

**JAK2 V617F in
chronic myeloproliferative and
myelodysplastic/myeloproliferative
diseases**

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JAK2

Janus kinase (JAK)

- family of non-receptor tyrosine kinases
- 4 members: JAK1, 2 and 3, TYK2 (tyrosine kinase 2)
- 2 tyrosine kinase domains: one active and one catalytically inactive but autoinhibitory
- 2-headed Roman god of beginnings and doorways
- binding to type I cytokine receptors
- cloning in 1989

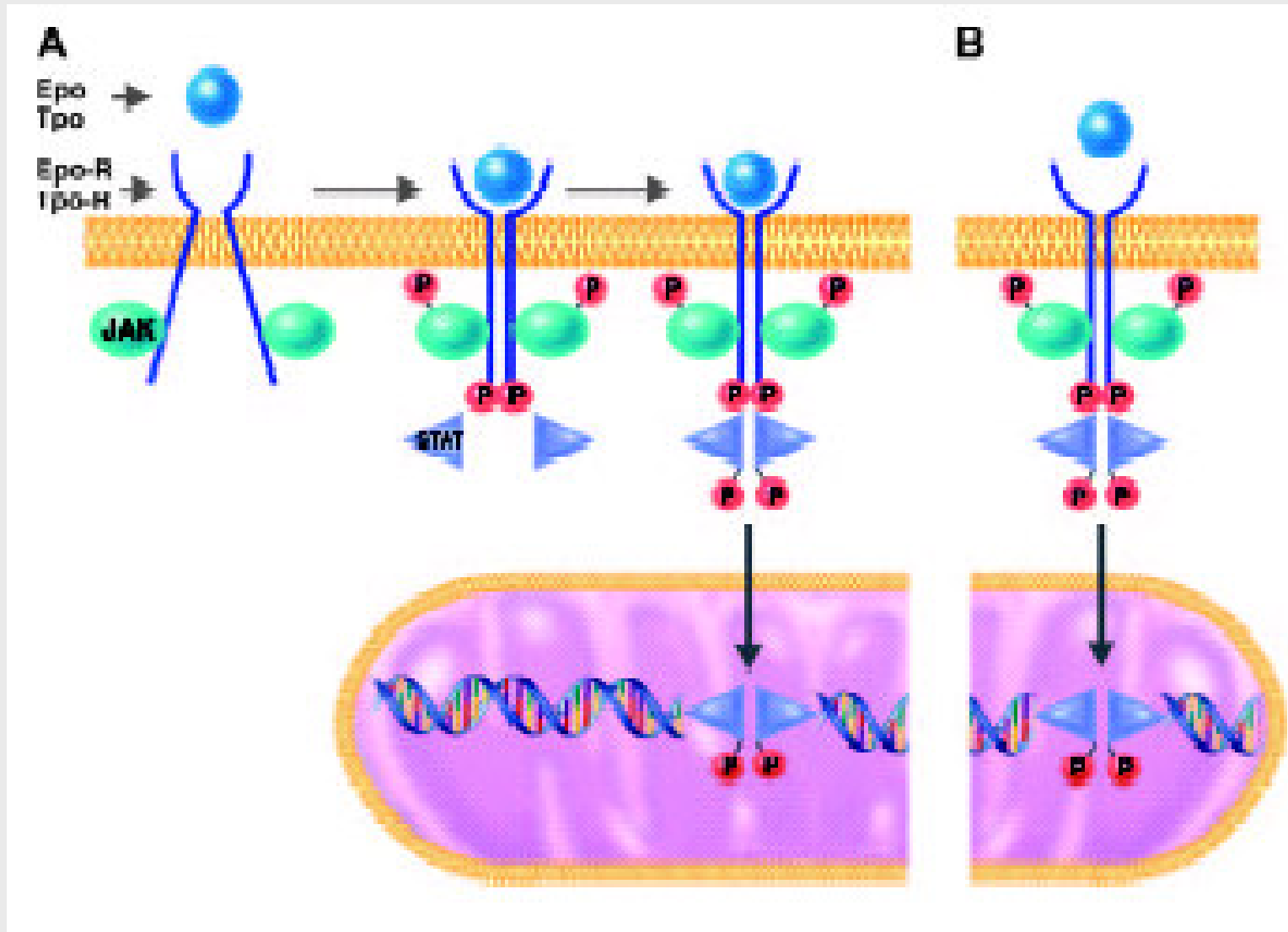
JAK2

- binding to EPO, TPO, GM-CSF, IL3 and IL5 receptors

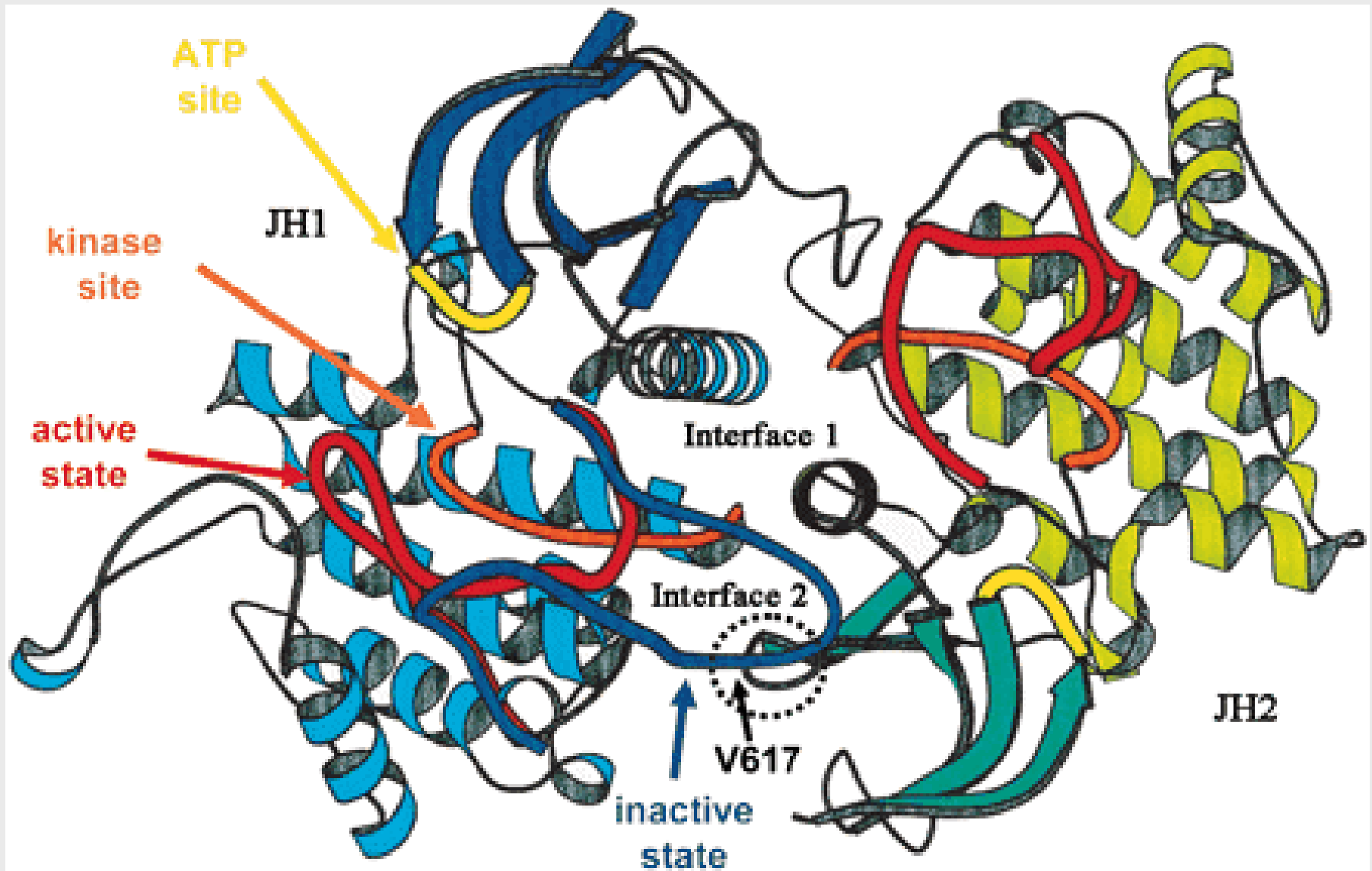
JAK2 V617F

JAK2 Val617Phe

- reported by 4 groups March/April 2005 in polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIM)
- bp 1849 G to T in exon 12, valine to phenylalanine in AA 617
- located in the pseudokinase autoinhibitory domain resulting in constitutive activation
- acquired: heterozygous, homozygous
- induces erythrocytosis (James et al, 2005) and a PV-like disease (Wernig et al, Lacout et al, 2006) in a murine bone marrow transplant model



Schafer A. Blood 2006;107:4214



Kaushansky K. Blood 2005;105:4187

Frequency in PV, ET and CIM

