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Original

Clinical significance of optimal red cell mass and plasma volume estimation methods

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Abstract

BACKGROUND: The aim of this study was to present and compare the results of proposed methods for optimal red cell mass and plasma volume (RCM&PV) estimation, and their influence on the interpretation of obtained results.

MATERIAL AND METHODS: In 120/280 patients with polycythaemia rubra vera, subjected to RCM&PV determination with autologous erythrocytes *in vitro* labelled with ^{51}Cr-sodium chromate, optimal volumes were determined using:

1. traditional ml/kg of:
— the real body weight method (ml/kg RBW);
— the optimal body weight method (ml/kg OBW).
2. the body weight, height, and sex based method (Retzlaff's tables),
3. the method recommended by the International Council for Standardization in Haematology (ICSH), based on body surface area.

RESULTS: Different interpretation of the same results of 120 RCM&PV measurements was registered in 48/120 patients (40%). The greatest disagreement existed between ml/kg RBW and ml/kg OBW methods (in 39/120 subjects, 32.5%). In underweight patients the ml/kg RBW method, and in overweight patients the ml/kg OBW method, offered better agreement with ICSH&Retzlaff's methods. The ml/kg RBW method disagreed with ICSH&Retzlaff's methods and ml/kg OBW in 25% and 19.2% of patients respectively. ICSH and Retzlaff's methods disagreed in 10/120 patients (8.3%). The ICSH method yielded significantly lower optimal volumes than Retzlaff's.

CONCLUSION: Three methods for optimal RCM&PV estimation lead to different interpretations of the same results of RCM&PV measurements with ^{51}Cr-erythrocytes in 40% of patients. Two ml/kg body weight methods show greater disagreement in comparison with ICSH and Retzlaff's methods, which differ significantly. The ICSH method yields lower optimal values compared to Retzlaff's.

Key words: **labelled red blood cells, labelled erythrocytes, blood volume, ^{51}Cr-sodium chromate, optimal plasma volume, optimal red blood cells mass, optimal erythrocytes volume**

Introduction

The Nobel Prize laureate, George de Hevesy, was the first investigator who used radioactive nuclides for labelling of erythrocytes [1]. He introduced ^{32}P sodium phosphate for red blood cell labelling in order to determine the blood volume of patients. Since then many radionuclides have been used for the same purpose ^{51}Cr, ^{99m}Tc, ^{111}In, ^{113m}In, ^{11}C, ^{67}Ga, ^{68}Ga.

It has been shown that ^{99m}Tc elutes from red blood cells at a greater rate than ^{51}Cr following the labelling procedure [2]. Elution of ^{99m}Tc from erythrocytes is 2% for the first 30 minutes, while it is less than 1% per day for ^{51}Cr [3]. The elution becomes important especially in patients with splenomegaly, referred for blood volume measurement, where mixing time within the spleen is prolonged, and blood sampling has to be delayed until 60 minutes following the injection of labelled erythrocytes [2].

The radionuclide that still remains in use for red blood cell labelling for the purpose of blood volume and red blood cell mass measurement is ^{51}Cr [4–6], thanks to its fairly stable bond to the

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β-globin chain of hemoglobin (after a reduction from a hexavalent to a trivalent state).

The recommended methods for the measurement of red cell mass and plasma volume (RCM&PV) have been drawn up by the Radionuclide Panel of the International Committee for Standardization in Haematology [7, 8].

The problem that still remains is how to interpret the obtained results of RCM&PV measurements. They have to be compared to the normal values, or optimal values for the investigated person.

Several methods for optimal/normal RCM&PV estimation have been used:
- traditionally, results and optimal values have been expressed in terms of ml per kilogram real body weight. Polycythaemia Vera Study Group (Berlin, 1975) criteria include RCM values expressed in ml/kg. Polycythemia is defined as an increase in RCM equal or greater than 32 ml/kg for females and 36 ml/kg for males [9];
- formulae/tables based on both height and body weight (including Retzlaf's tables) obtained following measurements of RBC&PV in normal population [10]. For the diagnosis of absolute erythrocytosis (polycythemia) it is necessary that RCM is increased more than 25% above the predicted value [9];
- formulae based on body surface area recommended by the International Council for Standardization in Haematology [11].

The aim of this work was to present and compare:
- the proposed methods for optimal RCM&PV estimation and
- their impact on the interpretation of obtained results in patients referred for RCM&PV measurements.

