

1 **Abstract**

2 Masked polycythaemia vera (PV) has been proposed as a new entity with poorer
3 outcome than overt-PV. In the present study, the initial clinical and laboratory
4 characteristics, response to treatment and outcome of masked and overt-PV were
5 compared using red cell mass and haemoglobin or haematocrit levels for the distinction
6 between both entities. Sixty eight out of 151 PV patients (45%) were classified as
7 masked-PV according to World Health Organisation diagnostic criteria, whereas 16
8 (11%) were classified as masked-PV using the British Committee for Standards in
9 Haematology (BCSH). In comparison with overt-PV, a higher platelet count and a
10 lower *JAK2V617F* allele burden at diagnosis were observed in masked-PV. Patients
11 with masked-PV needed lower phlebotomies and responded faster to hydroxycarbamide
12 than those with overt-PV. Complete haematological response was more frequently
13 achieved in masked than in overt-PV (79% versus 58%, $p=0.001$). There were no
14 significant differences in the duration of haematological response, the rate of resistance
15 or intolerance to hydroxycarbamide and the probability of molecular response according
16 to type of PV (masked versus overt). Overall survival, rate of thrombosis and major
17 bleeding, and probability of transformation was superimposable among patients with
18 masked and overt-PV.

19

Introduction

In the current World Health Organization (WHO) and British Criteria for Standards in Haematology (BCSH) classifications, haemoglobin and haematocrit cut-offs are used as major diagnostic criteria, allowing to establish the diagnosis of polycythaemia vera (PV) without the need of red cell mass measurement (Tefferi et al 2007, McMullin et al 2007). However, the drawback of this approach is the possibility of PV misdiagnosis due to the lack of sensitivity of the selected thresholds. Using the WHO criteria up to 45% of true PV patients would not meet the diagnostic criteria if red cell mass is not measured (Alvarez-Larrán et al 2012a, Barbui et al 2014a Barbui et al 2014b). The sensitivity of the BCSH cut-offs improved the diagnostic accuracy of PV but a proportion of patients with true PV still showed a haematocrit below 52% in men or 48% in women as required in the major criteria (Ancochea et al 2014).

Recently, it has been proposed the term "masked PV" for those patients not meeting the cut-offs of haemoglobin and haematocrit established in the WHO and the BCSH criteria, but showing histological data characteristic of PV (Barbui et al 2014a, Barbui et al 2014b). Furthermore, it has been reported that such patients with masked PV have differences in the initial clinical characteristics and a higher probability of transformation to myelofibrosis (MF) or acute leukemia, as well as a poorer survival (Barbui et al 2014a, Barbui et al 2014b). These data, not confirmed by independent groups, have generated uncertainty among clinicians since the term masked PV has been classically ascribed with an initial or pre-clinical phase of the disease (Shih & Lee 1995, Thiele et al 2005, Kvasnicka & Thiele J. et al 2010). In addition, the diagnosis of PV in such studies relied on histology, with red cell mass not measured in those cases with haemoglobin or haematocrit values below the PV criteria.

1 In the present study, 151 PV patients in whom an increased red cell mass had
2 been demonstrated in those cases with haemoglobin or haematocrit values below the cut
3 offs established by the WHO, were stratified as overt PV or masked PV according to
4 WHO and BCSH criteria. The aim of this study was to compare the presenting clinical
5 characteristics, response to treatment and complications during follow-up between
6 patients with masked and overt PV.

7

1 **Patients and methods**

2 **Selection of cases and original diagnosis**

3 One hundred and fifty one patients with polycythaemia vera consecutively
4 diagnosed and homogeneously treated since from 1985 at the Haematology Department
5 of the Hospital del Mar were included. The original diagnosis of PV was established
6 according to the PVSG before 2001 and the WHO criteria thereafter. An increased red
7 cell mass was demonstrated in all patients diagnosed with PV but not achieving the
8 haemoglobin threshold defined in the WHO classification.