	PV	ET	CIM
Baxter et al (a)	71/73 (97)	29/51 (57)	8/16 (50)
Levine et al (b)	121/164 (74)	37/115 (32)	12/46 (35)
James et al (b)	40/45 (89)	9/21 (43)	3/7 (43)
Kralovics et al (b)	83/128 (65)	21/93 (23)	13/23 (57)
Zhao et al (b)	20/24 (83)	-	-
Jones et al (a)	58/72 (81)	24/59 (41)	15/35 (43)
Total	393/506 (78)	120/339 (35)	51/127 (40)

(a) allele-specific PCR, (b) sequencing as detection method mutation
absent in 45 secondary erythrocytosis and 668 healthy

Mutation status

- homozygous: predominance (variable cut-off) of mutant signal heterozygosity/mixed clonality
- homozygosity results of duplication of mutant allele (during mitotic recombination) and not of deletion of wild type allele
- frequency of homozygosity (Baxter-Levine-James-Kralovics):
PV 109/410 (27 %), ET 6/280 (2 %), CIM 12/92 (13%)
- correlation with duration of disease suggesting a two-step process?: yes (Levine, Kralovics) – no (Baxter, Tefferi 2006)
- clinical correlations in PV - homozygous vs heterozygous (Tefferi et al 2006)
 - with Hb at diagnosis, fibrotic transformation
 - not with PLT and WBC at diagnosis, bleeding, thrombosis and acute leukemia transformation

