)	1 (4%)	0 (0%)	6 (25%)	10 (42%)	8 (33%)	0	$P < 0.001$
Megakaryopoiesis									
Quantity	0 (0%)	11 (50%)	10 (45%)	1 (5%)	0 (0%)	3 (13%)	19 (79%)	2 (8%)	$P < 0.01$
Small megakaryocytes	5 (23%)	15 (68%)	2 (9%)	0 (0%)	0 (0%)	13 (54%)	10 (42%)	1 (4%)	$P < 0.001$
Giant megakaryocytes	1 (5%)	8 (36%)	12 (54%)	1 (5%)	3 (13%)	13 (54%)	8 (33%)	0 (0%)	$P = 0.06$
Loose clusters	0 (0%)	9 (41%)	12 (55%)	1 (4%)	2 (8%)	17 (71%)	5 (21%)	0 (0%)	$P < 0.05$
Dense clusters	21 (95%)	1 (5%)	0 (0%)	0 (0%)	1 (4%)	12 (50%)	11 (46%)	0 (0%)	$P = 0.001$
Maturation defects	6 (27%)	15 (68%)	1 (5%)	0 (0%)	3 (12%)	18 (75%)	3 (13%)	0 (0%)	$P < 0.001$
Nuclear hyperlobulation	0 (0%)	7 (32%)	14 (64%)	1 (4%)	5 (21%)	14 (58%)	5 (21%)	0 (0%)	$P < 0.01$
Bulbous nuclei	18 (82%)	4 (18%)	0 (0%)	0 (0%)	2 (8%)	19 (79%)	3 (13%)	0 (0%)	$P < 0.001$
Naked nuclei	4 (18%)	17 (77%)	1 (5%)	0 (0%)	0 (0%)	18 (75%)	6 (25%)	0 (0%)	$P < 0.01$
Bone remodeling	0 (0%)	19 (86%)	3 (14%)	0 (0%)	0 (0%)	5 (52%)	14 (37%)	5 (11%)	$P < 0.001$
Reticulin fibers	22 (100%)	0 (0%)	0 (0%)	0 (0%)	24 (100%)	0 (0%)	0 (0%)	0 (0%)	

Non esiste un parametro morfologico patognomonico !

