

# Evaluation of mathematical indices as tools for distinguishing $\beta$ -thalassemia trait from iron deficiency anemia in Portuguese females with microcytic anemia

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## Introduction

Microcytic anemia is a common condition frequently caused by iron deficiency anemia (IDA) or  $\beta$ -thalassemia trait (BTT). Some mathematical indices have been described as fast and inexpensive tools for distinguishing these two conditions. This approach is very useful in mass screening programs especially in countries with limited resources.<sup>1</sup>

**AIM:** This study aimed to evaluate the diagnostic performance of 13 distinct indices: RBC, England&Fraser, Mentzer, Srivastava, Shine&Lal, RDW, Ricerca, Jayabose (RDWI), Green&King (G&K), MDHL, MCHD, Sirdah and Ensani.

## Materials and Methods

We investigated 102 adult Portuguese females, presenting anemia (Hemoglobin<12g/dL) and microcytosis (Medium Corpuscular Volume, MCV<80fL). The *HBB* gene was screened for pathogenic variants by ARMS or PCR following Sanger sequencing. The iron status was evaluated by standard approaches. IDA was considered when serum ferritin<12 $\mu$ g/L and/or transferrin saturation<15%.

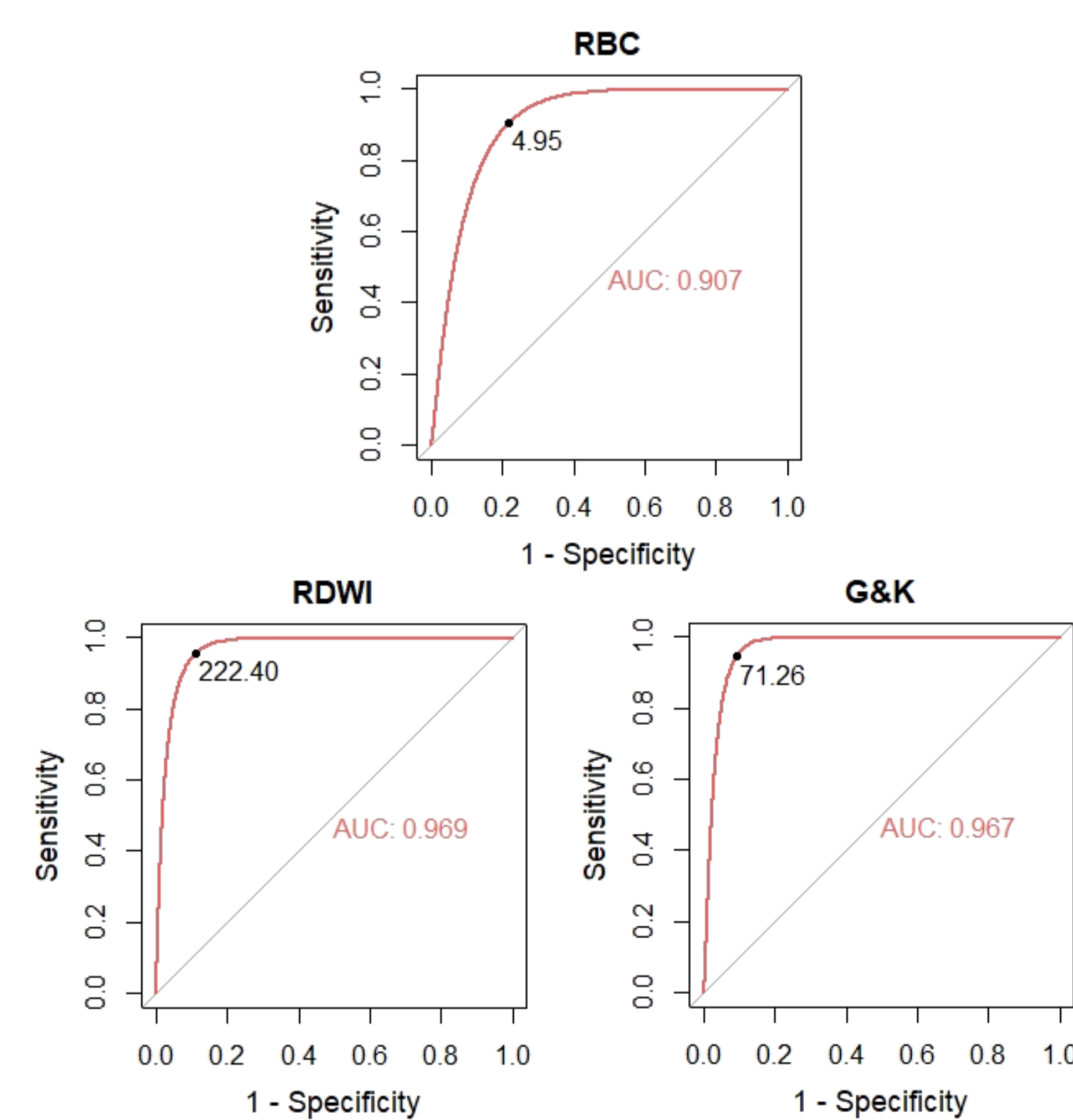
Two groups were generated: 51 BTT (with one *HBB* variant: c.92+1G>A; c.92+6T>C; c.92+110G>A or c.1188C>T) and 51 IDA, being assured that no individual had simultaneously the two conditions (Table 1 and Figure 1). To determine the performance of the indices, sensitivity, specificity, Youden index (YI) and receiver operating characteristic (ROC) curves were calculated. Due to the high values of AUC (Area Under the Curve) from ROC analysis, a cutoff of 0.70 for the YI was established in order to determine the best formulas.