Clinical and pathologic correlations in ET

Wolanskyj et al (W), Br J Haematol 2005, n=150

Campbell et al ©, Lancet 2005, n=806

JAK2 V617F in ET is associated

- with: higher Hb and WBC, venous thrombosis ©, PV transformation
- not with: bleeding, arterial thrombosis ©, thrombosis (W), MF and AML transformation

Interpretation: JAK2 V617F + ET and PV form a biological continuum with the degree of erythrocytosis determined by physiological and genetic modifiers

Also association with increased erythro- and granulopoiesis, lower serum EPO and ferritin, sensitivity to hydraea ©

JAK2 V617F and PRV1 over-expression

Consensus

- in PV: homozygous JAK2 V617F is correlated with higher PRV1 expression
- in PV: JAK2 V617F is correlated with increased PRV1 expression

No consensus

- in ET and CIM on the correlation between JAK2 V617F and increased PRV1 expression
- In PV, ET and CIM: is PRV1 over-expression a direct consequence of JAK2 activation or represents it a general state of granulocyte activation (not necessarily linked to JAK activation)?

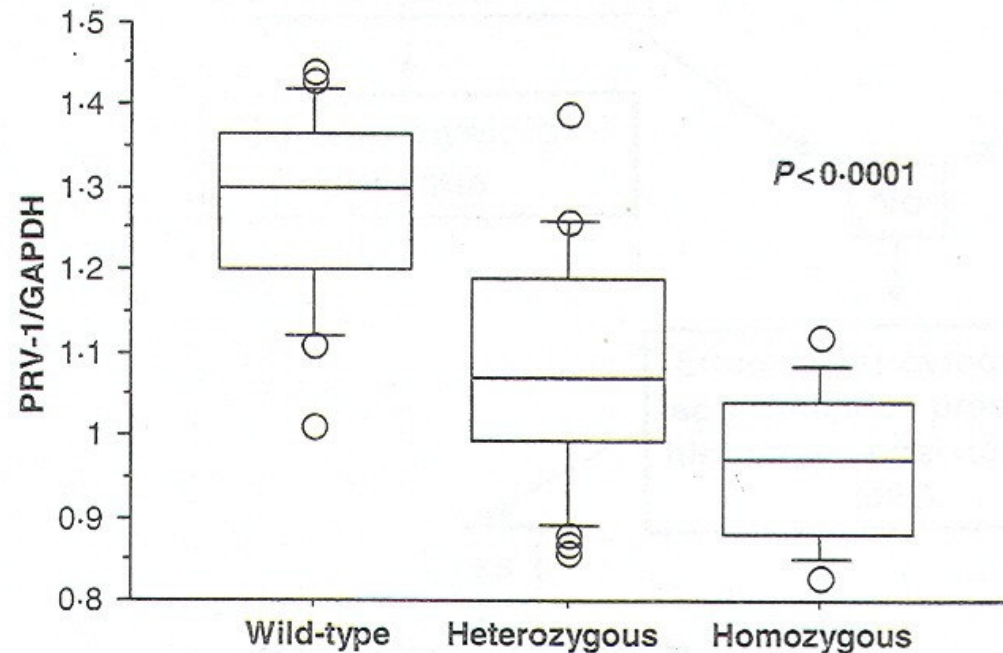


Fig 1. Distribution of granulocyte polycythaemia rubra vera-1 (PRV-1) and glyceraldehyde-3-phosphate dehydrogenase ratio in 70 patients with myeloproliferative disorders including 38 with polycythaemia vera, 22 with essential thrombocythemia and 10 with agnogenic myeloid metaplasia. The figure displays a significantly altered PRV-1 expression based on mutation status for *JAK2*; wild-type versus heterozygous *JAK2*^{V617F} versus homozygous *JAK2*^{V617F}. Boxes enclose values within the first and third quartile ranges divided by a line representing the median. Whiskers indicate 1.5 × the interquartile range above and below the 75th and 25th percentile.

PRV-1 expression in erythrocytosis RQ-PCR literature

	Cells	Vaquez	SE/RE
Kralovics, 2003	granulocytes	21/23 (91%)	
Liu, 2003	granulocytes	9/13 (69%)	
Klippel, 2003	granulocytes	68/68 (100%)	0/7
Fruehauf, 2003	granulocytes	12/12 (100%)	0/7
Tefferi, 2003	granulocytes	25/30 (83%)	4/22 (18%)*
Florensa, 2004	granulocytes	10/11 (91%)	
Spinelli, 2002	WBC	15/15 (100%)	
Ricksten, 2002	WBC	23/26 (89%)	
Cilloni, 2004	WBC	34/34 (100%)	0/12
Total	granulocytes/WBC	217/232 (94%)	4/48 (8%)
our study	WBC	14/16 (88%)	0/7

