Masked polycythemia vera: analysis of a single center cohort of 2480 red cell masses

Classical Philadelphia-negative myeloproliferative neoplasms (MPN) are characterized by the presence of driver mutations (*JAK2, CALR* or *MPL*) and comprise polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Recently masked PV (mPV)[1] was defined in the 2016 World Health Organisation classification of MPN[2] as a new JAK2V617F-positive entity with a phenotypic presentation mimicking ET (apparently isolated thrombocytosis) but associated to endogenous erythroid colony formation (EEC) as found in PV or histological findings of PV (initially described as latent or inapparent PV).[3,4] Distinguishing ET from mPV is impossible based on hemoglobin or hematocrit values but is of the utmost importance since patients with mPV may have a high risk of thrombosis.[5,6] If mPV is misdiagnosed as ET, therapy is based on anti-aggregating agents (AA) only whereas phlebotomy + AA or cytoreductive therapy + AA would be more adequate.

We previously showed that another proportion of JAK2+ ET patients have early-stage polycythemia when red blood cell measurement (RCM) is systematically measured.[7] Recently, Alvarez-Larran *et al.* and others showed that mPV could be identified using red blood cell mass (RCM) measurement[8,9] (masked PV presenting an increased RCM above 125%) in place of bone marrow histology.[10] This study included a small number of patients (83 overt PV and 68 mPV) and did not compare mPV to ET patients with hematocrit levels within the same order of magnitude and thus failed to explain why mPV present sub-normal hematocrit and hemoglobin levels.

To further analyze these novel World Health Organisation entities and the role of RCM in their diagnosis, we retrospectively analyzed 2,480 consecutive patients in whom a RBCM was performed in our center for suspected polycythemia during the last 7 years. We first compared JAK2V617F positive and negative patients (*Online Supplementary Table S1*). Then, we analyzed the characteristics at diagnosis of the following groups of patients: "PV" (RCM >125%, presence of *JAK2*V617F mutation, hemoglobin higher than 18.5/16.5g/dL [male/female]; "masked PV" (presence of *JAK2*V617F mutation, RCM >125% and hemoglobin 16.5-18.5/15-16.5g/dL [male/female]; *"JAK2V617F ET with hemoglobin level identical to mPV"* (presence of *JAK2*V617F mutation, RCM<125%, hemoglobin 16.5-18.5/15-16.5g/dL [male/female]). We note that in this cohort 37.3% of the 2480 patients had an RCM >125% confirming polycythemia. All but one *JAK2*V617F-positive patients with hemoglobin >16.5/18.5g/dL (female/male) (n=117) had a RCM >125%. Patients with lower hemoglobin levels (15-16.5g/dL (56 females) and 16.5-18.5g/dL (112 males)) could be further classified according to the RCM level between masked PV (n= 113) when RCM was > 125% or *JAK2*V617F-positive ET (n=55) when RCM was <125% of predicted value (Figure 1). Variables were summarized as frequencies and percentages or means ± standard deviation, min-max or median and lowest datum still within the 1.5 interquartile range of the lower quartile, and the highest datum still within the 1.5 interquartile range of the upper quartile (Tukey boxplot), as appropriate. Comparisons were performed using Student *t*-test. Mean ages (min-max) were 63.8 years (y) (32.1-91.1) in PV, 60.3y (18.3-96.9) in mPV (p PV *vs.* mPV=not significant (NS)) and 56.6y (18.3-87.6) in ET (PV *vs.* mPV *P*=0.003; ET *vs.* mPV *P*=NS). Characteristics of patients are represented in Figure 2. According to the definitions of patients subgroups, mean hemoglobin levels were statistically different between PV and mPV or ET: 18.6g/dL (16.5-22.3) in PV *versus* 16.6g/dL (15-18.4) in mPV and