Material and methods

Autologous red blood cells were labelled *in vitro* using ^{51}Cr-sodium chromate (Na_2CrO_4) in 280 patients with polycythaemia vera, referred for RCM&PV measurement, according to the recommendations of the International Committee for Standardization in Haematology [7, 8]. RCM and plasma volume were determined in all of them. For the interpretation of the obtained results, optimal RCM&PV were estimated and compared with measured RCM&PV values.

In 120 consecutive patients optimal RBC&PV values were estimated by means of three different methods:
1. Ml per kilogram of:
   a. real body weight (ml/kg RBW),
   b. optimal body weight, (ml/kg OBW).
2. The method based on both height and body weight (for men and women).
3. The body surface area method.

1. a. For the ml/kg RBW method, the obtained values for RCM&PV (using ^{51}Cr-Er), expressed in millilitres, were divided by the real body weight expressed in kilograms;
   b. For the ml/kg OBW method, the optimal body weight had to be calculated. Thereafter, values obtained for RCM&PV (using ^{51}Cr-Er), expressed in milliliters, were divided by the optimal body weight expressed in kilograms.

The resulting values for ml/kg RBW and ml/kg OBW were compared to the normal values for RCM, PV and blood volumes for males and females (Tab. 1) [12]. Absolute polycythaemia was the conclusion in the case when RCM was equal or greater than 36 ml/kg for males and 32 ml/kg for females. Pathologic finding was present in the case when RCM or PV values were outside the range of normal values (Tab. 1).

Table 1. Normal values for RCM, PV and blood volumes [12]

Normal values	ml/kg body weight [ml]	
	Males	Females
RCM	25–35	20–30
PV	30–45	30–45
Blood volume	55–80	50–75

2. For optimal RCM&PV estimation method based on both height and real body weight (for males and females), Retzlaff's tables were used (Tab. 2A and 2B). They offer information about optimal blood volume (in milliliters) in males and females.

Optimal RCM has to be calculated by multiplying optimal blood volume (in milliliters) by optimal hematocrit value:
- 0.42 for males, and
- 0.38 for females.

If the measured RCM was at a level more than 25% above the optimal value absolute erythrocytosis was deemed to be present. If the measured RCM was at a level more than 25% under the optimal value, anaemia was diagnosed. Pathologic findings were also present when measured plasma volume values were higher or lower than 25% of the optimal values.

3. For body surface area method, we used the method recommended by the Expert panel of the International Council for Standardization in Haematology (ICSH) [11].

For males:

Mean normal RCM [ml] = $(1486 \times S) - 825$

Mean normal PV [ml] = $1578 \times S$

Mean normal blood volume [ml] = Mean normal RCM + + Mean normal PV

For females:

Mean normal RCM [ml] = $(1.06 \times \text{years of age}) + (822 \times S)$

Mean normal PV [ml] = $1395 \times S$

where S is the body surface area expressed in m^2.

$$S = W^{0.425} \times h^{0.725} \times 0.007184,$$

where W is the real body weight [kg] and h is the body height [cm]. Deviations from the optimal RCM or PV values greater than 25% were considered as pathologic findings.

The optimal values for RCM, blood and PV, estimated using all three methods, were compared. The impact of these methods on optimal volume estimation of the obtained results of RCM and PV interpretation was analysed.

Results

The group of 120 consecutive PRV patients consisted of 39 females (aged from 32–81 years; mean value = 58.5 years) and 81 males (aged from 22–76 years, mean value = 55 years). There was no significant difference in the age of the investigated persons ($p > 0.05$).

Body weight in females ranged from 43.5–105 kg (mean value = 66.6 kg), and in males from 52–139 kg (mean value = 83.3 kg). Forty-four (36.7 %) patients (19 females and 25 males) were under-

