Low Mean Cell Haemoglobin is a Valuable Parameter of Thrombotic Risk Stratification in Patients with Polycythemia Vera

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Abstract

Objectives: Thrombosis is a leading cause of morbidity and mortality in patients with Philadelphia negative chronic myeloproliferative neoplasms (MPNs). There are many thrombosis risk stratifications used in this patient group taking into consideration the age, thrombosis history and cardiovascular factors (hypertension, hypercholesterolaemia, hyper-triglyceridaemia, thrombocytosis, smoking and diabetes mellitus). In this work we evaluated the possible role of iron deficiency in thrombotic events (TE) of the polycythaemia vera (PV) patients. Methods: We considered the low mean cell haemoglobin (MCH <28 pg) value as a parameter to assess the iron deficiency in the multicentre database (15 Hungarian haematology centres) of our HUMYPRON GROUP (Hungarian MPN Working Group). The MCH values, recorded at the time of diagnosis of 296 patients with polycythaemia vera, were retrospectively analysed.

Results: The low MCH, at the diagnosis, was found to be a risk factor for thrombotic events occurring after diagnosis (OR: 1.966). It was also shown as an additive and independent parameter in the Tefferi high-risk patient groups, and combining it with Tefferi risk stratification an extremely high thrombotic risk group could be determined (Nagelkerke R square: 0.084). We have supposed that low MCH in PV reveals a disease form featured with a high proliferation activity. Our hypothesis was confirmed with a sub-study (n=52) showing that the high JAK2V617F allele burden was significantly correlated with the low MCH (p=0.005) and the high white blood cell count (WBC) (p=0.001).

Conclusions: Iron deficiency, existing at the time of diagnosis of PV, was proven to be a risk factor for imminent thrombotic events. The low MCH was found to be a strong additive factor when it was combined with the known thrombotic risk stratification systems. The low MCH showed significant correlation with the high JAK2V617F allele burden.

Keywords: Iron deficiency; Mean cell haemoglobin; Myeloproliferative neoplasms; Polycythemia vera; Thrombotic risk

Introduction

Philadelphia negative chronic myeloproliferative neoplasms (polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF)) are associated with higher thrombotic risk leading to excessive cardiovascular mortality [1-4].

A comprehensive epidemiological study involving PV patients, the European collaboration study on low dose aspirin in polycythaemia (ECLAP), revealed that 41% of all mortality was of cardiovascular origin (1.5 deaths per 100 person per year). Coronary heart disease was responsible for 15% of all deaths, whereas congestive heart failure, non-haemorrhagic stroke and pulmonary embolism accounted for 8-8% each. The cumulative incidence of nonfatal thrombosis was 3.8 events per 100 people per year, without a difference between arterial and venous thrombosis [5].

The thrombophilic behaviour observed in these conditions manifests in microcirculatory disturbances, arterial and venous thrombosis. The possible mechanism resulting in thrombophilia in MPN patients has been widely investigated over the last decades, nevertheless there is no definite answer that could explain this phenomenon. Data from earlier studies are often conflicting or difficult to interpret. For instance, both the iron deficiency and the excess have been associated with increased thrombotic risk [6].

Epidemiological studies support the hypothesis of Sullivan [7], that elevated level of stored iron associates with higher incidence of cardiovascular morbidity [8-10]. However, iron deficiency was recognised as a risk factor of cerebral sinus thrombosis [11], carotid artery thrombosis [12], central retinal vein occlusion [13,14] and superior sagittal sinus thrombosis [15].

Donovan et al. [16] found that PV patients treated with phlebotomy alone suffered significantly more thrombotic events than those who received myelosuppressive regimens [17]. Iron deficiency is commonly detected in patients with PV. The phlebotomy can contribute to the further worsening of iron deficiency [18].

There is different risk stratifications used to assess the thrombotic risk at the time of diagnosis in clinical practice of Philadelphia negative MPNs. The risk stratification proposed by Tefferi estimates the
possibility of a recurrent thrombosis in PV patients. There are two risk
categories, the high (age >60 years or prior thrombotic event) and the
low (age ≤ 60 years and no thrombosis history) [19]. The risk analysis
by Landolfi, beside these variables, also takes general vascular factors
into consideration [20].

The transferrin saturation, serum ferritin level, reticulocyte
haemoglobin content and bone marrow iron content are the most used
and suitable parameters to measure the body’s iron status nowadays.
On the other hand, more reports raised attention that red blood cell
hypochromia and decreased red blood cell haemoglobin content
indicate the presence of iron deficiency [21-26]. The low mean cell
haemoglobin content (MCH) is an appropriate parameter which notes
iron deficiency [21], and it is more available in retrospective databases
in comparison to other iron status parameters.

The aim of our retrospective multicentre study was to evaluate the
impact of MCH, white blood cell count (WBC), platelet count (PLT),
age and thrombotic events at the time of diagnosis and compare our
findings to Tefferi and Landolfi thromboembolic risk stratifications in
PV patients registered in the Hungarian Myeloproliferative Neoplasia
Working Group (HUMYPRON GROUP) database [27].

