Red cell mass measurement in patients with clinically suspected diagnosis of polycythemia vera or essential thrombocythemia

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ABSTRACT

The cut off for hemoglobin or hematocrit that indicates the need for an isotopic red cell mass study was investigated in 179 patients with a presumptive diagnosis of polycythemia vera or essential thrombocythemia. Hematocrit showed better diagnostic accuracy than hemoglobin. Hemoglobin over 18.5 g/dL in males or over 16.5 g/dL in females showed a high specificity indicating that red cell mass study could be avoided in such cases, but it showed low sensitivity leading to 46% false negatives. The best value of hematocrit to indicate a red cell mass study was 0.50 L/L in males (specificity 75%, sensitivity 87.5%) and 0.48 L/L in females (specificity 73%, sensitivity 94%). Lowering the hematocrit threshold to 0.48 L/L in males increased sensitivity up to 95%. A red cell mass study should be performed in patients with suspected diagnosis of essential thrombocythemia or polycythemia vera and with hematocrit between 0.48 L/L and 0.52 L/L.

Key words: polycythemia vera, essential thrombocythemia, red cell mass, hemoglobin, hematocrit.

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Introduction

The demonstration of an increased red cell mass (RCM) measured by isotopic methods was the main criterion for the diagnosis of polycythemia vera (PV) according to the Polycythemia Vera Study Group.[1] Recently, in the new WHO criteria,[2] hemoglobin (Hb) over 18.5 g/dL in males and over 16.5 g/dL in females is considered surrogate marker of an increased RCM; because of this, in many centers, RCM is no longer being performed. However, it is important to distinguish PV from essential thrombocythemia (ET) given that PV is one of the commonest myeloproliferative neoplasms and can often resemble ET. There are several reasons why the myeloproliferative neoplasm type should be correctly identified, including a reduced life expectancy in PV when compared to ET,[3] a higher probability of transformation to myelofibrosis in PV[4,5] and, lastly, a higher rate of thrombosis observed in PV than in ET.[6-8] In addition, PV management recommendations for PV and ET differ.[9]

It is well known that a proportion of patients suffering from PV do not reach the Hb threshold defined by the WHO.[10-12] This is extremely important in those cases with concomitant thrombocytosis. In these cases, if RCM is not measured, the majority of patients may be erroneously classified as ET. Therefore, isotopic RCM measurement could be a requisite for the differential diagnosis of ET or PV when WHO Hb criteria are not satisfied. However, which hemoglobin or hematocrit value should be applied for RCM in patients with a clinically suspected diagnosis of ET or PV has not been established.

The objective of the present study was to identify the cut off for hemoglobin (Hb) or hematocrit (Hct) that indicates the need for isotopic red cell mass evaluation in patients with a presumptive diagnosis of PV or ET.

Design and Methods

Between 1985 and 2011, 347 subjects were consecutively diagnosed with PV or ET at the Hematology Department of the Hospital del Mar of Barcelona, Spain. At our institution, routine initial evaluation for patients with a suspected diagnosis of ET or PV includes automatic blood cell counts, serum erythropoietin level, endogenous erythroid colony formation, *JAK2*V617F quantification, and red cell mass measurement when Hct is over 0.45 L/L in males and over 0.42 L/L in females. In patients diagnosed before 2005, *JAK2*V617F mutation was studied from DNA criopreserved samples obtained at diagnosis or fresh samples collected during follow up. RCM was measured using red blood cells labelled with Cr51 with red blood cell volume, plasmatic volume and sanguineous volume, calculated according to ICSH recommendations.[13] The diagnosis of PV and ET was established according to the PVSG or the WHO criteria before and after 2001, respectively. The final diagnosis was revised according to the current WHO criteria. Accordingly, the

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diagnosis of PV required hemoglobin level over 18.5 g/dL in males and over 16.5 g/dL in females, or an increased red cell volume of over 125% of normal values, the demonstration of a mutation in the *JAK2* gene, and one minor criteria (decreased erythropoietin serum level, endogenous erythroid colony formation or compatible bone marrow histology). JAK2-negative cases required the presence of at least two minor criteria to establish PV diagnosis. Bone marrow biopsy was performed in all patients with suspicion of ET but it was not routinely assessed in PV. Patients with early/pre-fibrotic primary myelofibrosis were not included in the present study. Informed consent was obtained for the scientific use of the patients' clinico-hematologic data and this was approved by the institutional review board of the Hospital del Mar.