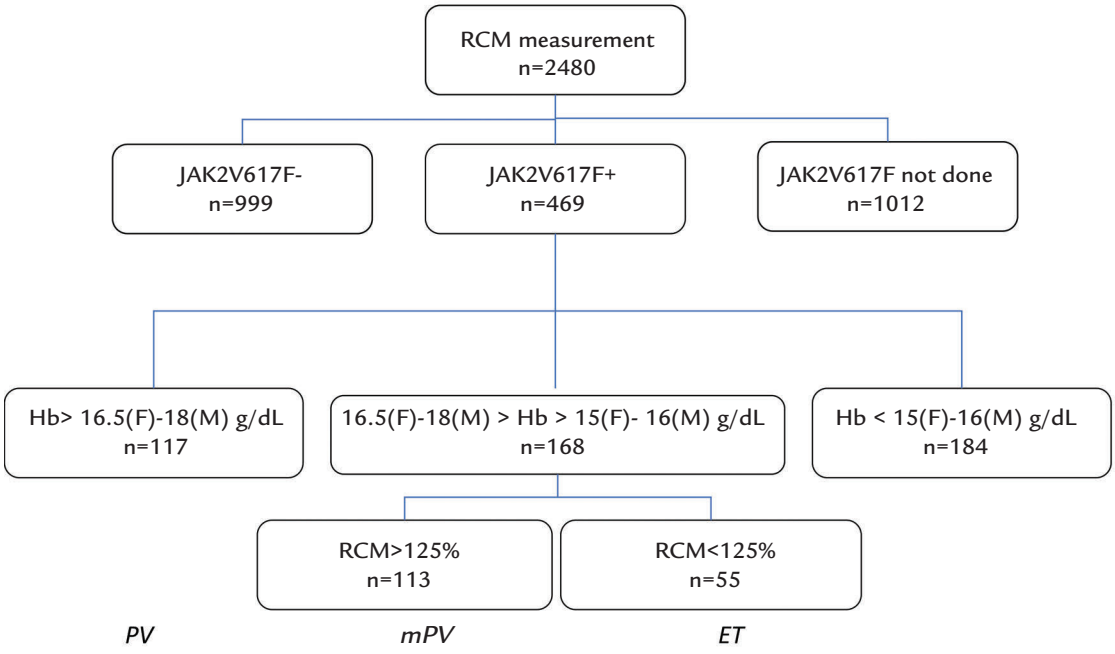

Figure 1. Diagnostic chart of suspected polycythemic patients. The number of patients concerned in each step of the chart is noted next to each item (n). RCM: red blood cell mass; Hb: hemoglobin; F: female; M: male, PV: polycythemia vera; mPV: masked PV; ET: essential thrombocythemia.