* 1 lung disease, 1 testosterone, 2 idiopathic

PRV-1 expression in thrombocytosis and myelofibrosis

RQ-PCR literature

		ET	secondary T	myelofibrosis
Kralovics, 2003	granulocytes	10/15 (67%)	hereditary T +!	4/6 (67%)
Liu, 2003	granulocytes	2/12 (17%)		
Fruehauf, 2003	granulocytes	1/8 (13%)		
Tefferi, 2003	granulocytes	3/14 (21%)		5/12 (42%)*
Florensa, 2004	granulocytes	10/17 (59%)		
Ricsten, 2002	WBC	14/52 (27%)		
Johansson, 2003	WBC	17/10 (24%)		
Cilloni, 2004	WBC	32/32 (100%)	0/16	
Klippel, 2004	WBC	14/53 (26%)		
	granulocytes	24/53 (46%)		
Total	WBC/granulocytes	103/273 (38%)	0/16	9/18 (50%)
our study	WBC	3/8 (38%)		

* Positives in “spent” phase of Vaquez

Conclusion of PRV-1

Quantification of PRV-1 mRNA

In erythrocytosis *

- highly sensitive (94%) for Vaquez
- highly specific (>92%) for Vaquez

WHO criterium? : surrogate for EEC or additional facultative major (A) criterium

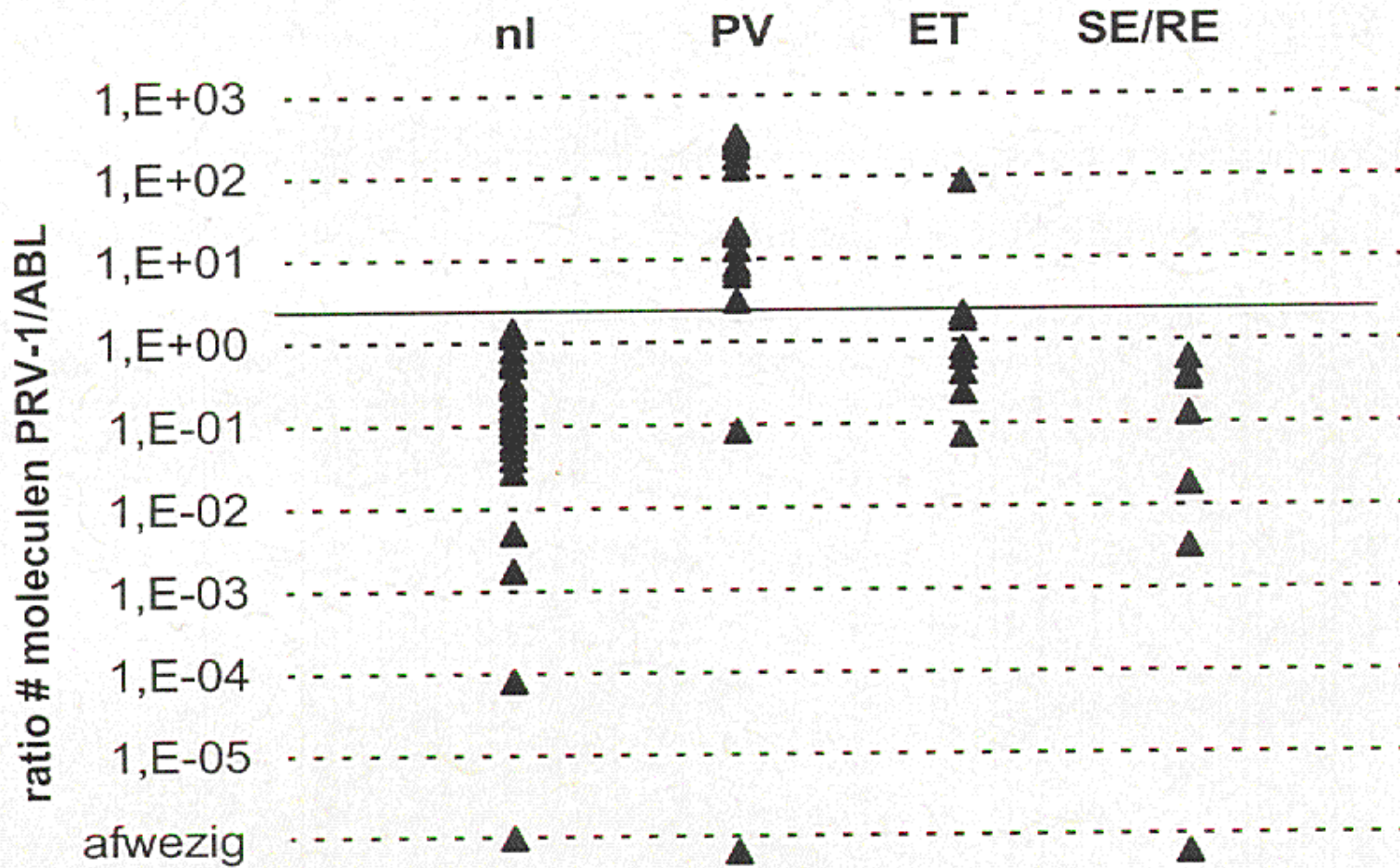
In thrombocytosis (Vaquez excluded) *

- highly specific (100%) for ET (or Vaquez?) – study of more cases
- sensitivity: 33% (13-100%) – technical standarization?
- good correlation with EEC, clonality and erythropoietin assays

PRV1+, EEC+, monoclonal ET: more aggressive subgroup – higher frequency for emergence of Vaquez?

