An elevated venous haemoglobin concentration cannot be used as a surrogate marker for absolute erythrocytosis: a study of patients with polycythaemia vera and apparent polycythaemia

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Summary

The diagnosis of polycythaemia vera (PV) has been established upon sets of clinical criteria, which require the presence of absolute erythrocytosis (AE). The most recent clinical criteria for PV, published by the World Health Organization (WHO) in 2001, also required AE, and stated that the measured red cell mass (RCM) could be replaced by a surrogate marker for AE; a haemoglobin (Hb) value of >18.5 g/dl in males and >16.5 g/dl in females. The present study evaluated the potential of venous haematocrit (Hct) and Hb values as possible surrogate markers for AE in a series of 77 consecutive patients with PV and 66 patients with apparent polycythaemia (AP), in all of whom the RCM had been previously determined. In only 35% of the male PV patients would Hb values >18.5 g/dl indicate the presence of AE. Conversely, 14% of male AP patients would be misdiagnosed as having AE. A Hb > 16.5 g/dl would predict the presence of AE in 63% of the female PV patients, but 35% of female AP cases would be misdiagnosed as having AE. However, when the Hct was ≥0.60 an AE was always present, and this was true for both male and female subjects.

Keywords: polycythaemia vera, apparent polycythaemia, absolute erythrocytosis, haemoglobin, haematocrit.

In the absence of molecular markers, the diagnosis of polycythaemia vera (PV) still relies upon sets of clinical criteria. More than three decades ago the Polycythemia Vera Study Group (PSVG) determined a set of diagnostic criteria (Berlin, 1975; Berk et al, 1986) upon which the diagnosis of PV should be established. These criteria offered clinical investigators guidance for the standardization of patient groups in clinical trials. Indeed, over recent decades the PSVG criteria formed the gold standard for the diagnosis of PV. One of the three key criteria, the A1 criterion, required the presence of a raised red cell mass (RCM), i.e. absolute erythrocytosis (AE). Herein, AE was defined as a measured RCM ≥36 ml/kg in male and ≥32 ml/kg in female subjects. The error of this method of expression has repeatedly been challenged because fat tissue, being relatively less vascular, results in a lower RCM for obese compared with thinner individuals. As a consequence thereof, the revised PV criteria proposed by Pearson and Messinezy (1996) defined AE as a measured RCM in excess of 25% above their individual normal predicted RCM (Pearson et al, 1995). In these revised PV criteria, AE was also considered to be an A1 criterion. Recently, the World Health Organization (WHO) criteria for the diagnosis of PV were published (Pierre et al, 2001). It is likely that these latter criteria will govern the diagnosis of PV over several years to come. In this latest set of criteria, the A1 criterion also requires AE, i.e. the measured RCM must exceed the normal predicted value by more than 25%. Nevertheless, it also states that a measured RCM may be replaced by a surrogate marker for AE, herein a haemoglobin (Hb) concentration in males >18.5 g/dl and in females >16.5 g/dl respectively.

Inasmuch as an increased value for venous haematocrit (Hct) or an elevated Hb concentration arouses the suspicion of a polycythaemic state, it would appear appropriate to measure the RCM and thereby assess whether or not a state of AE is present. The simultaneous measurement of the plasma volume is, in most cases, highly rewarding. The measurements of RCM and plasma volume should be preferably carried out by the 31Cr- and 125I-methods, respectively (International Committee for Standardization in Haematology (ICSH), 1980).
Even though the presence of AE, as assessed by a measured RCM, is required by the original PVSG criteria (Berlin, 1975) and the revised criteria referred to above (Pearson & Messinezy, 1996; Pierre et al., 2001), RCM measurements are no longer performed in many centres (Johansson et al., 2002a; Streiff et al., 2002; Klippen et al., 2003). Frequently, RCM measurements are considered to be cumbersome, costly and time-consuming, and are replaced by surrogate markers for AE, although the variables that constitute these markers have not been confirmed. The revised criteria referred to above do, however, include some valuable assays, i.e. clonality markers, not been confirmed. The revised criteria referred to above do, however, include some valuable assays, i.e. clonality markers, endogenous erythroid colony formation in vitro, and low serum erythropoietin. Most importantly, the value of histological examinations of bone marrow biopsies has recently been duly recognized (Pierre et al., 2001).

The aim of the present work was to evaluate the potential of Hct and Hb as possible surrogate markers in the evaluation of AE. We therefore reviewed a consecutive series of PV patients in whom RCM had always been measured and in whom AE invariably was present; the results were related to the simultaneous measurement of the venous Hb concentration and micro-Hct. As control subjects we employed a series of patients with apparent polycythaemia (AP) evaluated at our institution during the same time period. RCM had been measured in all of these AP patients and none of them demonstrated AE.