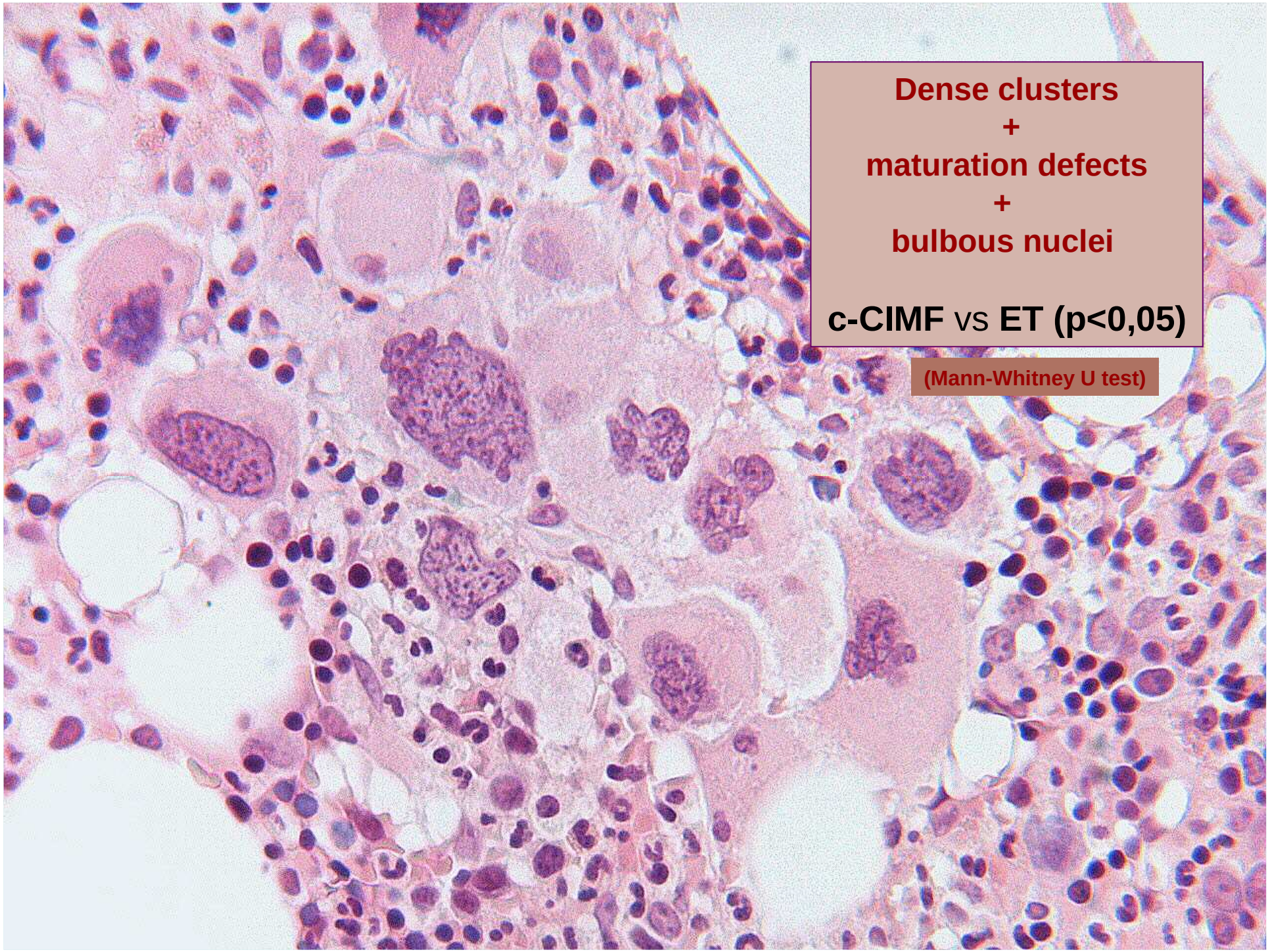
Valutazione della potenza diagnostica dell'associazione di più parametri morfologici (pattern istologico)