Table 2A. Optimal blood volumes in females

Wt (lb)	Height in inches 58	60	62	64	66	68	70	72	74	Wt (kg)
80	2.300	2.400	2.500	2.600	2.750	2.850	2.950	3.050	3.150	**36.3**
85	2.400	2.550	2.650	2.750	2.850	2.950	3.050	3.150	3.250	**38.6**
90	2.550	2.650	2.750	2.850	2.950	3.050	3.200	3.300	3.400	**40.8**
95	2.650	2.750	2.850	2.950	3.100	3.200	3.300	3.400	3.500	**43.1**
100	2.750	2.850	2.950	3.100	3.200	3.300	3.400	3.550	3.650	**45.4**
105	2.850	2.950	3.050	3.200	3.300	3.400	3.550	3.650	3.750	**47.6**
110	2.950	3.050	3.150	3.300	3.400	3.500	3.650	3.750	3.850	**49.9**
115	3.000	3.150	3.250	3.350	3.500	3.650	3.750	3.850	4.000	**52.2**
120	3.100	3.250	3.350	3.500	3.600	3.750	3.850	3.950	4.100	**54.4**
125	3.200	3.350	3.450	3.600	3.700	3.850	3.950	4.050	4.200	**56.7**
130	3.300	3.400	3.550	3.650	3.800	3.950	4.050	4.200	4.300	**59.0**
135	3.350	3.500	3.650	3.750	3.900	4.000	4.150	4.250	4.400	**61.2**
140	3.450	3.600	3.700	3.850	4.000	4.100	4.250	4.350	4.500	**63.5**
145	3.550	3.650	3.800	3.950	4.050	4.200	4.350	4.450	4.600	**65.8**
150	3.600	3.750	3.900	4.050	4.150	4.300	4.450	4.550	4.700	**68.0**
155	3.700	3.850	3.950	4.100	4.250	4.400	4.500	4.650	4.800	**70.3**
160	3.750	3.900	4.050	4.200	4.350	4.450	4.600	4.750	4.900	**72.6**
165	3.850	4.000	4.150	4.250	4.400	4.550	4.700	4.850	4.950	**74.8**
170	3.900	4.050	4.200	4.350	4.500	4.650	4.800	4.900	5.050	**77.1**
175	4.000	4.150	4.300	4.450	4.600	4.700	4.850	5.000	5.150	**79.4**
180	4.050	4.200	4.350	4.500	4.650	4.800	4.950	5.100	5.250	**81.6**
185	4.150	4.300	4.450	4.600	4.750	4.900	5.000	5.150	5.300	**83.9**
190	4.200	4.350	4.500	4.650	4.800	5.000	5.100	5.250	5.400	**86.2**
195	4.250	4.450	4.600	4.750	4.900	5.050	5.200	5.350	5.450	**88.5**
200	4.350	4.500	4.650	4.800	4.950	5.100	5.250	5.400	5.550	**90.7**
205	4.400	4.550	4.700	4.900	5.050	5.200	5.350	5.500	5.650	**93.0**
210	4.450	4.650	4.800	4.950	5.100	5.250	5.400	5.550	5.700	**95.3**
215	4.550	4.700	4.850	5.000	5.150	5.350	5.500	5.650	5.800	**97.5**
220	4.600	4.750	4.900	5.100	5.250	5.400	5.550	5.700	5.850	**99.8**
225	4.650	4.850	5.000	5.150	5.300	5.450	5.650	5.800	5.950	**102.1**
230	4.700	4.900	5.050	5.200	5.400	5.550	5.700	5.850	6.000	**104.3**
235	4.800	4.950	5.100	5.300	5.450	5.600	5.750	5.950	6.100	**106.6**
240	4.850	5.000	5.200	5.350	5.500	5.700	5.850	6.000	6.150	**108.9**
245	4.900	5.100	5.250	5.400	5.600	5.750	5.900	6.050	6.250	**111.1**
250	4.950	5.150	5.300	5.500	5.650	5.800	6.000	6.150	6.300	**114.4**
255	5.000	5.200	5.350	5.550	5.700	5.900	6.050	6.200	6.350	**115.7**
260	5.100	5.250	5.450	5.600	5.750	5.950	6.100	6.300	6.450	**117.9**
265	5.150	5.300	5.500	5.650	5.850	6.000	6.150	6.350	6.500	**120.2**
270	5.200	5.350	5.550	5.700	5.900	6.050	6.250	6.400	6.600	**122.5**
275	5.250	5.450	5.600	5.800	5.950	6.150	6.300	6.450	6.650	**124.7**
280	5.300	5.500	5.650	5.850	6.000	6.200	6.350	6.550	6.700	**127.0**
285	5.350	5.550	5.700	5.900	6.100	6.250	6.450	6.600	6.750	**129.3**
290	5.400	5.600	5.800	5.950	6.150	6.300	6.500	6.650	6.850	**131.5**
295	5.450	5.650	5.850	6.000	6.200	6.400	6.550	6.750	6.900	**133.8**
300	5.500	5.700	5.900	6.100	6.250	6.450	6.600	6.800	6.950	**136.1**
305	5.600	5.750	5.950	6.150	6.300	6.500	6.650	6.850	7.050	**138.3**
310	5.650	5.800	6.000	6.200	6.350	6.550	6.750	6.900	7.100	**140.6**
315	5.700	5.850	6.050	6.250	6.450	6.600	6.800	6.950	7.150	**142.9**
Height in meters	**1.52**	**1.57**	**1.63**	**1.68**	**1.73**	**1.78**	**1.83**	**1.88**	**1.93**	