9 For the purpose of the study patients were retrospectively classified as WHO-
10 masked PV if they did not fulfil the haemoglobin thresholds defined in the WHO
11 criteria (haemoglobin < 18.5 g/dL in men or < 16.5 g/dl in women) while the remaining
12 patients were classified as WHO-overt PV. According to BCSH criteria, patients were
13 classified as masked PV when the haematocrit was < 0.52 L/L in men or < 0.48 L/L in
14 women. The diagnosis of postpolycythaemic myelofibrosis was established when
15 constitutional symptoms, anaemia, increasing splenomegaly, leukoerythroblastic picture
16 and fibrosis of the bone marrow were present according to the criteria proposed by the
17 International Working Group for Myelofibrosis Research and Treatment (Barosi *et al*,
18 2008).

19 The following initial clinical and laboratory characteristics were compared
20 between masked PV and overt PV: age, gender, history of thrombosis, haematological
21 parameters including red blood cell count, haemoglobin, haematocrit, mean corpuscular
22 volume, leucocyte count, platelet count, ferritin, serum erythropoietin, lactate
23 dehydrogenase, megakaryocytic and erythroid endogenous colony formation and
24 *JAK2V617F* allele burden. *JAK2V617F* allele burden was assessed in 103 patients at
25 diagnosis in DNA obtained from purified granulocytes by real-time allele-specific

1 polymerase chain reaction with probes specific for the mutated and the wild type forms.
2 The *JAK2V617F* allelic ratio was calculated as the percentage of the number of copies
3 of *JAK2V617F*/total number of *JAK2* copies (*V617F*+ wild type).

4 Patients younger than 60 years without history of thrombosis were managed with
5 phlebotomies and low-dose acetyl salicylic acid. Cytoreductive therapy was started in
6 patients older than 60 years or in those with thrombosis history. Other indications of
7 cytoreduction included progressive splenomegaly and leucocytosis, disease-related
8 symptoms not controlled by phlebotomies or antiplatelet therapy and high phlebotomy
9 requirements.

10 In those patients treated with hydroxycarbamide, the haematological parameters
11 included in the 2013 European LeukemiaNet (ELN) response criteria were used to
12 define complete haematological response (CHR) as the achievement of haematocrit <
13 0.45 L/L without phlebotomy, leukocyte count $\leq 10 \times 10^9/L$ and a platelet count <
14 $400 \times 10^9/L$ (Barosi et al 2013). Taking into account the retrospective nature of the
15 study, assessment of disease-related symptoms and palpable splenomegaly were not
16 considered in the definition of CHR. Partial response was defined as the absence of
17 CHR but achievement of a haematocrit less than 0.45 L/L without phlebotomy.
18 Molecular response was evaluated in 77 patients. Quantitative allele specific PCR for
19 the *JAK2V617F* mutation was performed prior to hydroxycarbamide and every 6
20 months thereafter. In these cases, definition of molecular response was categorized
21 according to 2013 ELN criteria with complete molecular response defined as
22 eradication of the *JAK2V617F* mutation and partial response as > 50% decrease in allele
23 burden provided that the allele burden is > 20% (Barosi et al 2013). Resistance and
24 intolerance to hydroxycarbamide was evaluated according 2010 ELN criteria (Barosi et
25 al 2010)

1 Informed consent for the scientific use of the patients' clinicohaematological
2 data and biological samples was obtained in accordance with the requirements of the
3 Hospital del Mar ethics committee.

4

5 **Statistical analysis**

6 Categorical and continuous variables were compared using the Chi-square test
7 and the student test, respectively. Time to haematological and molecular response,
8 durability of response, survival and time to thrombosis, major bleeding and
9 transformation were assessed using the Kaplan-Meier method. Variables predicting time
10 to response, time to event or survival were analysed using the log rank test.

11

Results

Clinical and laboratory characteristics at diagnosis

Sixty eight out of 151 patients (45%) did not reach the haemoglobin thresholds defined by the WHO criteria and were classified as WHO-masked PV whereas 16 (11%) were classified as masked PV according to the haematocrit cut-offs defined by the BCSH. The main clinical and laboratory characteristics at diagnosis of masked and overt PV using WHO and BCSH criteria are shown in table I. As can be seen there were no statistically significant differences among masked and overt PV regarding age, gender, history of thrombosis, microvascular symptoms, pruritus, thrombosis at diagnosis, or presence of palpable splenomegaly with either of the classifications. The mean corpuscular volume of red blood cells and the serum ferritin level was similar among masked and overt PV. Masked PV showed significantly higher platelet counts than those with overt PV whereas no significant differences in the leucocyte counts were observed. Low serum EPO level was more frequently observed in patients with overt PV than in patients with masked PV according to WHO criteria but not with BCSH criteria.