* exclusion of acute neutrophilia (polytrauma, surgery, infection)

Results PRV-1 expression study

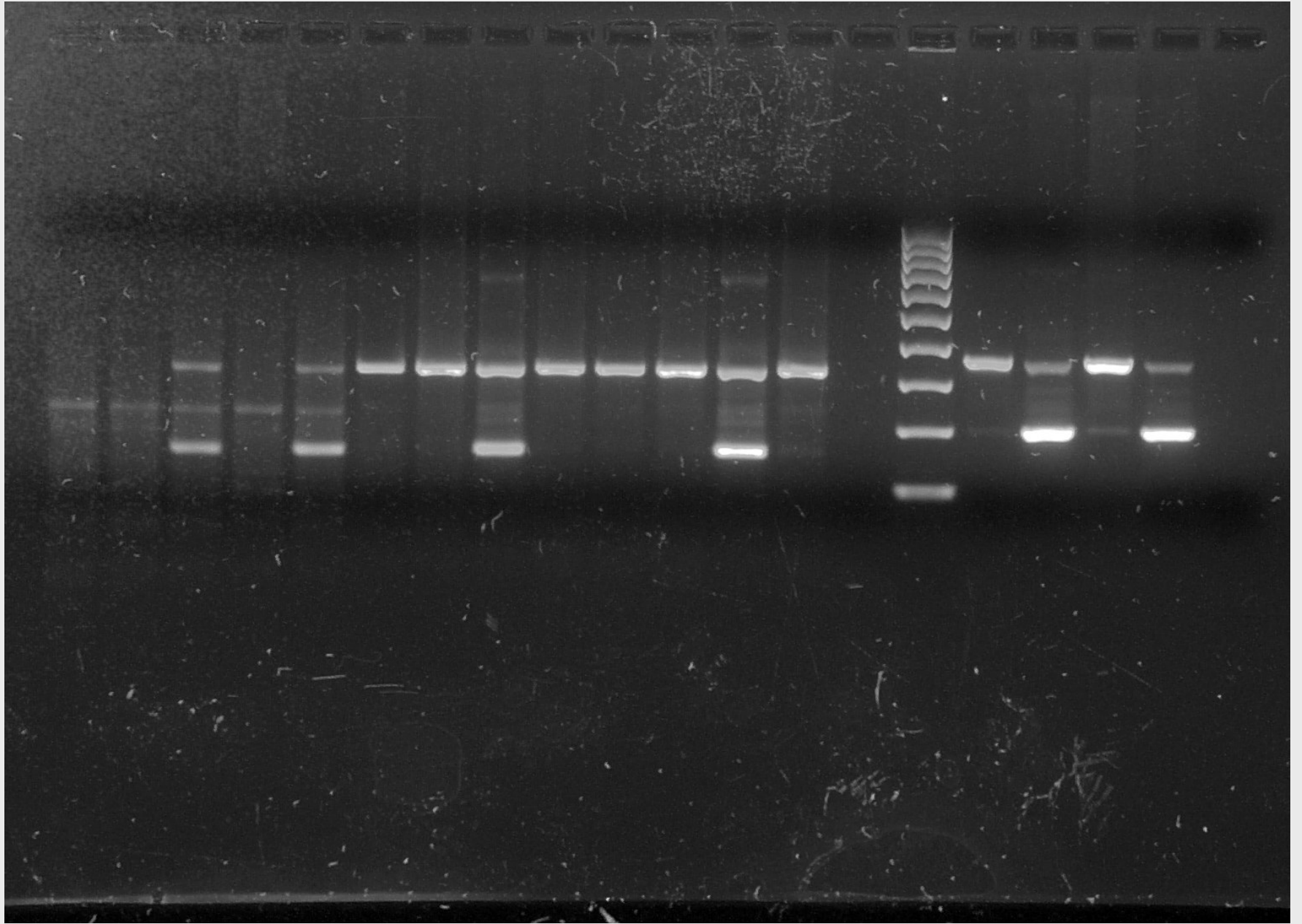


Mutation Detection

- *blood* or bone marrow
- Granulocytes or *all cells*
- DNA or RNA
- *Allele specific PCR* or RFLP or sequencing

Baxter et al, Lancet 2005;365:1054 and Nollet

- reverse primer: common
forward primer 1: mutant specific: 203 bp
forward primer 2 mutant+WT (internal control): 364 bp
(amplicon lengths for DNA primers)
- DNA primer seq. (Baxter), RNA primer seq. (Nollet)
- Agarose electrophoresis
- Sensitivity: +/- 1%



our results in PV and ET

	PV	ET
JAK2+	21/24 (88 %)	5/14 (36 %)
PRV1+	18/21 (86 %)	5/13 (38 %)
JAK2+ PRV1+	16	3
JAK2+ PRV1-	2*	1
JAK2- PRV1+	1	2
JAK2- PRV1-	1	7

* (1 congenital PRV1 deficiency?)