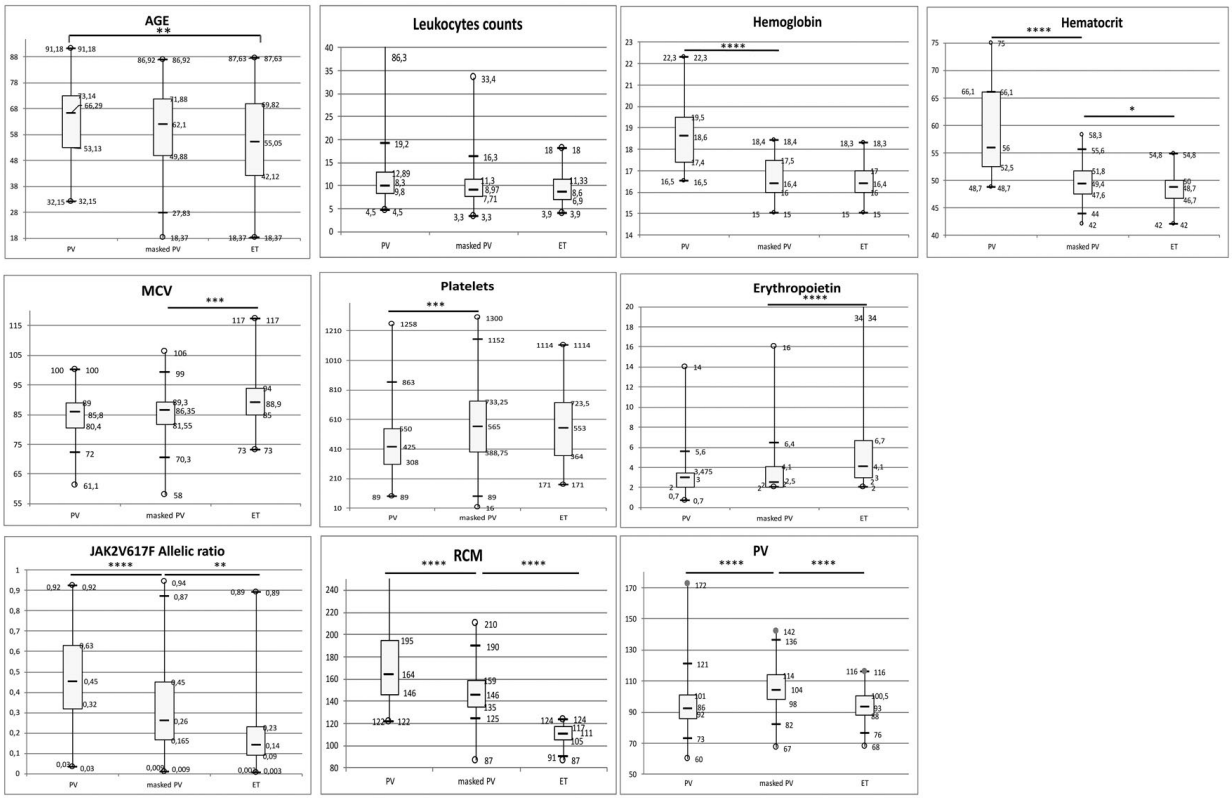

Figure 2. Characteristics of essential thrombocythemia (ET), masked polycythemia vera (mPV) and overt polycythemia vera (PV) patients. Box and whisker plots show median, first and third quartiles, and maximum and minimum values. Results shown are for ET, masked PV and overt PV respectively. Results of hypothesis testing (*P*-values) for differences between groups are shown where thresholds are met. Age in years; Leukocytes: g/L; hemoglobin: g/dL; Hematocrit (%), mean corpuscular volume: MCV; (Fl), platelets (g/L), erythropoietin (mUI/mL), *JAK2*V617F allele ratio (mutated/(mutated+wild-type), red cell mass (RCM) (% of the normal value), plasma volume (% of the normal value). **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

16.4g/dL (15-18.3) in ET (PV *vs.* mPV *P*<0.0001; mPV *vs.* ET *P*=NS and PV *vs.* ET *P*<0.0001). Accordingly, hematocrit levels were different between PV and both of the other groups of patients. In addition statistically different hematocrit levels were observed between mPV and ET patients (*P*=0.015). Of note, mean corpuscular volume (MCV) were lower in PV and in mPV when compared to ET patients but was not statistically different between PV and mPV patients: respectively 90fL (+/-8.1fL) in ET, 84.7fL (+/-5.54fL) in PV and 84.7fL (+/-8.1fL) in mPV (PV *vs.* mPV *P*=NS; mPV *vs.* ET *P*=0.00018 and PV *vs.* ET *P*<0.0001). Mean leukocyte counts for PV, mPV and ET patients were 11.4 (4.5-86.3), 10.01 (3.3-33.4) and 9.28 (3.9-18) g/L, respectively (not statistically different). Importantly, the mean platelet levels were significantly lower in PV patients compared to both mPV and ET patients while no difference was observed between mPV and ET patients. Mean platelet numbers were 460 g/L(+/-222 g/L) in PV, 560 g/L(+/-245g/L) in mPV and 563 g/L (+/-252 g/L) in ET patients (PV *vs.* mPV *P*=0.0014; PV *vs.* ET *P*=0.0075 and mPV *vs.* ET *P*=NS). The proportion of patients with platelets above 500g/L, 600g/L and 1000g/L in PV, mPV and ET are summarized in the *Online Supplementary Table S2*. These results confirm that platelet counts of mPV patients are highly similar to those of ET patients. We then compared circulating EPO levels among the three groups of patients. Mean serum EPO levels were comparable in mPV and PV and lower than in ET patients (PV: 3.41+/-2.3 mUI/mL; mPV: 3.42+/-2.2 mUI/mL and ET 6.22+/-4.09 mUI/mL, respectively; PV *vs.* mPV *P*=NS; mPV *vs.* ET *P*=0.0003 and PV *vs.* ET *P*=0.0002). *JAK2*V617F allele burden was significantly different between the three groups. Mean mutant allelic ratios +/- standard errors were as follows: 46% +/-22%, 31% +/-23% and 19.9% +/-20% in PV, mPV and ET, respectively (PV *vs.* mPV *P*<0.0001; mPV *vs.* ET *P*=0.0094 and PV *vs.* ET *P*<0.0001). Thus, it appeared that the three groups of patients were different from a molecular point of view. Red cell mass and plasma volume (measured in all patients) were different in the three groups with the higher RCM in PV (170% +/-33%), an intermediate RCM in mPV (149% +/-19%) and normal RCM (by definition) in ET patients (109.9%=/-9.6%) (Respectively PV *vs.* mPV *P*<0.0001; mPV *vs.* ET *P*<0.0001 and p PV *vs.* ET <0.0001). Interestingly, as plasma volumes are not calculated but directly measured by I125-labelled albumin injection in our department,[11] we could identify a clear increased plasma volume in mPV compared to PV and ET patients (plasma volumes in PV, mPV and ET were 95.9% +/-15%, 105.8%+/-12%, and 93.6% +/-9.2%, respectively; p PV *vs.* mPV <0.0001, p mPV *vs.* ET <0.0001 and p PV *vs.* ET =NS). Moreover, the proportion of patients with an hemodilution (plasma volume>110%) was 14% in PV, 2% in ET and 35% in mPV (PV *vs.* mPV *P*<0.001; p mPV *vs.* ET <0.001 and p PV *vs.* ET=NS). This difference between PV and mPV patients could not be attributed to