The *JAK2* mutational status was available in 143 patients, with the V617F mutation and exon 12 mutations being detected in 138 and 5 cases, respectively. The *JAK2V617F* allele burden at diagnosis was available in 103 patients, being lower in patients with masked than in those with overt PV, however the difference was statistically significant only when using the BCSH criteria (table 1).

Treatment

First line treatment with phlebotomies was indicated in 53 patients whereas 119 were started on cytoreduction. In 15 additional cases complete therapy data was not

1 available. Antiplatelet therapy was administered in 125 cases, 87 as primary prophylaxis
2 and 38 as secondary prophylaxis. Twenty-six patients received oral anticoagulation.

3 In those patients initially managed with phlebotomies, phlebotomy requirements
4 were significantly lower in WHO-masked PV than in WHO-overt PV, especially in the
5 first three years after diagnosis. Thus, median number of phlebotomies in the first year
6 was 8 in overt PV and 5 in masked PV ($p=0.001$), in the second year 5 in overt PV and
7 2 in masked PV ($p=0.002$) and in the third year 4 procedures in overt PV and 1 in
8 masked PV ($p=0.04$). From fourth to tenth years of treatment, phlebotomy requirements
9 decreased in both groups of patients, with WHO-masked PV patients being easily
10 managed with a median of one annual phlebotomy, however the difference with overt
11 PV was not statistically significant. Regarding BCSH-masked PV, significant
12 differences in phlebotomy requirements were only observed in the first year of
13 treatment (median number of 7 procedures in overt-PV versus 4 in masked PV, $p=0.02$)
14 but not afterwards.

15 Hydroxycarbamide was started in 112 patients, 79 as first line and 33 as second-
16 line-treatment, usually after a variable period of phlebotomies. The median starting dose
17 was 857 mg/day (Range: 500-1500) and the median duration of treatment 3.4 years
18 (Range: 17 days-25 years). Median starting dose in patients with WHO-masked PV was
19 750 mg /day (range: 500-1000) in comparison with 1000 mg/day (Range: 500-1500) in
20 WHO overt PV, $p=0.06$.

21 Two patients had a haematocrit below 0.45 L/L at the time of hydroxycarbamide
22 start. Eighty-five out of the 110 (77%) remaining patients achieved a stable haematocrit
23 < 0.45 L/L without phlebotomy requirement after a median of 173 days on therapy.
24 Time to response in haematocrit was significantly shorter in patients with WHO-masked
25 PV than in those with WHO-overt PV (128 days versus 287 days, $p<0.001$, figure 1a).

1 Regarding patients classified as BCSH-masked or BCSH-overt PV, there were no
2 significant differences in time to haematocrit response (172 days in masked PV versus
3 173 days in overt PV, $p=0.4$). Twenty four patients lost the response in haematocrit
4 during follow-up with the probability of maintaining the haematocrit response at 2 years
5 being 75%. There were no statistically significant differences in the duration of
6 haematocrit response according to type of PV using both WHO and BCSH criteria.

7 Response in leucocyte and platelet count was observed in 89% and 88% of
8 patients, respectively. Median time to response in leucocyte count was 91 days whereas
9 response in platelet count was achieved after a median of 99 days. Regarding leucocyte
10 and platelet count response, there were no significant differences in time to response or
11 in the duration of response according to type of PV (masked versus overt) using both
12 WHO and BCSH criteria.

13 CHR was achieved in 68% of patients after a median time of 228 days on
14 hydroxycarbamide. Median dose at time of response achievement was 1000 mg
15 (Range: 250-1750) being similar among masked and overt PV patients. Seventy-nine
16 percent of WHO-masked PV achieved a CHR after a median of 173 days in comparison
17 with 58% of WHO-overt responders needing a median of 496 days ($p=0.001$, figure 1b)
18 According to BCSH criteria, CHR was achieved by 75% and 67% of patients with
19 masked and overt PV, respectively. No significant differences were observed in time to
20 CHR according to BCSH criteria (173 days in masked PV versus 241 days in overt PV,
21 $p=0.2$) or in the duration of CHR according to either WHO or BCSH criteria.