Clinical utility of JAK2 assay in PV and ET

Diagnosis of PV and ET

- diagnosis is complex and expensive
- JAK2 V617F assay
 - is simple
 - inexpensive
 - clonality marker independent of gender
 - clinical specificity for clonal disease 100% but not for type of disease
 - clinical sensitivity for PV 75 à 90%, for ET 33 à 50%

FRONTLINE TEST – PRV1: abandon or 2° line (in ET)

Prognosis of PV and ET:

- PV: none?
- ET: venous thrombosis? sensitivity to hydrea

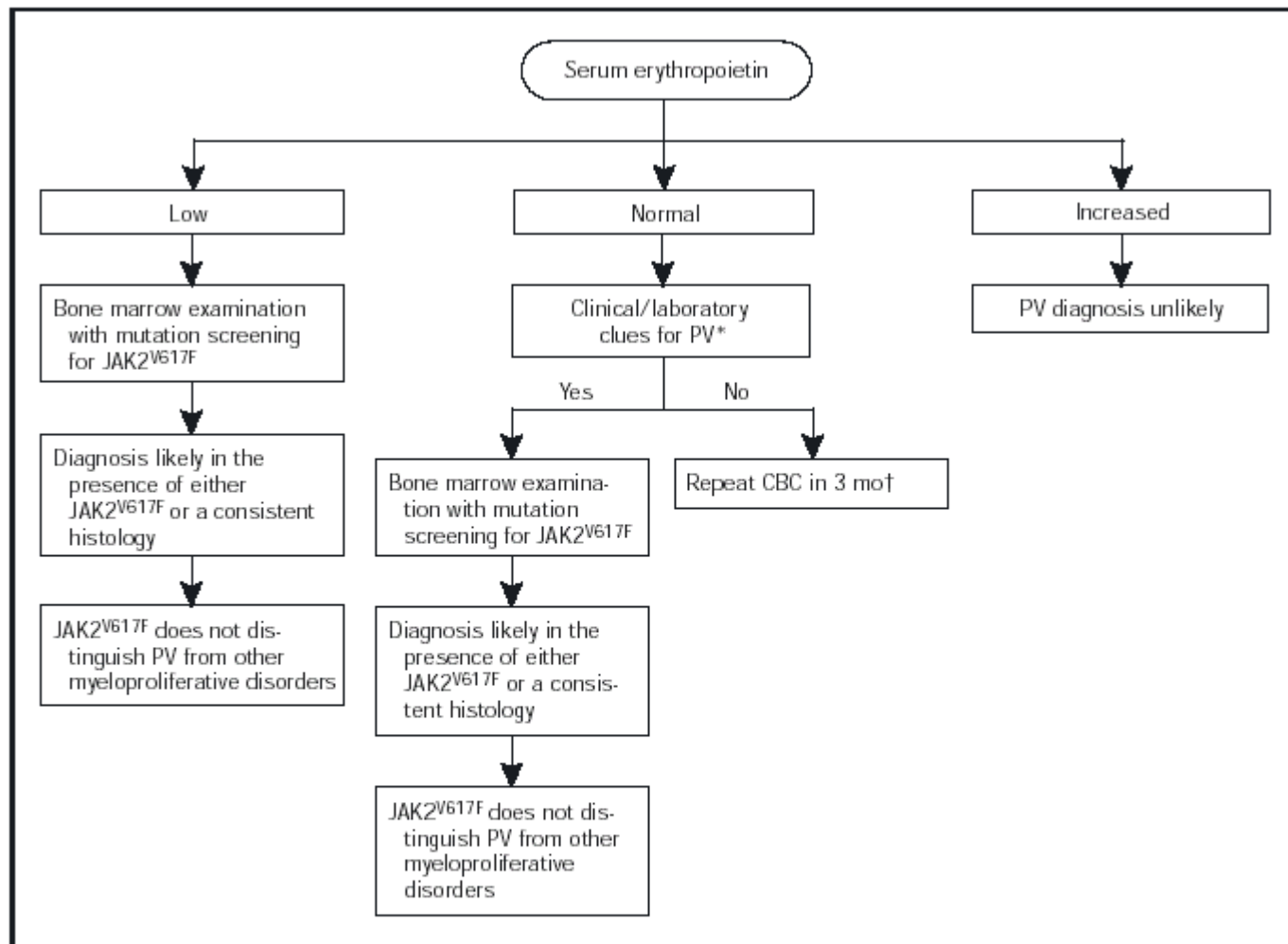


FIGURE 1. Diagnostic algorithm for polycythemia vera (PV).

*Clinical clues for PV include splenomegaly, thrombosis, aquagenic pruritus, and erythromelalgia. Laboratory clues for PV include thrombocytosis, leukocytosis, and increased leukocyte alkaline phosphatase score. Janus kinase 2 (JAK2) screening is to detect the V617F mutation that occurs in most patients with PV. BM = bone marrow; CBC = complete blood cell count; MPD = myeloproliferative disorders.

†Alternatively, one can consider mutation screening for JAK2^{V617F} to help decide necessity of BM examination.