Table 2B. Optimal blood volumes in males

Wt (lb)	Height in inches									Wt (kg)
	60	62	64	66	68	70	72	74	76	
90	3.150	3.250	3.350	3.450	3.550	3.650	3.750	3.850	3.950	**40.8**
95	3.250	3.350	3.450	3.550	3.650	3.750	3.850	4.000	4.100	**43.1**
100	3.350	3.450	3.500	3.650	3.800	3.900	4.000	4.100	4.200	**45.4**
105	3.450	3.550	3.650	3.750	3.900	4.000	4.100	4.200	4.300	**47.6**
110	3.500	3.650	3.750	3.850	4.000	4.100	4.200	4.300	4.400	**49.9**
115	3.600	3.750	3.850	3.950	4.100	4.200	4.300	4.400	4.550	**52.2**
120	3.700	3.850	3.950	4.050	4.200	4.300	4.400	4.500	4.650	**54.4**
125	3.800	3.900	4.050	4.150	4.250	4.400	4.500	4.600	4.750	**56.7**
130	3.900	4.000	4.150	4.250	4.350	4.500	4.600	4.700	4.850	**59.0**
135	3.950	4.100	4.200	4.350	4.450	4.600	4.700	4.800	4.950	**61.2**
140	4.050	4.150	4.300	4.400	4.550	4.650	4.800	4.900	5.050	**63.5**
145	4.100	4.250	4.400	4.500	4.650	4.750	4.900	5.000	5.150	**65.8**
150	4.200	4.350	4.450	4.600	4.750	4.850	4.950	5.100	5.200	**68.0**
155	4.300	4.400	4.500	4.650	4.800	4.950	5.050	5.200	5.300	**70.3**
160	4.350	4.500	4.600	4.750	4.900	5.000	5.150	5.250	5.400	**72.6**
165	4.450	4.550	4.700	4.850	4.950	5.100	5.200	5.350	5.500	**74.8**
170	4.500	4.650	4.750	4.900	5.050	5.150	5.300	5.450	5.550	**77.1**
175	4.550	4.700	4.850	5.000	5.100	5.250	5.400	5.500	5.650	**79.4**
180	4.650	4.800	4.900	5.050	5.200	5.350	5.450	6.600	5.750	**81.6**
185	4.700	4.850	5.000	5.150	5.250	5.400	5.550	5.700	5.800	**83.9**
190	4.800	4.900	5.050	5.200	5.350	5.500	5.600	5.750	5.900	**86.2**
195	4.850	5.000	5.150	5.300	5.400	5.550	5.700	5.850	6.000	**88.5**
200	4.900	5.050	5.200	5.350	5.500	5.650	5.750	5.900	6.050	**90.7**
205	4.950	5.100	5.250	5.400	5.550	5.700	5.850	6.000	6.150	**93.0**
210	5.050	5.200	5.350	5.500	5.650	5.800	5.900	6.050	6.200	**95.3**
215	5.100	5.250	5.400	5.550	5.700	5.850	6.000	6.150	6.300	**97.5**
220	5.150	5.300	5.450	5.600	5.750	5.900	6.050	6.200	6.350	**99.8**
225	5.200	5.400	5.550	5.700	5.850	6.000	6.150	6.300	6.450	**102.1**
230	5.300	5.450	5.600	5.750	5.900	6.050	6.200	6.350	6.500	**104.3**
235	5.350	5.500	5.650	5.800	5.950	6.100	6.250	6.400	6.550	**106.6**
240	5.400	5.550	5.700	5.900	6.050	6.200	6.350	6.500	6.650	**108.9**
245	5.450	5.600	5.800	5.950	6.100	6.250	6.400	6.550	6.700	**111.1**
250	5.500	5.700	5.850	6.000	6.150	6.300	6.450	6.650	6.800	**113.4**
255	5.600	5.750	5.900	6.050	6.200	6.400	6.550	6.700	6.850	**115.7**
260	5.650	5.800	5.950	6.100	6.300	6.450	6.600	6.750	6.900	**117.9**
265	5.700	5.850	6.000	6.200	6.350	6.500	6.650	6.800	7.000	**120.2**
270	5.750	5.900	6.100	6.250	6.400	6.550	6.750	6.900	7.050	**122.5**
275	5.800	5.950	6.150	6.300	6.450	6.650	6.800	6.950	7.100	**124.7**
280	5.850	6.000	6.200	6.350	6.500	6.700	6.850	7.000	7.150	**127.0**
285	5.900	6.100	6.250	6.400	6.600	6.750	6.900	7.100	7.250	**129.3**
290	5.950	6.150	6.300	6.450	6.650	6.800	6.950	7.150	7.300	**131.5**
295	6.000	6.200	6.350	6.550	6.700	6.850	7.050	7.200	7.350	**133.8**
300	6.050	6.250	6.400	6.600	6.750	6.900	7.100	7.250	7.450	**136.1**
305	6.100	6.300	6.450	6.650	6.800	7.000	7.150	7.300	7.500	**138.3**
310	6.150	6.350	6.500	6.700	6.850	7.050	7.200	7.400	7.550	**140.6**
315	6.200	6.400	6.550	6.750	6.900	7.100	7.250	7.450	7.600	**142.9**
	1.52	**1.57**	**1.63**	**1.68**	**1.73**	**1.78**	**1.83**	**1.88**	**1.93**	
	Height in meters									