Receiving operating characteristic (ROC) curves were performed to evaluate the diagnostic accuracy of Hb and Hct in order to distinguish between normal and increased RCM measured by the Cr51 method. In ROC curves, the specificity and sensitivity of each Hb and Hct value is calculated. The area under the curve (AUC) of a perfect diagnostic test (sensitivity 100%, specificity 100%) is 1 whereas the AUC of a test without diagnostic accuracy is 0.5 (sensitivity 50%, specificity 50%). The best diagnostic test is that with a higher AUC. A diagnostic test is usually considered to have an acceptable diagnostic accuracy when the sensitivity and specificity is higher than 80%, resulting in an AUC of over 0.8. Since the purpose of the present study was to investigate which Hb or Hct value should indicate measurement of RCM (as opposed to not measuring it), the cut off was selected according to sensitivity prevailing over specificity in order to reduce the number of false negative cases. In the differential diagnosis of ET *versus* PV, false positives represent cases with an Hct or Hb value over a pre-determined threshold but with normal RCM leading to an erroneous diagnosis of PV if RCM is not measured. False negatives (cases with Hct or Hb values below the pre-determined threshold and an increased red cell mass) would correspond to those patients in whom a mistaken diagnosis of ET would be made if RCM was not measured.

Results and Discussion

Isotopic RCM was determined as part of the initial evaluation in 179 patients (88 males, 91 females) with a suspected diagnosis of PV or ET. Main hematologic values at diagnosis are shown in Table 1. The majority of patients showed a Hb level and/or platelet counts over the normal values and the *JAK2* mutation (V617F or exon 12) was present in 98% of the cases; a clinical picture compatible with PV or ET. RCM was increased in 114 patients establishing a PV diagnosis, whereas ET was diagnosed in 63 of the 65 remaining cases. Two cases with normal RCM did not fulfill ET nor PV criteria at time of evaluation but were diagnosed with PV later on during follow up.

The diagnostic accuracy of the WHO Hb criteria is shown in Table 2. WHO Hb criteria showed a high specificity indicating that a Hb value over 185 g/L in males or over 165 g/L in females could be used as an adequate surrogate of increased RCM in patients with a high suspicion of PV or ET. Considering this, only 2 females with Hb over 165 g/L presented a normal RCM, whereas no males with Hb over 185 g/L showed a normal RCM. In contrast, WHO Hb criteria showed very low sensitivity. Interestingly, a total of 53 of 114 (46%) patients with increased RCM had Hb values at diagnosis below those defined by the WHO for diagnosis of PV corresponding to 27 (30%) and 26 (29%) false-negatives in males and females, respectively. Forty out of these 53 (75%) patients presented concomitant thrombocytosis, with these cases eventually being misdiagnosed as ET if RCM were not measured.

ROC curves were performed in order to evaluate which automatic blood test is better in guiding the need for RCM measurement. The Hct showed a larger AUC (AUC 0.92, 95%CI: 0.88-0.96) than the Hb (AUC 0.88, 95%CI: 0.84-0.93) resulting in a better diagnostic accuracy in predicting RCM in the whole series. When the patients were stratified

Table 1. Main hematologic values according to red cell mass (RCM) measurement in 179 patients with a presumptive diagnosis of polycythemia vera or essential thrombocythemia.

	RCM > 125% n=114	Normal RCM n=65	*P*
Male, n. (%)	64 (56)	24 (37)	0.02
Age*	61 (20-84)	69 (29-93)	0.03
Palpable spleen, n. (%)	35 (31)	3 (5)	<0.001
RBC x10^{12}/L*	6.8 (4.9-9.9)	5.2 (4.4-6.7)	<0.001
Hemoglobin, g/L*	180 (137-238)	150 (131-172)	<0.001
Hematocrit, L/L*	0.56 (0.44-0.73)	0.47 (0.42-0.58)	<0.001
MCV, fL*	82 (66-101)	90 (76-101)	<0.001
WBC count x10^9/L*	10.4 (5.7-39.9)	8.6 (5.3-20)	0.005
Platelet count x10^9/L*	516 (155-1480)	606 (418-1744)	0.004
Low serum epo, n. (%)	68 (72)	23 (42)	<0.001
BFU-e, n (%)	67 (90)	47 (90)	ns
JAK2 mutation , n. (%)	110 (96)	65 (100)	ns
V617F, n. (%)	105 (92)	65 (100)	ns
Exon 12, n. (%)	5 (4)	ND	
% alleles V617F	59 (2-100)	26 (9-100)	<0.001