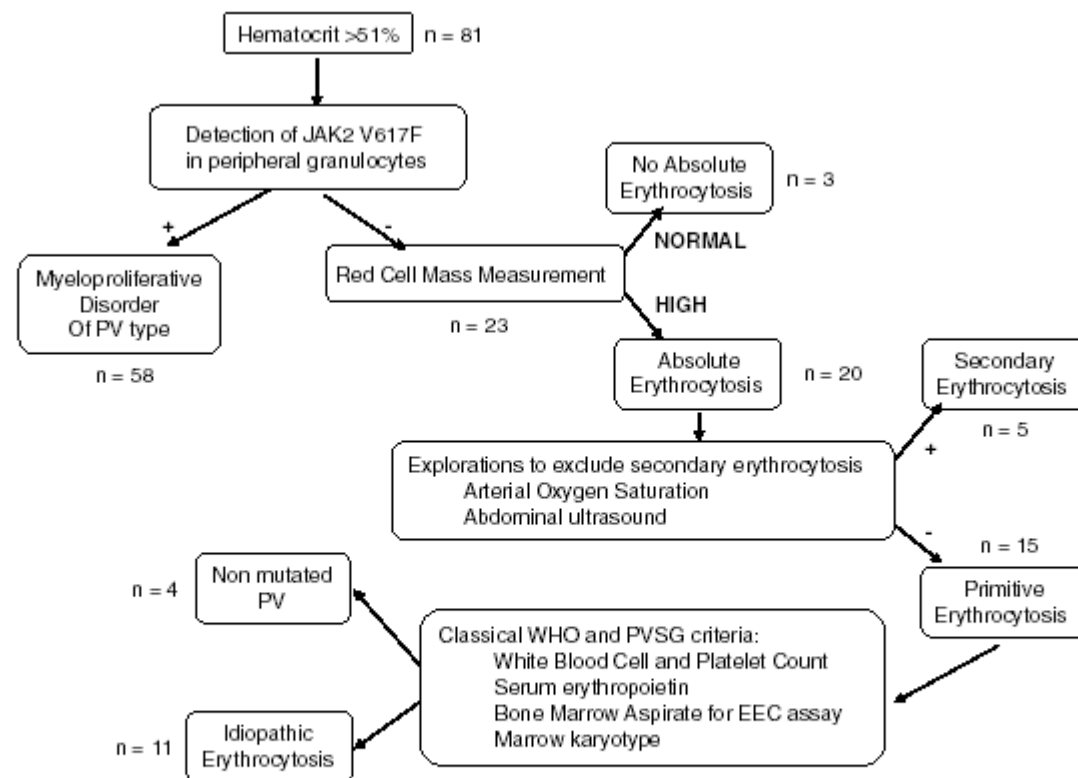


Figure 2 Diagnostic chart proposed for the diagnosis of an erythrocytosis (i.e. hematocrit value above 51%). The number of patients concerned at each step of the chart is written next to each item (n), only the patients having had all clinical data being listed here (n= 81). The detection of JAK2 V617F as a first intention diagnostic test would have avoided 58/81 patients to have other investigations to diagnose a myeloproliferative disorder of PV type.