Loose clusters
+
large / giant megakaryocytes
+
nuclear hyperlobulation

ET vs **c-CIMF (p<0,01)**

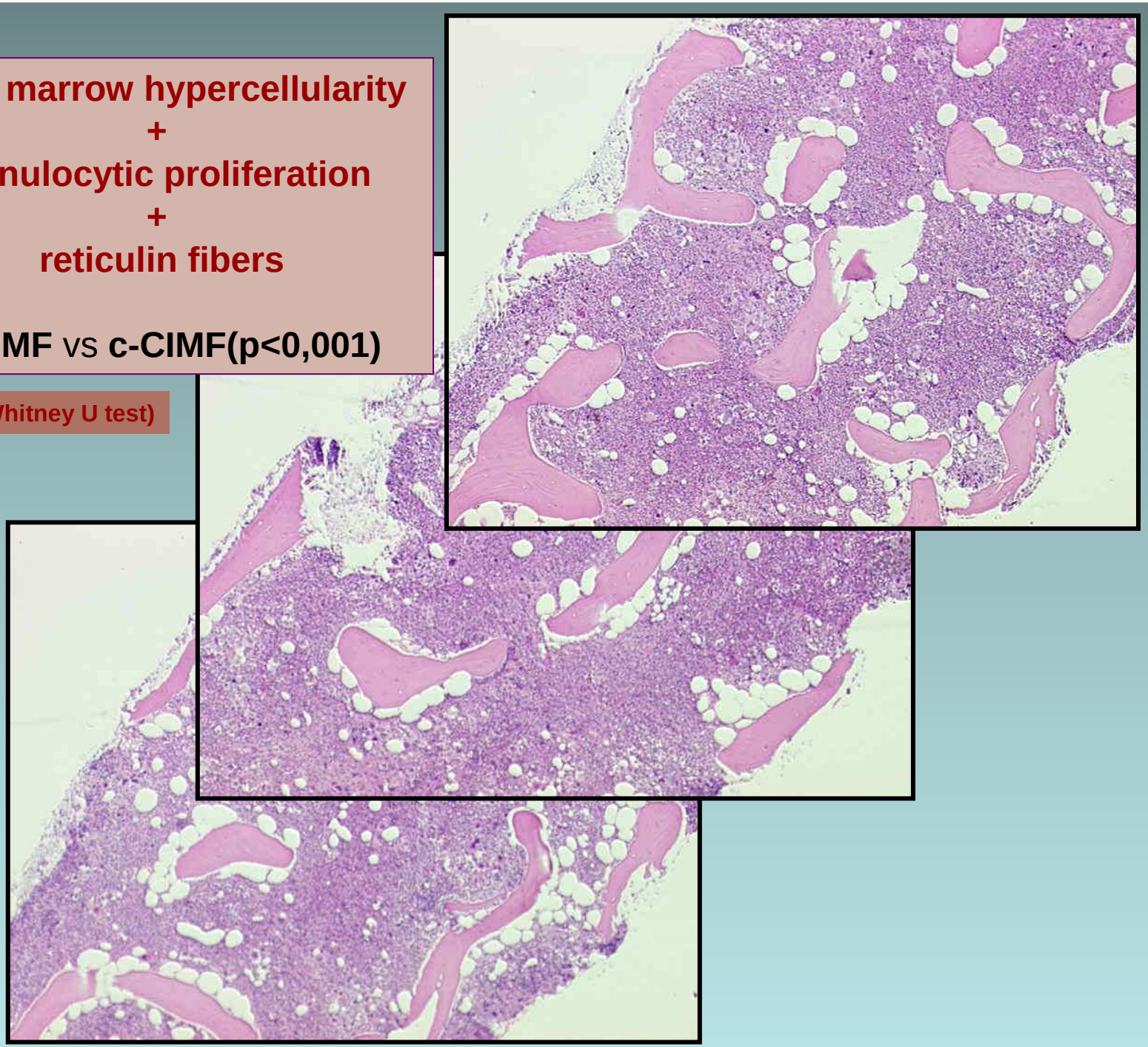
(Mann-Whitney U test)


Dense clusters
+