Materials and Methods

After its establishment in 2012, the Hungarian MPN Working
Group (HUMYPRON GROUP) introduced a simple, practical database
for Philadelphia negative MPN patients with clinical and laboratory
data collected from 15 Hungarian haematology centres [27].

Eligibility criteria

Eligibility criteria included the age, 18 years or older, and a
previous confirmation of PV according to WHO 2008 criteria
[28]. Clinical factors (age, gender, previous thrombotic history and
risk stratifications by Tefferi and Landolfi and family history
of thrombophilia) and laboratory parameters at diagnosis (white
blood count: WBC, haemoglobin: Hb, haematocrit: Htc, mean cell
haemoglobin: MCH, platelet: PLT and C-reactive protein: CRP) were
collected. We used MCH value to assess the iron status at the time of
MPN diagnosis. We excluded patients with thalassemia, myelofibrosis
and end-stage kidney disease whose MCH alterations might have been
associated with the original disease and not with the iron deficiency.

Diagnosis of thrombosis

Vascular thrombosis was defined according to Gisslinger et al.
[29]. Major events included the following complications: peripheral
vascular (peripheral arterial thrombosis, deep venous thrombosis
and pulmonary thromboembolism), cardiovascular (myocardial
infarction), central nervous system (stroke, retinal vessel thrombosis
and sinus thrombosis) and intra-abdominal vascular events (splenic-
portal vein thrombosis and Budd-Chiari syndrome). Minor events
were angina pectoris, transient ischemic attack and superficial
thrombophlebitis of the extremities, and they were taken into account
only in patients who had no other major thrombotic events. Visual
complaint, headache, dizziness, tinnitus or acroparesthesia were not
considered as thrombotic events.

JAK2V617F (c.1849G>T) activating mutation was screened by allele-
specific polymerase chain reaction (PCR) [30]. In a subgroup of the
JAK2 V617F mutant cases, real-time quantitative PCR was performed
to determine the V617F allele burden [31].

Ethics and study management

The study was conducted according to the good clinical practice
rules and the principles of the Helsinki Declaration. Written informed
consent was obtained from the subjects for using their data anonymously
after explaining them the purpose and nature of the study.

Statistical Analysis

Fisher’s exact test was used to compare dichotomous variables,
while Mann-Whitney test served to analyse continuous variables.

Results

Data of 296 PV patients were available from the date of diagnosis,
allowing us to identify thromboembolic events throughout the
following period of 61 months on average. Median age was 61.4 years
and male dominance was observed. Only JAK2V617F or exon 12 mutated
patients were included. We adopted both the Landolfi and Tefferi
risk analyses with the simplification of using only two categories (low and
high risks) (Table 1).

Altogether 99 thromboembolic events were observed in 82 patients
between date of diagnosis and date of study entry. Low MCH value

| PV (n=296) | No. of pts. | % |
|-----------|-------------|---|
| Sex       |             |   |
| Male      | 169         | 57.09% |
| Female    | 127         | 42.91% |
| Previous TE event |    |    |
| No        | 224         | 75.68% |
| Yes       | 72          | 24.32% |
| MCH       |             |   |
| Low (<28 pg) | 95      | 32.09% |
| Not low (≥ 28 pg) | 201 | 67.91% |
| WBC       |             |   |
| Not high (≤ 10 × 10^9/l) | 125 | 42.23% |
| High (>10 × 10^9/l) | 171 | 57.77% |
| PLT       |             |   |
| Not high (≤ 450 × 10^9/l) | 152 | 51.35% |
| High (>450 × 10^9/l) | 144 | 48.65% |
| Landolfi risk |         |    |
| Low (low+intermediate risk) | 69 | 23.31% |
| High (high+very high risk) | 227 | 76.69% |
| Tefferi risk |         |    |
| Low       | 113         | 38.18% |
| High      | 183         | 61.82% |
| Median age at the diagnosis (years) |             |
| Male      | 58.6        |   |
| Female    | 63.9        |   |

Table 1: Sex, TE history, WBC, MCH, PLT, Landolfi- and Tefferi-risk, median age at the diagnosis in patients with PV (n=296).
(<28 pg) was found in 36 patients. The male/female ratio, median age, median follow up, Tefferi- and Landolfi risk results were similar in the groups of low and normal/high MCH values (≥ 28 pg) (Table 2).

Univariate analysis found significant correlation between the thromboembolic events and the previous thrombosis history, high Tefferi and Landolfi risk groups, high WBC as well as low MCH value. No other parameter was found to correlate with thromboembolic events (Table 3).

After diagnosis, most patients were treated with the combination of hydroxurea and aspirin. No conclusion could have been drawn from the treatment effect on thrombotic risk, as the follow-up period was long and the patients usually received more than one therapies.

Multivariable analysis revealed that the low MCH is an independent risk factor for thrombotic events. An additive effect to Tefferi risk was observable regarding both WBC and MCH. Male gender, together with the other three factors, also has a significant effect (Table 4).