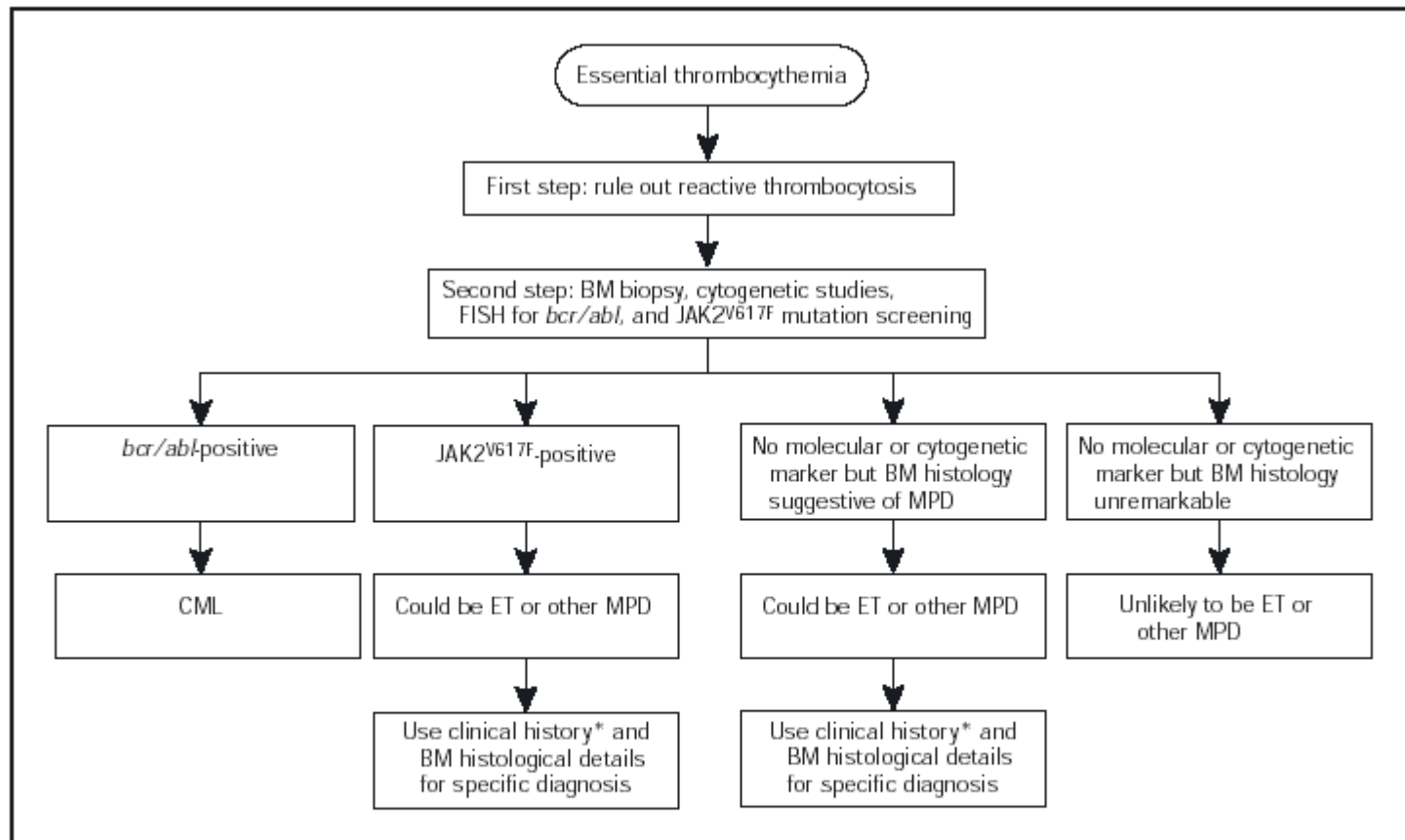


FIGURE 2. Diagnostic algorithm for essential thrombocythemia (ET) that incorporates *JAK2^{V617F}* mutation screening.

*In addition to clinical history, laboratory tests that are helpful in distinguishing reactive thrombocytosis from ET include serum ferritin, peripheral blood smear, and C-reactive protein. BM - bone marrow; CML - chronic myeloid leukemia; FISH - fluorescent in situ hybridization; *JAK2* - Janus kinase 2; MDS - myelodysplastic syndrome; MMM - myelofibrosis with myeloid metaplasia; MPD - myeloproliferative disorder; PV - polycythemia vera.

JAK2 V617F in other myeloid tumours

CMPD

- CML: 0/90, 0/99
- CNL: 1/6 (17%), 2 case reports, 1/6 (own results)
- HES: 0/11, 2/134

MDS/MPD

MDS

- total frequency (6 studies): 11/310 (3.5%)
- no association with fibrosis (Kremer et al, 2006)
- isolated 5q- syndrome: 6/97 (6.2%)

AML

- de novo AML: 1/219 (0.5%)
- AML, M0-M6: 0/28
- AML/M7: 2/11 (18%)

JAK2 V617F in lymphoid and non-hematological tumours

CLL: 0/5, 0/45

ALL: 0/20, B-ALL: 0/83, T-ALL: 0/93

NHL: 0/117

Hodgkin: 0

Non-haematological cell lines: 0/486

2005: Jones et al, Steensma et al, Levine et al, Scott et al,
Jelinek et al, Johan et al, Ohyashiki et al, Mc Lornan et al
Tono et al

2006: Kremer et al, Lea et al, Lee et al, Ingram et al, Melzner
et al

JAK2 V617F in aCML/CMML

Frequency

- aCML: 3/16 (19%), 0/8 (own results)
- CMML: 11/223 (4.9%, 4 studies), 2/40 (own results)
- aCML/CMML: 9/115 (8.0%, Levine et al)

Phenotype CMML (Levine - own results)

- WBC > $13.0 \times 10^9/l$: 5/8 – 2/2
- high PLT count: 0/8 – 2/2 (> $600 \times 10^9/l$)

The V617F JAK2 MUTATION IN
MYELOYDYSPLASTIC/MYELOPROLIFERTATIVE DISEASES IS
ASSOCIATED WITH MARKED THROMBOCYTOSIS

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Poster presented at the 2006 annual meeting of the BHS

Diagnosis	Hb (g/dl)	MCV (fl)	WBC (x 10E9/)	Monocytes (%)	PLT (x 10E9/)	Ringed sideroblasts (%)	karyotype
RARS-t	10.9	111	8.4	5	719	50	46,XY
RARS-t	9.2	96	6.1	4	1116	35	46,XX
RARS-t	14.4	92.5	10.5	6	773	50	46,XY
RARS-t	10.4	87.1	16.9	4	684	50	in process
CMML	14.5	83	44.2	16	741	0	46,XY
CMML	10.2	106.9	14.6	49	628	4	46,XY

JAK2 V617F in RARS with marked thrombocytosis (RARS-T)

- WHO provisional entity in the MDS/MPD, unclassifiable group
- Defined by
 - RARS ($\geq 15\%$ ringed sideroblasts) and
 - platelet count $> 600 \times 10^9/l$ (and megakaryocyte proliferation)
- Frequency of JAK2 V617F
 - 3/3 (Remacha et al, Haematologica May 2006)
 - 9/10 (Gattermann, Billiet et al, submitted to Blood)
- Truly mixed myelodysplastic/myeloproliferative disorder caused by a dysplastic mitochondrial mutation in JAK2 V617F myeloproliferative cells?