females
males

females
males

females
males

females
males

females
males

females
males

weight, while 75 (62.5 %) (20 females and 55 males) were overweight, and one male patient had optimal body weight (0.8%). 20/39 (51.3%) females and 55/80 (68.8%) males were overweight (Fig. 1).

The difference between real and optimal body weight ranged from 0.3–40.9% of optimal body weight for females (mean value = 9.6%), and for males from 0.2–62.0% (mean value 13.9%). The difference between real and optimal body weight was highly significant for males ($p < 0.01$), while it was not the case for female patients ($p > 0.05$). The mean measured body weight was higher than the mean optimal body weight in both males and females.

In the group of underweight patients, 52.6% females had more than a 10% deviation from OBW, while 56% of males had deviation of less than 10% (Fig. 2).

In the overweight group of patients, 55% of females had a deviation from OBW of less than 10%, while 61.9% males had a deviation of greater than 10% (Fig. 3).

The results of the RCM, blood and PV measurements (mean values) obtained using ^{51}Cr-erythrocytes are shown in Table 3, while the mean optimal values for RCM, blood and PV estimated using the ICSH method and Retzlaff's tables are displayed in Table 4.

The optimal RCM, PV and blood volume values estimated by ICSH and Retzlaff's methods differed highly significantly ($p < 0.01$) in male subjects. Optimal PV values estimated by ICSH and Retzlaff's methods differed significantly ($p < 0.05$) in female subjects. Optimal values obtained by ICSH method were significantly lower than optimal values obtained by Retzlaff's method.

Highly significant difference was registered between RCM optimal values, determined using ICSH and Retzlaff's method ($p < 0.01$) in patients with deviation of real body weight from optimal body weight:
— lower than 10%,
— from 10–20%, and
— greater than 20%.

Concordant results obtained using all three methods for optimal RCM&PV estimation were obtained in 72/120 (60%) patients (absolute erythrocytosis in 49/72, normal findings in 21/72 and depleted plasma volume in 2/72).

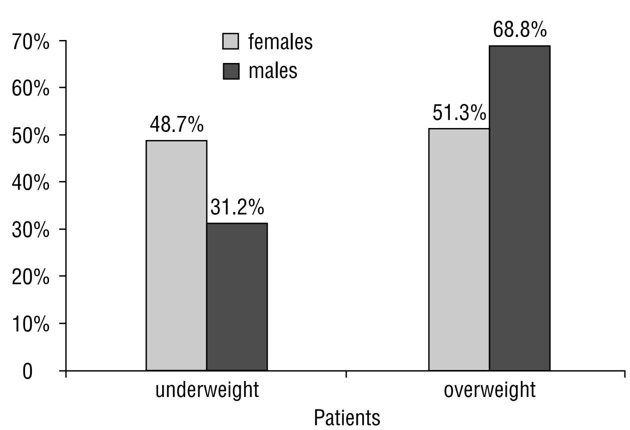

Figure 1. RBW aberration from OBW in 119 patients (39 females and 80 males) regarding their sex; RBW — real body weight; OBW — optimal body weight.

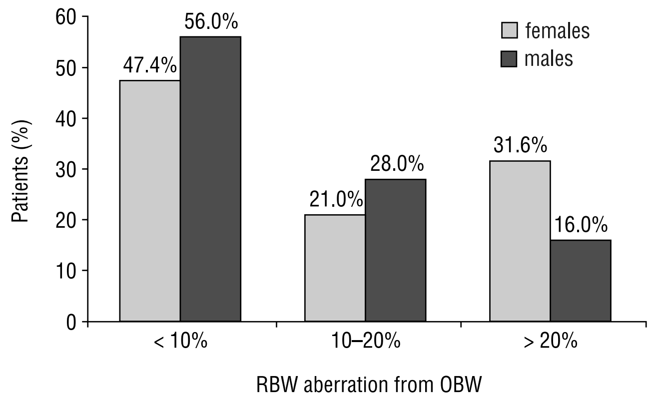

Figure 2. Underweight 44 patients regarding their sex; for aberrations see Figure 1.