Patients and methods

The study comprises a total of 77 consecutive patients (31 males and 46 females) with PV and 66 patients with AP (49 males and 17 females) seen at our outpatient Haematology Clinic during a 10-year period (1993–2002). RCM and plasma volume were measured in all PV and AP patients included in the study. RCM was not always measured in a number of PV patients with very high venous micro-Hct values (>0.60), and these patients were not included in the present paper. In each PV patient the diagnosis satisfied the PVSG criteria (Berlin, 1975) as well as the revised criteria established by Pearson and Messinezy (1996). All AP patients had repeatedly demonstrated elevated values for venous micro-Hct, i.e. >0.48 in females and >0.51 in males (Messinezy & Pearson, 1993). None of the AP patients had an RCM in excess of 25% of their individual normal predicted value (Pearson et al., 1995).

Additionally, histological examinations of bone marrow biopsies were carried out on all PV and AP patient included in the study. Spleen size was assessed by γ-camera scintigraphy, and plasma erythropoietin concentrations were measured. However, the results of these studies will not be reported in the present paper.

Patients with PV and AP

The mean age of the 31 male PV patients was 66 (range: 39–86) years, and was 68 (range: 21–91) years for the 46 PV females. Correspondingly, the mean age for the 49 male AP patients was 61 (range: 24–80) years and 64 (range: 51–75) years for the 17 AP females.

Sampling for Hb concentration and venous micro-Hct

All blood samples were taken between 08:00 and 11:30 AM. The Hb concentration was measured using Cell Dyn 4000, Abbott (Santa Clara, CA, USA). Venous micro-Hct was obtained by centrifugation of K2-EDTA-anticoagulated venous blood using the IEC Micro-MB centrifuge for 5 min at 10 000 rpm.

Measurements of RCM and plasma volume

Measurements of RCM and plasma volume were carried out according to current principles, using the 51Cr- and 125I-methods respectively (ICSH, 1980).

Statistics

Standard statistical methods were used for the calculation of mean values and SD. The difference between mean values was tested with Student’s t-test for unpaired observations and was considered statistically significant if P < 0.05.

Results

The mean Hb concentrations for male and female PV patients were 18.2 ± 2.1 and 16.6 ± 1.8 g/dl respectively. These values did not differ statistically from the corresponding mean values for AP subjects (17.7 ± 0.8 and 16.4 ± 1.2 g/dl respectively). The mean micro-Hct for male and female PV patients was 0.60 ± 0.07 and 0.55 ± 0.05 respectively. The difference between these mean values and the corresponding mean values for AP (0.54 ± 0.02 and 0.52 ± 0.03) were, however, highly significant (P = 1.23 × 10−6 and P = 0.013 respectively).

Figure 1 shows the relationships between venous Hb concentrations and the measured/predicted RCM ratios in 31 male PV and 49 male AP patients, assuming that a cut-off Hb value of >18.5 g/dl indicated the presence of AE. Herein, a RCM ratio limit of >1.25 defined the presence of an AE. Only 11 of 31 (35%) of the male PV patients would have an Hb value in excess of 18.5 g/dl, indicating that AE is present. The remaining 20 of 31 (65%) male PV patients would be misdiagnosed as not having an increased RCM. Conversely, seven of 49 (14%) of the male AP patients would be misdiagnosed as having AE. Likewise, Fig 2 shows the relationship between Hb concentrations and the measured/predicted RCM ratios in 46 female PV and 17 female AP patients, assuming that a cut-off Hb value of >16.5 g/dl would indicate the presence of AE. Also here, a RCM ratio limit of >1.25 defined the presence of AE. The results showed that, in 29 of 46 (63%) of the female PV patients, an Hb concentration in excess of 16.5 g/dl would predict the presence of AE. In the remaining 17 female PV patients, however, this Hb value

Fig 1. The relationship between haemoglobin (Hb) and the measured/predicted red cell mass (RCM) in 31 male polycythaemia vera (PV; •) and 49 male apparent polycythaemia (AP; ○) patients. The abcissa (x-axis) intersects the ordinata (y-axis) at 1.25. The ordinata intersects the abcissa at 18.5 g/dl.

Fig 2. The relationship between haemoglobin (Hb) and the measured/predicted red cell mass (RCM) in 46 female polycythaemia vera (PV; •) and 17 female apparent polycythaemia (AP; ○) patients. The abcissa (x-axis) intersects the ordinata (y-axis) at 1.25. The ordinata intersects the abcissa at 16.5 g/dl.

Fig 3. The relationship between venous micro-haematocrit (Hct) and the measured/predicted red cell mass (RCM) in 31 male polycythaemia vera (PV; •) and 49 male apparent polycythaemia (AP; ○) patients.
would fail to predict the presence of AE. As regards the female AP patients, six of 17 (35%) would be misdiagnosed as having an increased RCM.