maturation defects
+
bulbous nuclei
c-CIMF vs ET (p<0,05)
(Mann-Whitney U test)

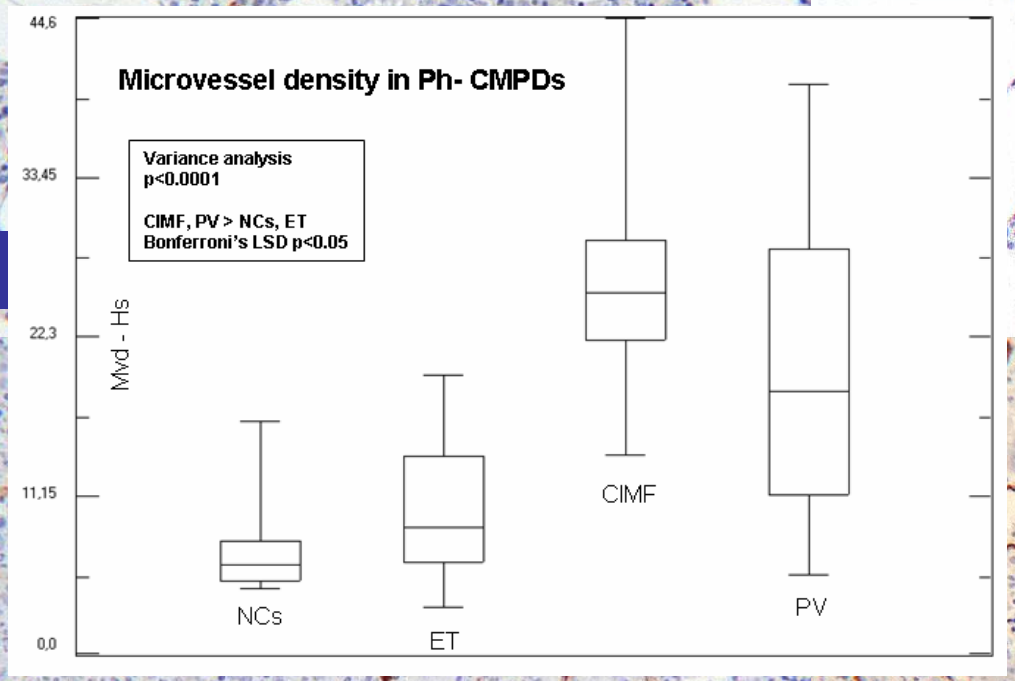
Bone marrow hypercellularity
+
granulocytic proliferation
+
reticulin fibers