** median (range). RBC: red blood cells; MCV: mean corpuscular volume; WBC: white blood cells; Epo: erythropoietin; BFU-e: endogenous growth of erythroid colonies. Epo level and BFU-e was assessed in 150 and 126 patients, respectively.*

Table 2. Diagnostic accuracy of WHO Hb criteria and different hematocrit thresholds as predictors of increased red cell mass in patients with a suspected diagnosis of PV or ET.

	True + n. of cases	True- n. of cases	False+ n. of cases	False- n. of cases	Specificity %	Sensitivity %
Male n=88						
Hb > 185 g/L	37	24	0	27	100%	58%
Hct > 0.48 L/L	61	10	14	3	42%	95%
Hct > 0.50 L/L	56	18	6	8	75%	87.5%
Hct > 0.52 L/L	52	23	1	12	96%	81%
Female n=91						
Hb > 165 g/L	24	39	2	26	95%	48%
Hct > 0.45	49	13	28	1	32%	98%
Hct > 0.48	47	30	11	3	73%	94%
Hct > 0.50	37	37	4	13	90%	74%
Hct > 0.52	29	39	2	21	95%	58%

True +: number of patients with the hematocrit level over the threshold and increased red cell mass. True -: number of patients with hematocrit level below the threshold and normal red cell mass. False +: number of patients with hematocrit level over the threshold and normal red cell mass. False -: number of patients with hematocrit level below the threshold and increased red cell mass.

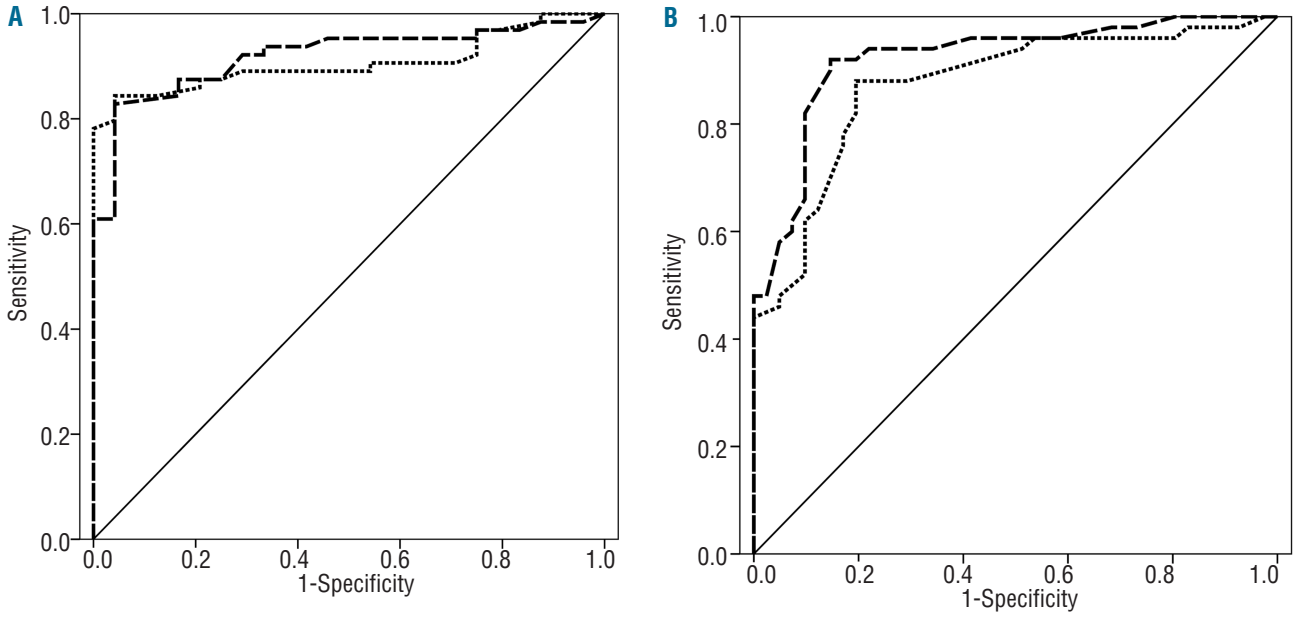

Figure 1. ROC curves for Hb and Hct in the diagnosis of an increased RCM in (A) males and (B) females. Dash line corresponds to Hct and dotted line to Hb.