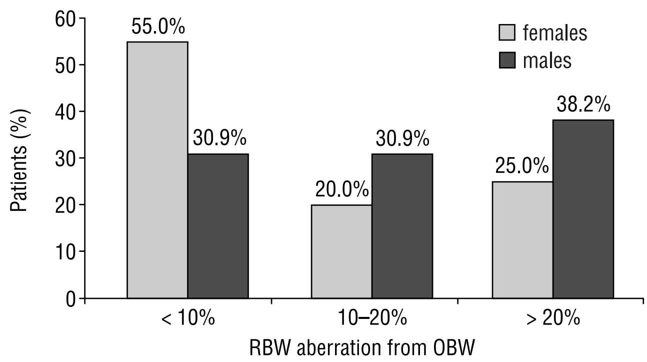

Figure 3. Overweight 75 patients regarding their sex; for aberrations see Figure 1.

Table 3. Results of RCM, blood and PV measurements using ^{51}Cr-erythrocytes

Parameter	Mean measured values [ml]	
	Males	Females
RCM	2870	2280
PV	2840	2160
BLOOD	5710	4440

Table 4. Results of optimal RCM and PV estimation

Parameter	Mean optimal values [ml]			
	Males		Females	
	ICSH method	Retzlaff's method	ICSH method	Retzlaff's method
RCM	2140	2240	1460	1490
PV	3140	3090	2380	2430
BLOOD	5280	5330	3840	3920

Table 5. Comparison between ml/kg RBW and ml/kg OBW methods in view of agreement/disagreement with ICSH and Retzlaff's methods, in the group of 48 patients with incongruent results

Patients		Method			
		ml/kg real body weight		ml/kg optimal body weight	
	n	Agreement	Disagreement	Agreement	Disagreement
Underweight	15	73.4 %	26.6 %	13.4 %	86.6 %
Overweight	33	21.2 %	78.8 %	69.7 %	30.3 %

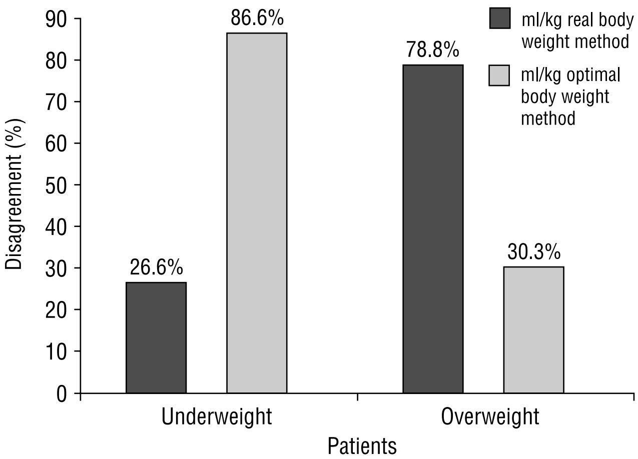

Figure 4. Comparison between ml/kg RBW and ml/kg OBW methods in view of disagreement with ICSH and Retzlaff's methods, in 48 patients with disagreement.

Disagreement of the results obtained using different methods for optimal RCM&PV estimation was found in 48/120 (40%) patients. It was greatest between ml/kg RBW and ml/kg OBW methods, and present in 39/48 (81.2%) patients with a disagreement in results and in 39/120 (32.5%) investigated persons. For patients whose body weight was lower than optimal (underweight), the ml/kg RBW method showed better agreement with the ICSH and Retzlaff's methods than the ml/kg OBW method (Tab. 5). For patients whose weight was over the optimal value (overweight), ml/kg OBW method showed better agreement with the ICSH and Retzlaff's methods (Fig. 4).

Disagreement between the ml/kg RBW method and the ICSH&Retzlaff's methods was registered in 30/120 patients (25%) (Tab. 6). Agreement with the ICSH method was found in 84/120 subjects (70%), and with Retzlaff's method in 89/120 patients (74.2%).

Disagreement between the ml/kg OBW method and the ICSH&Retzlaff's methods was noticed in 23/120 patients (19.2%). Agreement with the ICSH method was found in 95/ /120 patients (79.2%), and with Retzlaff's method in 93/120 subjects (77.5%).