f-CIMF vs **c-CIMF(p<0,001)**

(Mann-Whitney U test)



La densità microvascolare è significativamente aumentata nella CIMF e nella PV in confronto alla ET e ai casi di controllo (NCs)



NCs

ET

PV

CIMF

Gianelli et al. Am J Clin Pathol, 2007;128:966-73

Thiele J. *et al*.
Relevance of bone marrow features inthe differential diagnosis between Essential thrombocythemia and early stage idiopathic myelofibrosis. Haematologica 2000;85:1126-34.

Thiele J. *et al.*
Diagnostic differentiation of essential thrombocythaemia from thrombocythaemias associated with chronic idiopathic myelofibrosis by discriminate analysis of bone marrow features- a clinicopathological study on 272 patients. Histol Histopathol 2003;18:93-102

Florena AM. *et al.*
Value of bone marrow biopsy in the diagnosis of essential thrombocythaemia.
Haematologica. 2004;89:911-919.

Boveri E. *et al.*
Bone marrow microvessel density in chronic myeloproliferative disorders: a study of 115 patients with clinicopathological and molecular correlations. Br J Haematol. 2008; 140:162-8.

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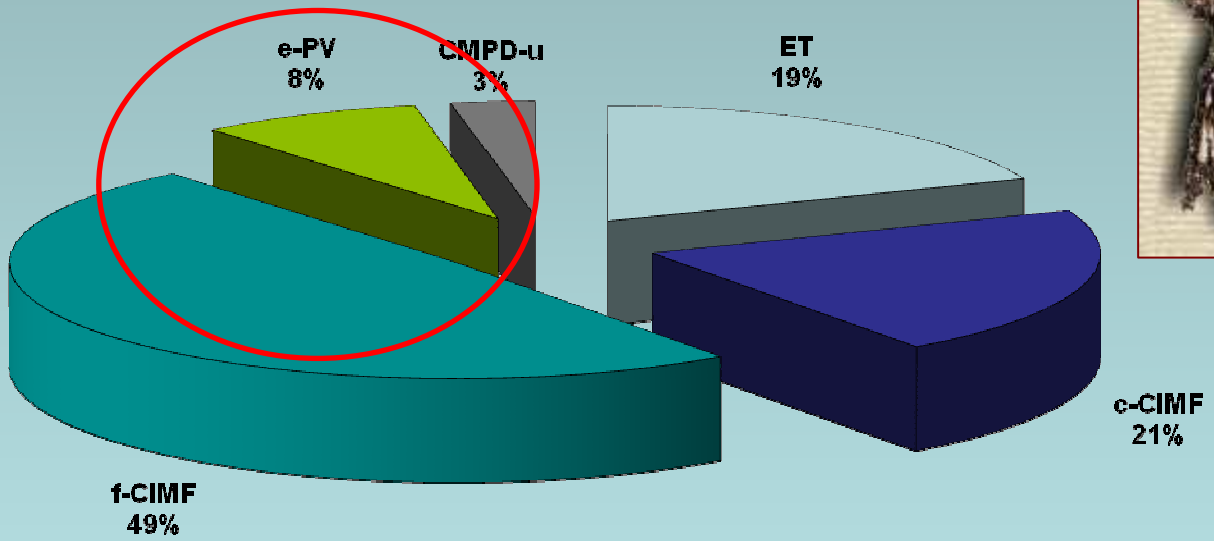