according to gender, in males, diagnostic accuracy of Hct and Hb was similar (males: AUC Hct 0.92, 95%CI: 0.86-0.98 vs. AUC Hb 0.91, 95%CI: 0.84-0.97); in females, the accuracy of Hct was also better than that for Hb (females: AUC Hct 0.92, 95%CI: 0.87-0.98 *vs.* AUC Hb 0.87, 95%CI: 0.80-0.95). ROC curves for Hb and Hct in the diagnosis of an increased RCM in males and females are shown in Figure 1. These findings indicate that, overall, Hct would be a better guide than Hb to decide if an isotopic RCM measurement should be performed or not.

The number of false negatives, false positives, sensitivity and specificity for different Hct points in males and females as a diagnostic test of increased RCM are shown in Table 2. In females, Hct over 0.52 L/L showed a specificity of 95% with only 2 cases of 31 with this value presenting a normal RCM. This feature indicates that Hct over 0.52 L/L could be used as a surrogate of increased RCM and, consequently, RCM measurement could be avoided in such cases. On the contrary, if RCM is not assessed in patients with Hct below 0.52, PV diagnosis could not have been established in 21 of 60 females in whom RCM was actually increased. Lowering Hct level to 0.45 L/L or 0.48 L/L increased sensitivity up to 98% and 95%, and the number of false positives to 28 and 11, respectively (Table 2). These results suggest that, in females, RCM would obtain the best diagnostic performance in the differential diagnosis of ET from PV in those cases with Hct ranging from 0.45 to 0.52 L/L.

In males, Hct over 0.52 L/L had a specificity of 96% and this could be considered equivalent to increased RCM. Patients with a presumptive diagnosis of PV or ET and Hct ranging from 0.48 L/L to 0.52 L/L would benefit from RCM measurement, as shown by a sensitivity of 95% for Hct over 0.48 L/L. If RCM had not been assessed in patients with Hct below 0.52 L/L, 12 cases would not have been diagnosed as PV. Performing a RCM study in those cases with Hct between 0.48 L/L and 0.52 L/L would have avoided misdiagnosis in up to 3 patients (Table 2).

It must be pointed out that the present series was restricted to patients with a final diagnosis of PV or ET. The distinction between PV and apparent polycythemia was not evaluated in the present study. As a consequence, the proposed cut offs for Hb and Hct may not be appropriate in patients with isolated erythrocytosis and without features suggesting the presence of a myeloproliferative neoplasm, such as thrombocytosis or the *JAK2* mutation. Another aspect that must be taken into consideration is that Hct was measured by automatic analyzers, in contrast to previous studies in which Hct was measured by direct centrifugation of anticoagulated blood.[10]

A possible criticism of the methodology used in our study is that we systematically carried out RCM as a diagnostic procedure in patients clinically suspected of having PV and ET when the Hct was over over 0.45 L/L in males and over 0.42 L/L in females. It could be argued that such criteria for RCM measurement limit the possibility of identifying the lower Hb or Hct value at which RCM must be assessed. In this regard, the presence of clinical data suggesting the presence of an occult PV, such as splenomegaly, leukocytosis, thrombocytosis or portal vein thrombosis, has been proposed as an indication for RCM evaluation in patients with normal Hct or Hb values.[11] In addition, considering the data from the present and previous studies, and the current WHO diagnostic criteria, the presence of microcytic red blood cells, a high *JAK*V617F alelle burden, a decreased serum erythropoietin (EPO) level, or the bone marrow histology may also help clinicians to decide whether RCM should be measured or not in cases with normal Hct or Hb levels.[14-17]

In conclusion, the WHO Hb criteria for PV diagnosis have a low sensitivity leading to an important number of cases of misdiagnosis. Such diagnostic inaccuracy could be improved by evaluating RCM in cases with a suspicion of ET or PV and Hct ranging from 0.48 L/L to 0.52 L/L.

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