No significant difference was noticed in agreement with the ICSH&Retzlaff's methods between the ml/kg RBW and ml/kg OBW methods ($p > 0.05$).

Agreement between the method based on body surface area (ICSH) and the method based on patients weight and height (Retzlaff's tables) was present in 110 of 120 (91.7%) investigated persons. Absolute erythrocytosis was present in 69/110 (62.7%), normal

Table 6. Concordance of ml/kg body weight methods with ICSH and Retzlaff's methods

	Concordant results with				n
Method	ICSH	Retzlaff	Both	Neither	
Ml/kg RBW	1	6	83	30	120
Ml/kg OBW	4	2	91	23	120

results in 37/110 (33.6%), and PV deplition in 4/110 (3.6%) subjects.

Disagreement between the method based on body surface area (ICSH) and the method based on patients' weight and height (Retzlaff's tables) was found in 10/48 patients with disagreements (20.8%). Incongruent interpretation of results of RCM and PV measurement using the ICSH and Retzlaff's methods were registered in 10/120 (8.3%) investigated persons. The ICSH method found absolute erythrocytosis in 7/10 patients, while Retzlaff's method indicated normal findings in all of them. In 1/10 patients, the ICSH method yielded normal findings, while Retzlaff's method showed plasma volume depletion (haemoconcentration). In 1/10 patients we found the opposite situation: Retzlaff's method indicated normal findings (with RCM at the lower border of normal values), and the ICSH method showed plasma volume depletion (plasma volume slightly lower than 25% of the optimal value — hemoconcentration at the borderline) (Tab. 7). In 1/10 patients, the ICSH method indicated anaemia, while Retzlaff's method showed normal finding of RCM, but it was at the border-line with anemia.

Discussion

The measurement of red cell mass (RCM) and plasma volume (PV) is indicated:
- in diagnosing of erythrocytosis, in order to differentiate true (absolute) erythrocytosis from pseudo- (relative) erythrocytosis,
- in therapy and management of polycythemia vera,
- in the diagnosing of anemias, in order to exclude pseudoanaemia (increased plasma volume),
- to assess the severity of anaemia in patients with splenomegaly (hematocrit may be falsely low in splenomegaly),
- in the postoperative state (after high blood-loss surgeries) where clinical indicators may be unreliable in the detection of oligemia or overtransfusion.

Hematocrit values over 50% in males and over 45% in females are abnormal and require further evaluation [13]. It is frequently

Table 7. Disagreement between ICSH and Retzlaff's method

	Method For optimal volumes estimation				Patient Body weight		sex
	ICSH	REZLAFF	Ml/kg RBW	Ml/kg OBW	Difference R&O (%)	Direction of difference	
1	N	HC	HC	AE	63.1	overweight	m
2	ANEM	N (BL ANEM)	N	ANEM	29.4	underweight	f
3	AE	N	HC	AE	36.1	overweight	f
4	AE	N	AE	HC	20.7	underweight	m
5	HC (BL)	N (BL ANEM)	ANEM	ANEM	5.3	overweight	m
6	AE	N	N	N	7.8	overweight	f
7	AE	N	N	N	15.9	overweight	m
8	AE	N	N	AE	21.3	overweight	m
9	AE	N	N	N	14.5	overweight	m
10	AE	N	N	AE	5.8	overweight	m

RBW — real body weight; OBW — optimal body weight; R&O — real and optimal body weight; N — normal finding; AE — apsolute erythrocytosis; HC — hemoconcentration; ANEM — anemia; BL ANEM — normal finding at the border-line with anemia

necessary to document absolute increase in red cell mass performing blood volume examination by direct red cell labelling with ^{51}Cr-sodium-chromate. Since hematocrit in over 60% in males and in over 55% of females is almost always associated with absolute erythrocytosis, the greatest need for blood volume, red blood cell mass and plasma volume determination is in male patients with hematocrit in the range from 50–60% and in females from 45–55% [13].

Elevated red cell mass can occasionally be present in the face of normal hematocrit:

— expanded plasma volume can mask an elevated red cell mass (splenomegaly due to portal hypertension, portal or hepatic vein thrombosis);
— iron deficiency can lead to a fall in hematocrit in patients with polycythemia vera [14].

Therefore, some authors suggest RCM determination in patients with normal hemoglobin and hematocrit values with the intention of diagnosing "unapparent" polycythemia vera [13, 14]. This includes patients with portal or hepatic vein thrombosis, splenomegaly, leukocytosis, or thrombocytosis [14].