The low MCH level may be resulted by an interaction between the high proliferation activity and the iron demand. Supporting our hypothesis, we analysed a random subgroup (n=52) of 296 patients. The consistency of this subgroup did not differ in age, gender or any other parameters from the group which had been originally examined. The average age was 63.9 years. The distribution of female and male patients was 46% and 54%, respectively. Comparing JAK2V617F allele burden to MCH value and white blood cell count, we found that, the incidence of low MCH and high WBC was significantly higher in cases featured with the high JAK2V617F burden (Figures 1 and 2 and Table 5). In those patients whose JAK2V617F allele burden has exceeded 20% the low HCH occurred in a significantly higher proportion (p=0.005).

A similar rise, though a less remarkable, was found regarding WBC count (p<0.001).

26 of the 34 patient with elevated WBC (>10×10⁹/l) had low MCH (<28 pg), while only 8 of 18 patient with low WBC (≤10 ×10⁹/l) had low MCH (p=0.021).

### Discussion

According to our current knowledge, the thrombophilic state observed in myeloproliferative neoplasms is of multifactorial origin. The previous thrombotic event, elevated WBC, high JAK2V617F allele burden, advanced age, high body mass index (BMI), hypertension, type II diabetes mellitus and hyperlipidaemia are acknowledged as contributors of the increased thrombotic risk [20].

Clinical observations indicate that the course of PV is individually different. The proliferation rate of erythroid precursors in PV is higher in some patients than in the others. The low MCH suggests that the erythropoesis in PV exceeds the amount of available iron thus the
produced erythroid mass becomes iron depleted. Our analysis has proved that the low MCH is a sign of the high proliferation rate in PV.

We consider that the iron deficiency exists in the background of the low MCH level [21-26]. The incidence of thromboembolic events was significantly higher in the low MCH group, supporting our hypothesis that low MCH is an independent risk factor for TE morbidity.

Possible pathogenic mechanisms which explain the role of iron deficiency in thromboembolic events include reactive thrombocytosis caused by iron deficiency [6], blood flow pattern alterations due to the reduced deformability and increased viscosity of microcytic red blood cells [32] and the metabolic stress increase because of the anaemic hypoxia [33]. Thus, alterations in platelet count and function may
In our former article, we have hypothesized that elevated lipocandin2 (LCN2) expression level in PV and ET may also play a crucial role in the development of arterial and venous thrombosis [35]. Examining LCN2 gene expression levels, we found that, the higher relative expression correlated with the occurrence of the thrombotic events. LCN2 is a small 25 kDa glycoprotein, which was first identified as a bacteriostatic agent produced by activated neutrophils, that acts by sequestering bacterial ferric sideropheres and interfering with bacterial iron uptake [36].

LCN2 binds iron particles, the sideropheres, and transports them into the cells producing increased cytoplasmic iron levels [37]. Experimentally induced iron deficient anemia resulted a marked elevation of LCN 2 expression. After phlebotomy and alimentary iron depletion in the murine model [38-40], elevated LCN 2 levels were observed in blood, spleen and liver, and at the same time, a decrease in the overall iron levels was detected. Presumably, this mechanism may be responsible for the higher efficacy of cytotereduction compared to phlebotomy and therefore frequent phlebotomy is not recommended.

According to our hypothesis, the excessive cell turnover found in PV patients (erythropoesis) consumes iron and decreases its level. This leads to overexpression of LCN2 in the neutrophil granulocytes transporting iron into endothelium. The resulted significant increase in endothelial cytoplasmic iron levels (labile iron) leads to oxidative stress induction in the endothelial cells and contributes to the development of thrombosis.

Conclusions

Earlier publications proved that PV patients had an iron deficiency because of elevated red cell mass cell, and this condition could be corrected with cytoreductive therapy while phlebotomy might worsen it [18]. However we did not find any data in the literature if iron deficiency enhanced TE risk in patients with PV. In the HUMYPRON database, we found low MCH in 95 cases out of 296 PV patients. After excluding patients with HD because of uraemia, thalassemia and myelofibrosis we considered the low MCH as a sign of iron deficiency [21]. Comparing TE events in the two groups, the patients with low MCH were found to have significantly more thromboembolic events. Both of the internationally accepted risk stratifications, Tefferi and Landolfi, showed significant correlation with TE events in our analysis. Using multivariable analysis, the low MCH was found to be independent of and additive to the Tefferi risk stratification. We have shown that low MCH among PV patients reveals a disease with high proliferation activity with an iron demand that cannot be maintained by a normal diet. We confirmed our hypothesis with a sub-study showing that the high JAK2V617F allele burden was significantly correlating with the low MCH and the high white blood cell count.

Taking our findings in consideration we propose the phlebotomies to be performed with special attention to the iron level of those PV patients who had been diagnosed with low MCH (iron depletion).

**Limitations and Essentials**

The lack of a normal control group and its retrospective nature are the limitations of this publication. Polycythemia vera is often accompanied by thromboembolic events. We have investigated the potential role of the low mean cell haemoglobin content (MCH). Data of 296 patients was analysed retrospectively. Low MCH was found to be an independent risk factor for thrombosis.

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