22 Molecular response to hydroxycarbamide was assessed in 77 patients. With a
23 median molecular follow-up of 3.5 years (Range: 6 months- 14 years), no complete
24 molecular response was registered. Partial molecular response was observed in 24
25 (31%) patients, being the probability of response at 1 and 2 years 22% and 28%,

1 respectively. There were no significant differences in molecular response according to
2 type of PV (masked versus overt) either in WHO or BCSH-defined patients.

3 Maintenance hydroxycarbamide dose at last visit was 857 mg (250-1500) with
4 44 patients being in CHR, 28 in partial response and 40 with no response. Resistance to
5 hydroxycarbamide according to ELN criteria was observed in 22 cases, including 1
6 patient with haematocrit > 0.45 L/L despite 2 g/day of hydroxycarbamide and 21
7 patients with cytopenias at the minimum dose to achieve response. Median time to
8 resistance in these 22 patients was 5 years (Range: 56 days-15 years). There were no
9 significant differences in time to resistance according to type of PV (overt versus
10 masked). Intolerance to hydroxycarbamide was registered in 24 patients (leg ulcers
11 $n=12$, oral ulcers $n=9$, other $n=3$) Median time to intolerance in these 24 patients was 4
12 years (range: 17 days-14 years). There was a trend towards a shorter time to intolerance
13 in patients with WHO-overt PV than in those with WHO-masked PV (Kaplan-Meier
14 estimated median time to intolerance: 14 years versus 9 years, $p=0.08$). No significant
15 differences were observed according to BCSH criteria.

16 17 Survival, vascular complications and transformation

18 With a median follow-up of 6.4 years (Range: 0.1-26), a total of 40 patients died
19 resulting in a median projected survival of 18 years. There was no difference in survival
20 according to type of PV (masked versus overt) using both WHO and BCSH criteria
21 (figure 2). The probability of thrombosis and major bleeding after diagnosis is shown in
22 table 2, without significant differences observed according to type of PV. Twenty-two
23 patients evolved into myelofibrosis being the probability of transformation at 10 years
24 11% and the projected median time to myelofibrosis 18 years. The probability of
25 myelofibrotic transformation was similar among patients with masked and overt PV

1 (table 2). Transformation to acute leukaemia/myelodysplastic syndrome was observed
2 in three additional patients, all of them belonging to the group of overt PV.

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Discussion

In the present work we have analysed baseline characteristics, response to therapy and clinical outcomes in a cohort of 151 patients with PV controlled at a single institution. Patients were classified into masked and overt PV using both the WHO and the BCSH criteria. As previously reported, patients with masked PV showed higher platelet counts than patients with overt-PV (Barbui et al 2014a, Barbui et al 2014b). Of note, iron deficiency could be discarded as an explanation of this feature, since no significant differences were observed between both groups of patients in terms of mean corpuscular red blood cell volume and serum ferritin level. By contrast, patients with masked PV showed a lower *JAK2V617F* mutational load and more frequent normal erythropoietin levels than those with overt PV suggesting that masked PV represents an intermediate clinical picture between *JAK2V617F*-positive essential thrombocythaemia (ET) and PV.

With regard to treatment, patients with WHO masked PV reached a stable haematocrit below 0.45 L/L under a shorter time on hydroxycarbamide and needed less phlebotomies to control the disease. These results were predictable given the lower values of haemoglobin at time of treatment start. In addition, treatment with hydroxycarbamide in patients with masked PV normalised the blood counts faster and more frequently than in those with overt. PV. It can be discarded that these differences could be attributed to the hydroxycarbamide dose as it was similar between both groups of patients and rather be explained by a reduced proliferative capacity of masked PV. Rates of resistance and intolerance to hydroxycarbamide were similar to previously reported (Alvarez-Larran et al, 2012b), with no differences observed among masked and overt PV.

1 A recent retrospective study including 538 young patients with *JAK2V617F*
2 myeloproliferative neoplasms showed that the probability of thrombosis was higher in
3 patients with masked PV in comparison to those with *JAK2V617F*-positive ET or overt-
4 PV (Lussana et al 2014). The authors explained these differences because most patients
5 with masked PV were managed as low-risk ET patients without receiving phlebotomies
6 or cytoreduction. In our series, including a substantial proportion of high-risk patients,
7 no differences in the frequency of thrombosis have been observed between masked and
8 overt PV, but it must be mentioned that both masked and overt PV patients were
9 managed according to PV guidelines. These data reinforce the need of maintaining the
10 haematocrit below 0.45 L/L in patients with masked-PV as it is recommended in overt
11 PV.