an increased spleen volume as the proportions of patients with splenomegaly were 19% in PV, 19% in mPV and 5.7% in ET patients (PV *vs.* mPV *P*=NS; mPV *vs.* ET *P*=0.02 and PV *vs.* ET *P*=0.02).

This study shows that mPV shares clinical and biological features with both ET and PV. Masked PV patients present a median age, platelet, hemoglobin and leukocyte levels comparable to those of ET patients. However, mPV present also some features of PV (besides increased RCM) including lower Epo level and lower MCV, and have more frequently splenomegaly than ET patients. Some authors previously hypothesized that mPV could be a consequence of iron deficiency in PV patients.[1,2] Despite thrombocythemic patient MCV was in the normal range and not different from true PV patients, we can not totally exclude in this study that the platelet increase observed for these patients could be related, at least in some cases, to iron deficiency since ferritin level was not measured systematically for all patients. However, our findings are not in favor of this hypothesis since MCV level were not lower in mPV compared to true PV. Interestingly, this study demonstrates that mPV patients present an increased plasma volume that is observed neither in ET nor in PV, whether or not they have splenomegaly (*Online Supplementary Table S3*). According to these results and to the Lamy *et al*. publication,[3] RCM and plasma volume measurement seem to be very important in patients with a normal hematocrit and splenomegaly or to exclude PV. This hemodilution explains the apparent lower levels of hemoglobin/hematocrit in mPV. For this reason, they are not appropriate parameters for PV diagnosis and should not be used as diagnosis criteria but rather as orientation markers towards RCM measurement since in our cohort only when hematocrit was higher than 55%, RCM was not usefull to demonstrate an increased RCM (*Online Supplementary Table S4*). However, mPV cannot be summarized only as a "hemodiluted PV". For example, compared to true PV, mPV display a higher platelet count and a lower *JAK2*V617F allelic ratio suggesting an underlying biological difference.

Despite the lack of clinical follow-up that does not allow us to correlate patient's evolution to biological data, this study further suggests that in clinical practice, when neither bone marrow biopsy nor RCM are performed in patients presenting with apparently isolated thrombocytosis, a large proportion of them with a moderate increase in hemoglobin level may falsely been diagnosed as ET when they have in fact true or mPV. In addition, one should also cautiously interpret data derived from cohorts of ET patients not fulfilling World Health Organization criteria that probably include such mPV patients.

In conclusion, this study demonstrates that masked PV can be distinguished from PV and ET based on RCB measurement even when bone marrow biopsy is not performed. Our findings may explain why mPV present some characteristics of ET, but also confirm that this entity probably needs to be managed like PV as initially suggested by Barbui *et al*.

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