Currently, the most widely used method for blood volume and RCM measurement employs ^{51}Cr-labelled red blood cells. We performed autologous red blood cells labelling (erythrocytes of the investigated patients with polycythemia vera were labelled) with ^{51}Cr-sodium chromate. Some of them (with normal blood volume and RCM findings, or border-line anaemia) were examined after phlebotomies.

The problem that is encountered nowadays is how to interpret the obtained results of RCM&PV. Different approaches to this problem exist. In order to estimate their influence on the interpretation of measured RCM&PV results, we used several approaches and applied all of them to the same results of 120 RCM&PV measurements. Concordant interpretations were achieved in 60% of cases, while disagreements existed in 48/120 (40%) of patients.

The interpretation of the measured RCM&PV required the comparison of the obtained values:

— with the range of normal values or
— with optimal values estimated for each person.

Traditionally, RCM&PV results on one side, and range of normal values on the other, were expressed in terms of ml/kg of body weight. This approach can be misleading, since adipose tissue is less vascular than lean tissue [11, 13]. In obese subjects, the measured RCM values expressed in terms of ml/kg RBW are frequently lower than optimal, which leads to erroneous interpretation. In our group of overweight patients, this approach to the interpretation of obtained RCM&PV results showed disagreement with other approaches in 78.8% of cases, while in underweight persons disagreement was registered in only 26.6% of cases. The traditional ml/kg RBW method was better for our group of underweight rather than for overweight patients.

The majority of our patients were overweight (72.5%) with more than 50% of males (67.9%) and females (51.3%) falling into this category. Since there has been a suggestion that optimal body weight might be better solution than real body weight in such circumstances [3], we used the ml/kg OBW method for the interpretation of obtained results, too. This method resulted in a disagreement with other approaches in 30.3% of our overweight patients, which was better than 78.8%, that we registered with the ml/kg RBW method. On the other hand, in our underweight patients ml/ /kg OBW method disagreed with other methods in 86.6%, which was much worse than 26.6%, that we noticed with the ml/kg RBW method. In our group of patients, ml/kg OBW showed better results for overweight patients, while the ml/kg RBW method was better for underweight subjects.

The ml/kg RBW and ml/kg OBW methods disagreed in 39/120 (32.5%) of our patients and led us to form different conclusions.

When the whole group of our patients was taken into consideration, regardless of the difference between real and optimal body weight, no significant difference was obtained between the ml/kg RBW and ml/kg OBW methods. The concordance with the other two methods that we used (ICSH and Retzlaff's) was similar.

Methods based on both patient body weight and height are supposed to be more accurate than ml/kg body weight methods [8, 11]. The implementation of this approach is suggested due to the high incidence of obesity in the population [9]. In our group of patients with polycythemia vera 20/39 (51.3%) females and 55/80 (68.8%) males were overweight. Therefore, we used this approach by implementing Retzlaff's tables for optimal volumes estimation. An elevated RCM is now defined as being 25% greater than the mean predicted value of RCM for that individual [9]. The same definition is valid for the recently recommended method for optimal RCM&PV estimation by the International Council for Standardization in Haematology [9], based on patient surface area. The proposed reference ranges of normal values of ± 25% for both methods have been selected to include at least 98% of normal population.

In our group of polycythemia vera patients optimal RCM values determined by ICSH and Retzlaff's method differed highly significantly ($p < 0.01$), regardless of the patient's weight. The ICSH method yielded significantly lower optimal values for RCM&PV than Retzlaff's method. This led to differences in the interpretation of the obtained results in 10/120 (8.3%) of our investigated subjects. Lower optimal values of the ICSH method resulted in diagnosing absolute erythrocytosis in a majority of our patients with disagreement, while Retzlaff's method indicated normal findings in all of them. Also, in one patient normal RCM with border-line hemoconcentration was registered with the ICSH method, while Retzlaff's method showed border-line anaemia. Nevertheless, ICSH and Retzlaff's method showed better mutual agreement in comparison with the agreement they had with ml/kg body weight methods in our group of patients.

Conclusion

Different methods for optimal RCM&PV estimation (the ml/kg RBW method, the ml/kg OBW weight method, Retzlaff's method, and the ICSH recommended method) influence the interpretation of obtained results of RCM&PV measurement, and therefore their choice has great clinical significance. The four methods that we analysed using the same results of RCM and PV measurements (using autologous ^{51}Cr-Er) in 120 subjects, lead to incongruent interpretation in 40% of our polycythemia vera patients. We obtained the most congruent results with the ICSH and Retzlaff's methods, which also disagreed in 8.3% of our patients. The ICSH method yielded somewhat lower optimal RCM values in comparison with Retzlaff's method.

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