Classification according to the WHO (2001)

The Significance of Bone Marrow Biopsy and *JAK2*^{V617F} Mutation in the Differential Diagnosis Between the "Early" Prepolycythemic Phase of Polycythemia Vera and Essential Thrombocythemia

Gianelli et al. Am J Clin Pathol 2008;130:336-342

early-PV (*) n = 17

Gender
9 M / 8 F
Age
median 53 yrs. (20-69)
Follow-up
medium 16 years (5-26)
Evolution to PV
median age 9 years (1-12)

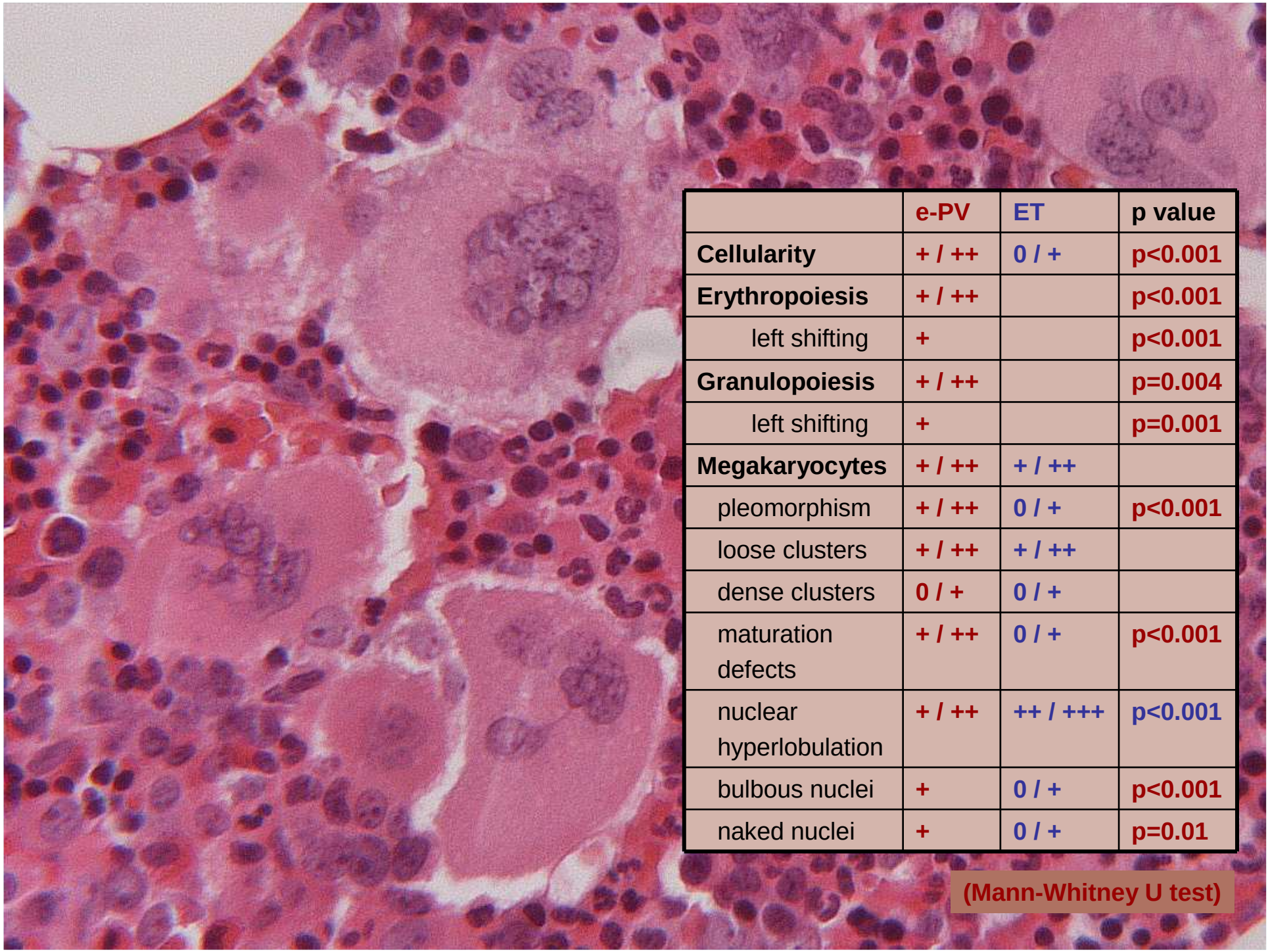
PV n=19

ET n=14

(*) patients presenting at the first observation with thrombocytosis in the range of ET and increased Hb levels, but lower than necessary for a PV diagnosis (WHO), developing during the course of the follow-up a well-characterized PV

Clinical characteristics of the patients

	e-PV (n = 17)	pp-PV (n = 19)	ET (n = 14)
Age (y)	53 (20-69)	63 (38-85)	62 (18-78)
M/F (ratio)	9/8 (1.1)	11/8 (1.4)	7/7 (1.0)
Hemoglobin concentration (g/dL)			
Male	15.95 (14.8-16.9)	18.6 (18.5-22.6)	14.1 (13.3-14.7)
Female	15.2 (13.4-16.4)	16.7 (16.5-17.4)	13.3 (10.7-14.6)
RBC count ($\times 10^{12}$/L)			
Male	5.21 (4.87-5.93)	6.6 (6.21-8.92)	4.59 (3.91-5.21)
Female	5.06 (4.3-5.43)	6.94 (6.15-7.95)	4.61 (3.29-5.05)
Hematocrit value (%)			
Male	47.3 (43.1-49)	56 (53.9-69.2)	42.1 (39.6-44)
Female	45.5 (39.3-47.6)	51 (49-53.3)	42.1 (31.7-43.8)
MCV (fL)			
Male	88.5 (81.2-97)	83.4 (57.8-102)	90.1 (89.2-117.4)
Female	90.1 (86.1-94.3)	72 (64.9-87.3)	89.5 (84-99.3)
Platelet count ($\times 10^9$/L)	720 (614-2,000)	609 (600-1,550)	989 (614-1,383)
WBC count ($\times 10^9$/L)	8 (5.9-11.7)	10.8 (7.6-17.8)	7.8 (6-11.1)
LDH (U/L)	379 (250-581)	510 (159-855)	435 (288-886)
LAP (IU/L)	114 (46-238)	156 (14-307)	77 (39-151)
Palpable splenomegaly, No. (%)	8 (47)	12 (63)	1 (7)
Palpable hepatomegaly, No. (%)	8 (47)	11 (58)	2 (14)