## Results and Discussion

We find that the three best performing indices to differentiate the 2 groups (IDA versus BTT) were RBC (YI=0.71; AUC=0.902), RDWI (YI=0.84; AUC=0.973) and G&K (YI=0.82; AUC=0.972) (Table 2, Table 3 and Figure 2).

**Table 2:** Formula and summary of sensitivity and specificity findings for the three best hematologic indices tested in distinguish BTT cases from IDA cases respectively, Red Blood Cells (RBC), Jayabose (RDWI) and Green and King (G&K), based on a cutoff of 0.70 for the Youden Index.

Indices Cutoffs	BTT (n= 51)	IDA (n=51)	Correctly diagnosed	Accuracy (Efficiency)	SENS	SPEC	PPV	NPV	YI	AUC
<b>RBC<sup>2</sup></b>										
BTT > 5	40	4	87	0.85	0.78	0.92	0.91	0.81	0.71	0.902
IDA < 5	11	47			0.92	0.78	0.81	0.91		
<b>Jayabose (RDWI) - MCVxRDW/RBC<sup>3</sup></b>										
BTT > 220	44	1	94	0.92	0.86	0.98	0.98	0.88	0.84	0.973
IDA > 220	7	50			0.98	0.86	0.88	0.98		
<b>Green and King (G&amp;K) - MCV<sup>2</sup> x RDW/Hb x 100<sup>4</sup></b>										
BTT < 65	42	0	93	0.91	0.82	1.00	1.00	0.85	0.82	0.972
IDA > 65	9	51			1.00	0.82	0.85	1.00		



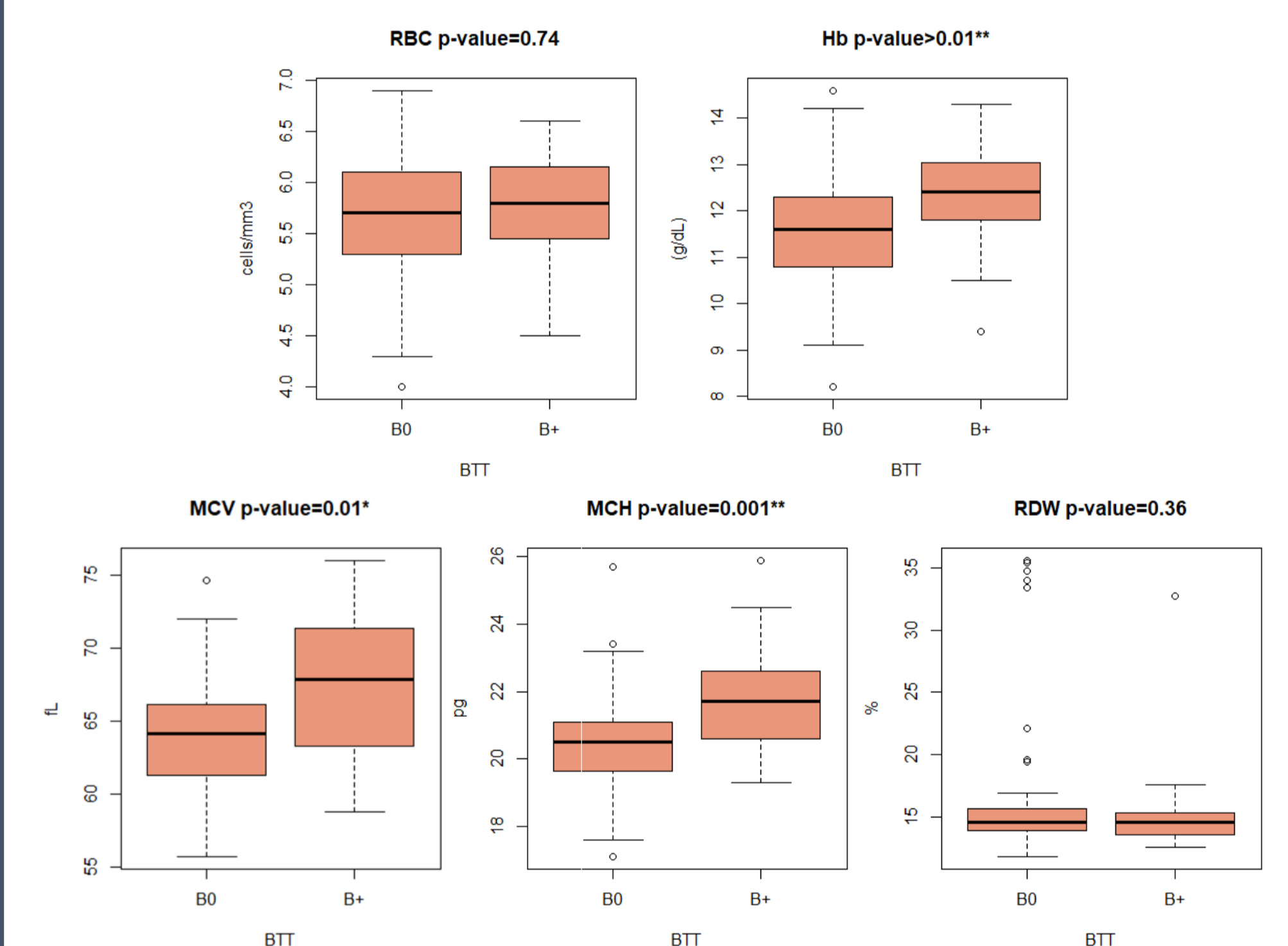
**Table 3:** Summary of sensitivity and specificity findings for Red Blood Cells (RBC), Jayabose (RDWI) and Green and King (G&K) and the respective estimated new best thresholds.