Figure 3 shows that none of the male patients with AP had a venous micro-Hct that was more than 0.59. However, in the PV group, all of whom had a measured increase in the RCM (i.e. AE) by definition, the range for micro-Hct was considerable (0.50–0.73). The same pattern was seen for the female AP and PV patients (Fig 4). None of the female AP patients had a micro-Hct in excess of 0.59. Indeed, the majority of the female AP patients had considerably lower micro-Hcts than their male counterparts. Female PV patients also showed a wide range of micro-Hct values (0.44–0.68).

The mean values for the ratios of measured/predicted plasma volumes for male PV and AP subjects were 0.92 and 0.81, respectively, and these mean values differed significantly (P = 0.001). Likewise, the corresponding mean values for the ratios of measured/predicted plasma volumes for female PV and AP subjects (1.01 and 0.89, respectively) differed significantly (P = 0.04).

Discussion

It appears more appropriate to define an erythrocytosis in terms of the Hct rather than Hb concentration. Iron deficiency can occur in erythrocytoses, and the Hct may then be disproportionately higher than the Hb value. It is well recognized that most patients referred for the investigation of polycythaemia (Hct >0.51 in males, >0.48 in females) (Messinezy & Pearson, 1993) are found to have a RCM within normal reference intervals. Thus, in the study of Pearson et al (1984), this group of patients constituted 102 of 188 (54%) male subjects referred for investigation of polycythaemia. It was also demonstrated that when the venous Hct was ≥0.60 an AE was invariably present. Conversely, however, more than 17.5% of patients with Hct ≤0.52 also demonstrated AE. Also, in an unselected group of 322 males, it was shown that the incidence of an AE rose as the Hct increased above normal levels. Thus, an AE was present in only 18% with a Hct of 0.50–0.52, rising to 65% at 0.56–0.58 and to 100% at 0.60 and above (Pearson, 1991). Males and females with Hct values above 0.60 and 0.56, respectively, can be assumed to have AE (Pearson & Guthrie, 1984; Pearson, 1991; Pearson et al, 2000).

One of the aims of the present work was to investigate whether a Hb value of >18.5 g/dl in males and >16.5 g/dl in females could serve as surrogate markers for AE, as suggested by the recent WHO criteria for PV (Pierre et al, 2001). The present results do not support this concept. Herein, it was clearly shown that an Hb concentration >18.5 g/dl supported the presence of AE in only 35% of the male PV patients. An Hb concentration >16.5 g/dl correctly predicted the presence of AE in 63% of female PV patients. However, 14% of the male AP patients would be misdiagnosed with AE and as many as 35% of the female patients with AP would be misdiagnosed with an increased RCM. It can thereby be concluded that the Hb values suggested by the WHO criteria for PV (Pierre et al, 2001) are certainly not able to act as surrogate markers for AE and should therefore be deleted or, alternatively, be replaced by more useful variables.

Our results do, however, support the carefully conducted work carried out by Pearson and colleagues in a number of studies (e.g. Pearson, 1991). The results of the current study clearly demonstrated that no male or female AP patient presented with a micro-Hct ≥0.60 (cf. Pearson, 1991). Indeed, one female AP patient had a venous micro-Hct as high as 0.59 (Fig 4). It could therefore be questioned whether this set of patients showed a difference between male and female subjects as regards Hb and Hct values. However, our results clearly demonstrated that differences between the sexes are present in both PV and AP patients. The female AP patient in Fig 4 (with a venous micro-Hct of 0.59) should therefore be considered as

Fig 4. The relationship between venous micro-haematocrit (Hct) and the measured/predicted red cell mass (RCM) in 46 male polycythaemia vera (PV; ●) and 17 female apparent polycythaemia (AP; ○) patients.

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an outlier (she was a heavy smoker and had an ileostomy in 1989 due to Crohn disease) but not be withdrawn from the present results. All other female AP patients had micro-Hct values ≤0.56. It could therefore be concluded that, in those instances where the venous micro-Hct in males or females was ≥0.60, then an AE was always present, and in such instances there is no need to measure the RCM. In instances when these micro-Hct values are not met, a diagnosis of AE cannot be established, and we feel that RCM and plasma volume should be determined.

For the diagnosis of PV, useful molecular markers will hopefully be available in the future. Meanwhile, in the opinion of the present authors, the simultaneous measurements of RCM and plasma volume will continue to be most helpful in the evaluation of various polycythaemic states. Thereby, indispensable information is provided, e.g. for a correct understanding of PV (and other states of AE) and AP (Johansson et al, 2002b). Finally, in view of the work-up of a disorder that may require lifelong follow up, the costs and time-consumption for measurements of RCM and plasma volume appear most reasonable.

References