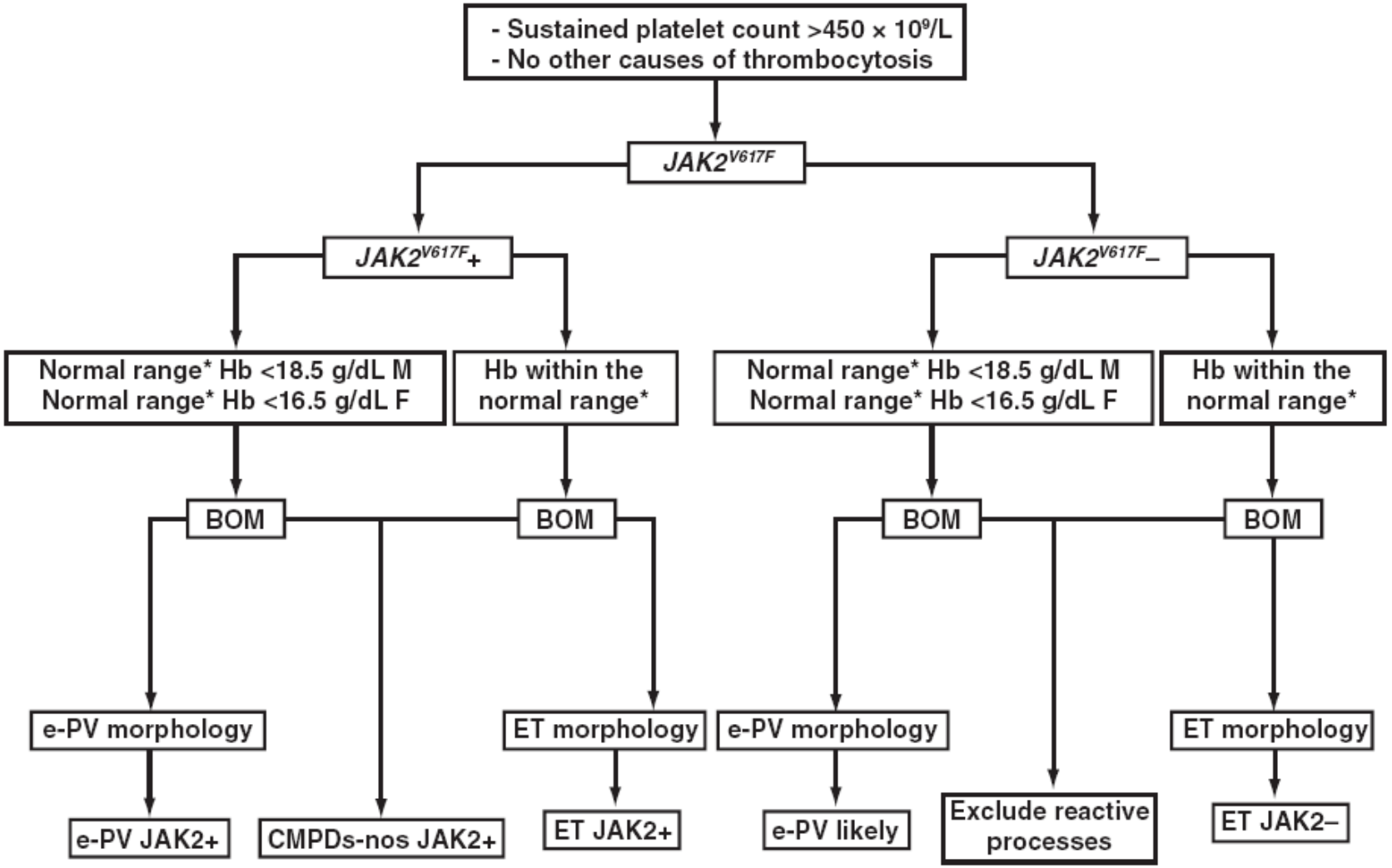


	e-PV	ET	p value
Cellularity	+ / ++	0 / +	p<0.001
Erythropoiesis	+ / ++		p<0.001
left shifting	+		p<0.001
Granulopoiesis	+ / ++		p=0.004
left shifting	+		p=0.001
Megakaryocytes	+ / ++	+ / ++	
pleomorphism	+ / ++	0 / +	p<0.001
loose clusters	+ / ++	+ / ++	
dense clusters	0 / +	0 / +	
maturation defects	+ / ++	0 / +	p<0.001
nuclear hyperlobulation	+ / ++	++ / +++	p<0.001
bulbous nuclei	+	0 / +	p<0.001
naked nuclei	+	0 / +	p=0.01

(Mann-Whitney U test)

Analisi dello stato mutazionale di JAK2 nelle BOM all'esordio

	e-PV	pp-PV	ET	*p*
V617F mutational status n (%)	16 /16 (100)	18 / 19 (95)	7 / 13 (54)	0.0007
heterozygous, *n* (%)	10 (63)	13 (73)	6 (85)	
homozygous, *n* (%)	6 (37)	5 (27)	1 (15%)	