12 Finally, overall survival and probability of transformation were similar in
13 patients with masked and overt-PV using either WHO or BCSH diagnostic criteria. This
14 findings contrast with the worst survival and higher probability of transformation
15 reported by Barbui in patients with masked PV (Barbui et al 2014a, Barbui et al 2014b).
16 Such discrepancy could be explained by the different criteria used in patient selection.
17 In previous series, selection of patients relied on histological findings. It must be taken
18 in consideration the low reproducibility of histological criteria which may result in low
19 sensitivity and therefore in a selection bias (Peterson & Ellis 1995, Wilkins et al 2008,
20 Brousseau et al 2010, Alvarez-Larrán et al 2014). By contrast, in the present study only
21 patients with increased red cell mass or haemoglobin/haematocrit values above the
22 thresholds established by the WHO and BCSH criteria were included. This approach
23 allows the inclusion of all *JAK2V617F*-positive patients with a well-documented
24 expansion of red cell mass irrespective of the histological findings.

1 Given the current tendency to abandon the red cell mass measurement in the
2 diagnosis of PV and the diagnostic limitations of bone marrow histology, a relevant
3 question is whether it is necessary to identify patients with masked-PV or not. In this
4 sense, the use of the BCSH instead of WHO 2008 criteria would minimize this dilemma
5 since the number of masked PV would be restricted to 10-15% of patients (Barbui et al
6 2014c). In addition, there is a new proposal of modification of WHO criteria including
7 haematocrit cut-offs closer to the BCSH ones, but the final proposal of new diagnostic
8 criteria of PV has not been published yet (Tefferi et al 2014). Alternatively, a practical
9 approach could be targeting the haematocrit response to <45 L/L in all *JAK2V617F*-
10 positive myeloproliferative neoplasms including ET, masked-PV and overt-PV.

11 In conclusion, patients with masked PV have a similar clinical outcome than
12 those with overt PV and are easily managed with the standards of treatment established
13 for PV.

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No relevant conflict of interest to declare regarding this article

Authorship

Alberto Alvarez-Larrán designed the study, collected the data, performed the statistical analysis, analysed and interpreted the results and wrote the paper. Anna Angona, Agueda Ancochea and Alicia Senín collected the data and approved the final version. Francesc García-Pallarols performed the statistical analysis and approved the final version. Concepción Fernández and Raquel Longarón performed the molecular studies and approved the final version. Beatriz Bellosillo performed the molecular studies and wrote the paper. Carlos Besses designed the study, analysed and interpreted the results and wrote the paper

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Table I: Clinical and laboratory characteristics at diagnosis in 151 patients with polycythaemia vera						
	WHO 2008			BCSH		
	Masked N=68	Overt N=83	p	Masked N=16	Overt N=135	p
Age, years*	70 (20-87)	64 (29-94)	0.2	62 (34-83)	68 (20-94)	0.2
Male sex, n (%)	33 (48)	50 (60)	0.1	12 (75)	71 (53)	0.1
Thrombosis, n (%)						
Previous	14 (21)	18 (22)	0.9	5 (31)	27 (20)	0.3
At diagnosis	6 (9)	6 (7)	0.7	1 (6)	11 (8)	0.8
Palpable spleen, n (%)	20 (29)	26 (31)	0.7	5 (31)	41 (30)	0.9
Microvascular symptoms, n (%)	23 (34)	19 (23)	0.1	5 (31)	37 (27)	0.7
Pruritus, n (%)	12 (18)	15 (18)	0.9	3 (18)	24 (18)	0.9
RBC count x10 ⁶ /μL*						
Males	6.5(5.3-9.9)	7.5(5.8-9.4)	<0.001	6.1(5.3-7.1)	7.3 (5.7-8)	0.001
Females	5.8 (5.3-7.6)	6.5 (5.6-8.5)	0.001	5.7 (5.3-6.4)	6.4 (5.4-8.5)	0.09
Haemoglobin g/L*						
Males	173 (150-183)	199 (185-235)	<0.001	161 (150-182)	192 (157-238)	<0.001
Females	157 (140-164)	181 (166-217)	<0.001	152 (140-156)	166 (145-217)	0.02
Haematocrit L/L*						