Indices	Cutoff	Predicted Cutoff	SENS	SPEC	PPV	NPV
RBC	5	4.95	0.92	0.80	0.82	0.91
RDWI	220	222.40	0.98	0.90	0.91	0.98
G&K	65	71.26	0.94	0.92	0.92	0.94

**Figure 2:** Smoothed ROC curves constructed to visualize the Area Under the Curve (AUC) for each of the three best tested hematologic indices to distinguish BTT cases from IDA cases respectively. Marked on the ROC curve is the new estimated best threshold.

**Table 1:** Comparison of hematologic parameters and iron status of the  $\beta$ -thalassemia trait (BTT) and iron deficiency anemia (IDA) populations, taking into consideration the reference values.

Parameters	Adult female Reference	BTT (N=51)	IDA (N=51)	P
Age (y)	> 16	45 $\pm$ 18	41 $\pm$ 15	0.034
RBC (x10 <sup>12</sup> /L)	3.90-5.20	5.28 $\pm$ 0.48	4.41 $\pm$ 0.41	0.003
Hb (g/dL)	11,80-14,80	11.05 $\pm$ 0.91	10.2 $\pm$ 1.43	0.071
MCV (fL)	82.00-98.00	65.66 $\pm$ 4.29	72.6 $\pm$ 7.91	0.006
MCH (pg)	27,30-32,60	21.00 $\pm$ 1.58	23.26 $\pm$ 3.48	0.038
RDW (%)	9,90-15,50	14.61 $\pm$ 1.40	22.79 $\pm$ 11.38	<0.001
HbA2 (%)	2,20-3,50	4.17 $\pm$ 0.48	-	-
Fe ( $\mu$ g/dL)	33.00-102.00	106.51 $\pm$ 33.88	34.38 $\pm$ 18.63	<0.001
Tf (mg/dL)	181.00-416.00	270.04 $\pm$ 46.71	370.02 $\pm$ 64.02	0.001
TIBC ( $\mu$ g/dL)	228.00-428.00	341.27 $\pm$ 64.27	462.46 $\pm$ 79.94	0.001
TSAT (%)	16.00-45.00	32.39 $\pm$ 11.86	7.69 $\pm$ 4.75	<0.001
Ft ( $\mu$ g/L)	10.00-160.00	129.55 $\pm$ 220.22	7.45 $\pm$ 7.88	0.001



**Figure 1:** Comparison of hematologic parameters of the  $\beta$ -thalassemia trait (BTT) population subgroups according to the severity of the mutation ( $\beta^0$  or  $\beta^+$ ). \*p-value  $\leq$  0.01 \*\*p-value  $\leq$  0.001

→ Our results suggest a similarity with other Mediterranean countries such as Spain<sup>5</sup> and Greece<sup>6</sup>, where G&K and RDWI also performed above our set cutoff. The same is observed in Brazil<sup>7</sup> probably due to its Portuguese ancestry.

## Conclusions

- We conclude that aiming to diagnosis the condition underlying a microcytic anemia in a female population, there is value in using this method to recognize the individuals suspected of BTT and forward them for HbA<sub>2</sub> measurement or *HBB* molecular test.
- In the future, a robust group of male patients should be added to the analysis in order to extrapolate which of these indices would best apply to the whole adult Portuguese population.

## References

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