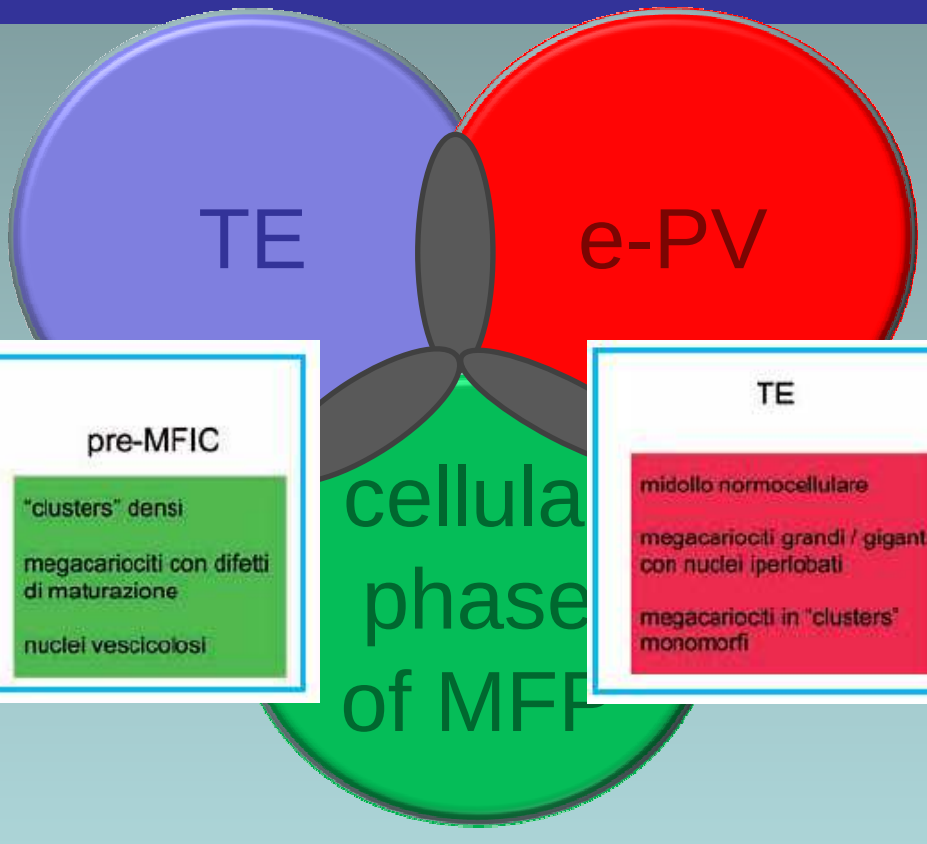
Gianelli et al. Am J Clin Pathol 2008;130:336-342

Conclusioni

- Gli algoritmi diagnostici proposti dalla WHO (2008), basati sulle informazioni cliniche, sullo stato mutazionale di JAK2 e sul quadro istologico consentono di classificare correttamente i pazienti con NMP nella grande maggioranza dei casi

- Esiste una “zona grigia” nella diagnosi differenziale delle NMP che è legata soprattutto alla precocità della diagnosi e che può essere in parte chiarita attraverso un’analisi accurata della morfologia osteomidollare

Conclusioni



TE		pre-MFIC
"clusters" lassi megacariociti grandi / giganti nuclei iperlobati	≠	"clusters" densi megacariociti con difetti di maturazione nuclei vescicolosi

TE		pre-PV
midollo normocellulare megacariociti grandi / giganti con nuclei iperlobati megacariociti in "clusters" monomorfi	≠	perplasia tri-lineare iperplasia e "left shifting" serie eritro-e granulopoietica megacariociti in "clusters" polimorfi

In particolare attraverso il riconoscimento di "patterns" istologici !!!


GRAZIE !