Males	53 (48-62)	62 (54-73)	<0.001	49 (48-51)	61 (52-73)	<0.001
Females	50 (44-58)	56 (50-67)	<0.001	46 (44-47)	53 (48-67)	0.003
MCV fL*						
Males	84 (62-90)	85(69-97)	0.3	85(62-97)	84(70-92)	0.6
Females	84 (53-103)	82 (69-98)	0.3	77 (72-90)	82 (56-103)	0.4
Leukocyte count x10 ⁹ /L*	10.8 (4.6-38)	10.8 (6-35)	0.3	10.9 (6.6-16)	10.8 (4.6-38)	0.4
Platelet count x10 ⁹ /L*	620 (160-1103)	424 (155-1481)	<0.001	732 (210-948)	470 (155-1481)	0.001
Low ferritin, n (%)	18 (36)	28 (46)	0.3	5 (31)	41 (43)	0.4
Low EPO, n (%)	43 (68)	59 (84)	0.04	14 (87)	88 (75)	0.4
EEC, n (%)	39 (97)	53 (91)	0.2	7 (100)	85 (93)	0.3
<i>JAK2V617F</i> mutant load at diagnosis %*	49 (15-100)	64 (1-100)	0.3	34 (22-86)	60 (1-100)	0.02

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3 *Median (range). RBC: red blood cells. EPO: erythropoietin. EEC: erythroid
4 endogenous colony growth. Ferritin and EPO available in 111 and 98 cases,
5 respectively. EEC and *JAK2V617F* mutant load at diagnosis assessed in 98 and 103
6 cases, respectively.

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Table II: Probability of thrombosis, major bleeding and myelofibrotic transformation in 151 patients with polycythaemia vera						
	WHO 2008			BCSH		
	Masked N=68	Overt N=83	p	Masked N=16	Overt N=135	p
Thrombosis			0.7			0.9
No. of events	11	14		2	23	
Probability at 10 years	24%	22.5%		23%	22%	
Major Bleeding			0.6			0.9
No. of events	6	8		1	13	
Probability at 10 years	10%	11%		10%	11.5%	
Myelofibrosis			0.7			0.6
No. of events	6	16		2	20	
Probability at 10 years	11%	14%		18%	12%	
Median time	18 years	17 years		Not reached	18 years	

Footnotes for the figures

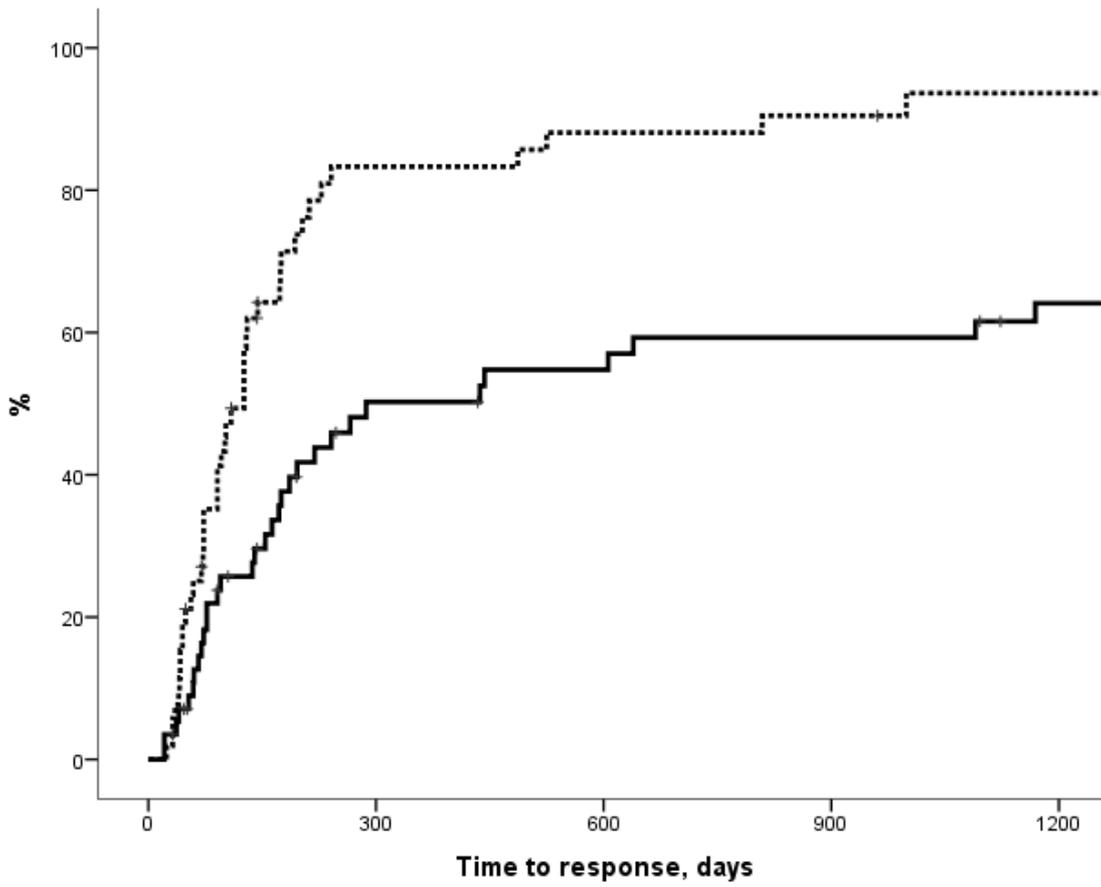
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Figure 1: a) Time to achieve a sustained haematocrit < 0.45 L/L without phlebotomies

b) Time to complete haematological response. Solid line corresponds to patients with WHO-overt PV, dotted line corresponds to patients with WHO-masked PV.

Figure 2: Overall survival of 151 patients with polycythaemia vera. Solid line corresponds to patients with WHO-overt PV, dotted line corresponds to patients with WHO-masked PV.

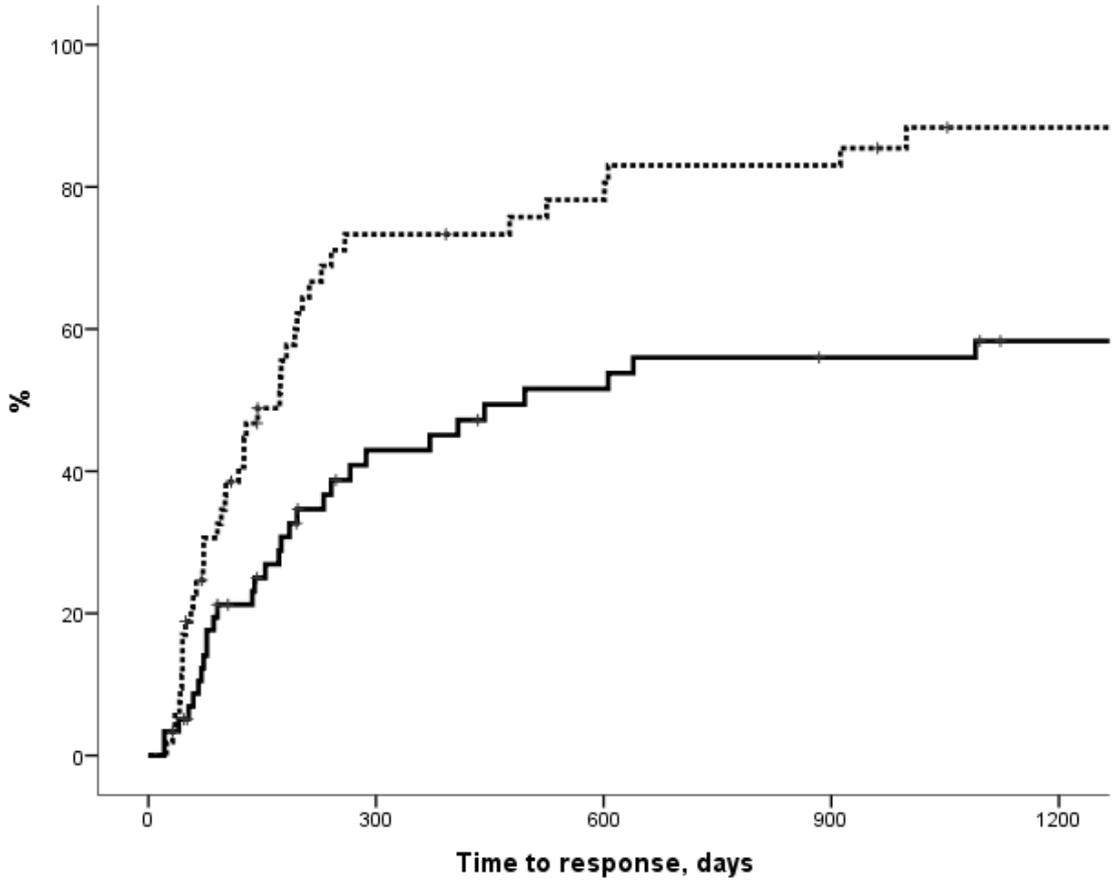
1 **Figure 1a**



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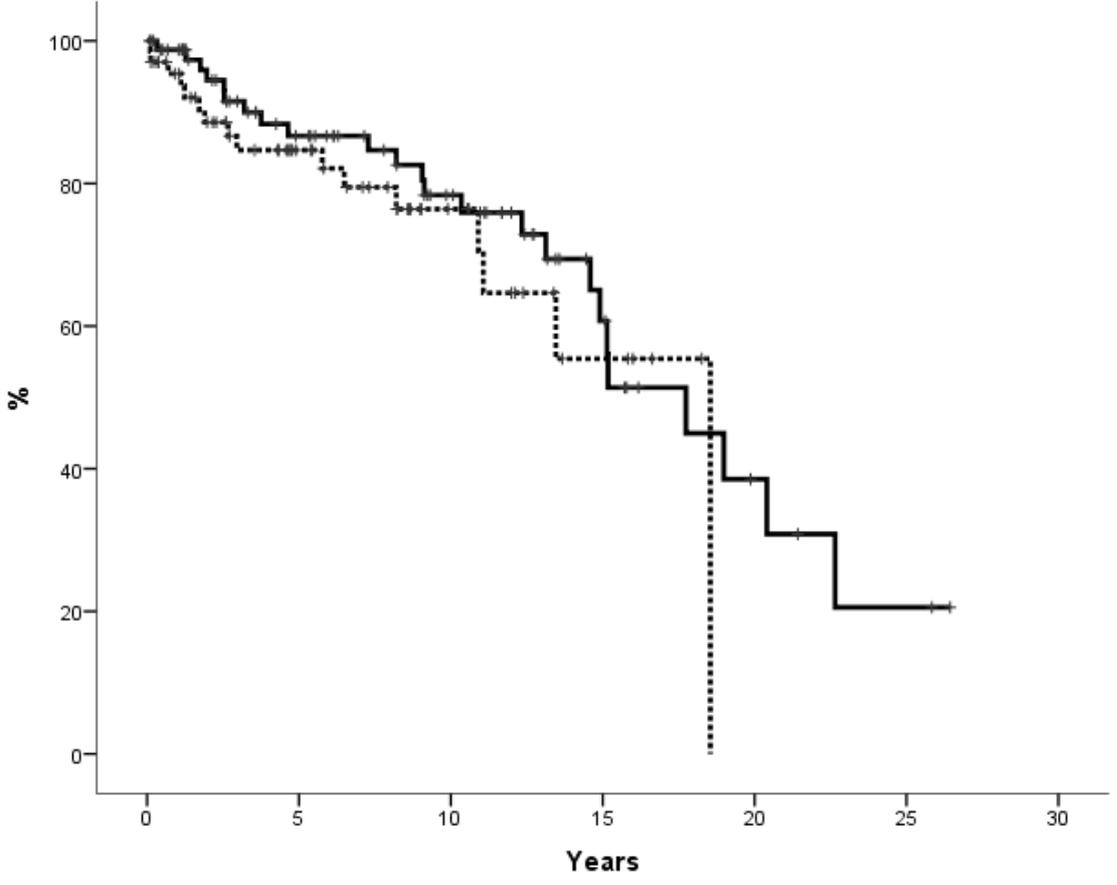
1 **Figure 1b**

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1 **Figure 2**
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