Intern Emerg Med (2007) 2:13–18
DOI 10.1007/s11739-007-0003-4

REVIEW

G. Finazzi • T. Barbui

The treatment of polycythaemia vera: an update in the JAK2 era

Received: 31 August 2006 / Accepted in original form: 17 October 2006 / Published online: 31 March 2007

Abstract The clinical course of polycythaemia vera is marked by a high incidence of thrombotic complications, which represent the main cause of morbidity and mortality. Major predictors of vascular events are increasing age and previous thrombosis. Myelosuppressive drugs can reduce the rate of thrombosis, but there is concern that their use raises the risk of transformation into acute leukaemia. To tackle this dilemma, a risk-oriented management strategy is recommended. Low-risk patients should be treated with phlebotomy and low-dose aspirin. Cytotoxic therapy is indicated in high-risk patients, with the drug of choice being hydroxyurea because its leukaemogenicity is low. The recent discovery of JAK2 V617F mutation in the vast majority of polycythaemia vera patients opens new avenues for the treatment of this disease. Novel therapeutic options theoretically devoid of leukaemic risk, such as alpha-interferon and imatinib, affect JAK2 expression in some patients. Nevertheless, these drugs require further clinical experience and, for the time being, should be reserved for selected cases.

Keywords Phlebotomy • Hydroxyurea • Interferon • Aspirin • JAK-2 mutation

G. Finazzi (✉) • T. Barbui
Department of Hematology
Ospedali Riuniti di Bergamo
Largo Barozzi 1, I-24128 Bergamo, Italy
e-mail: gfinazzi@ospedaliriuniti.bergamo.it

Introduction

Polycythaemia vera (PV) is a rare haematologic disorder characterised by clonal proliferation of bone marrow progenitors, leading to abnormal production of the erythroid cell line that is independent of physiological growth factor erythropoietin (EPO). This led investigators to search downstream receptor events, and therefore the pathophysiology of this disease has advanced considerably with the recent discovery of an acquired mutation of JAK2 (V617F) in the vast majority of PV patients, and in almost half of those with essential thrombocythaemia (ET) and idiopathic myelofibrosis (IMF) [1].

Earlier studies in untreated patients found a high incidence of thrombotic events and a life expectancy of about 18 months after diagnosis. Cytoreductive treatments of blood hyperviscosity by phlebotomy or chemotherapy have been shown to dramatically reduce the number of thrombotic events, even though haematological transformations towards IMF and acute leukaemia (AL) still represent a major cause of death [2].

Current recommendations for management of PV are based on a limited number of randomised clinical trials (RCTs), and a series of prospective and retrospective studies that have evaluated the effect of different treatments on the main outcomes of the disease, such as thrombotic complications, haematological evolution into AL or myelofibrosis and survival. The first studies were pioneered by the Polycythemia Vera Study Group (PVSG), and their results still have a significant impact on patient management [3, 4]. Recently, a prospective study called the European Collaboration on Low-dose Aspirin in Polycythemia (ECLAP) evaluated the current epidemiology of PV in Europe [5]. The strength of this survey is the huge number of patients included, and the assessment of events that are externally validated, as usually required for clinical trials.

In this article, we have reviewed PVSG and ECLAP studies with the aim to formulate updated guidelines for risk strat-

ification and patients' management. In addition, given the recent discovery of JAK2 mutation in most PV patients, we tackle the question of how these molecular advances may affect current treatment approaches and future clinical trials.

Classical studies

In the first study by the PVSG (01 trial) [3], 431 patients were randomised to one of the following treatments: (a) phlebotomy alone; (b) radiophosphorus (^{32}P) therapy plus phlebotomy or (c) chlorambucil plus phlebotomy. Patients treated with phlebotomy alone have a better median survival time (13.9 years) than those receiving ^{32}P (11.8 years) or chlorambucil (8.9 years). Causes of death are different in the 3 groups. Phlebotomised patients show an excess of mortality within the first 2–4 years, principally caused by thrombotic complications. Those patients in the two myelosuppression arms suffer higher rates of AL and other malignancies developing later during the follow-up. The incidence of IMF was virtually identical in the three arms.

In the late 1970s, the search for a nonmutagenic myelosuppressive agent led the PVSG to investigate hydroxyurea (HU), an antimetabolite that prevents DNA synthesis by inhibiting the enzyme ribonucleoside reductase. At that time, it was assumed that this agent would not be leukaemogenic or carcinogenic. In the last PVSG report [4], 51 PV patients treated with HU were followed for a median and maximum of 8.6 and 15.3 years, respectively. The incidence of AL, myelofibrosis and death were compared with the incidence in 134 patients treated only with phlebotomy in the PVSG-01 protocol. There are no significant differences in any of the 3 parameters, although the HU group shows a tendency to more ALs (9.8% *vs*. 3.7%), less myelofibrosis (7.8% *vs*. 12.7%) and fewer total deaths (39.2% *vs*. 55.2%).

Based on these studies, the PVSG produces the following recommendations that are shared by experts in the field [2, 3]: phlebotomy is suggested in all patients to keep the haematocrit (HCT) below 0.45. Stable patients at low risk for thrombosis (age <60 years, no history of thrombosis) may not require additional therapy. In patients at high risk for thrombosis, or with a very high phlebotomy requirement, the choice of a myelosuppressive agent is age-adapted. Older patients can be managed with ^{32}P, busulfan or pipobroman, whereas HU is considered the agent of choice in younger patients.

In a small size RCT, the PVSG evaluated the role of aspirin in PV [6]. A group of 166 patients was randomly assigned to the combination of high-dose aspirin (900 mg daily) plus phlebotomy and dipyridamole *vs*. ^{32}P. The trial was stopped because of excessive major bleeding without demonstration of efficacy in thrombosis prevention. This study has a significant impact on clinical practice: in a recent survey among American physicians it is reported that the use of aspirin is reserved to a minority of PV patients due to a concern for safety [7].

"The ECLAP papers"

General design

The ECLAP study includes a network of 94 haematological centres from 12 countries and an international coordinating centre in Italy (Consorzio Mario Negri Sud). Overall, 1638 PV patients were included in the study [5]. Five hundred and eighteen (32%) of these patients are entered into a parallel, double-blind, placebo-controlled, randomised clinical trial aimed at assessing the efficacy and safety of low-dose aspirin [8]. The remaining 1120 (68%) are registered in an observational, prospective, cohort study. The main reasons for excluding the patients from the randomised trial were: need for antithrombotic therapy (66%), contraindication to ASA (24%) or patients' unwillingness (18%).

Diagnosis of PV was based upon the criteria established by the PVSG [3] and patients were asked to adhere to the treatment recommended by the haematologist in charge of their care. The procedures in the study were planned to mimic the routine care of patients with PV. Data collection was specifically recorded at follow-up visits at 12, 24, 36, 48 and 60 months respectively. The mean duration of follow-up was 2.7 years (0–5.3).

The main outcome measures were fatality, and major and minor thrombosis. Major thrombosis includes cerebral ischaemic stroke, myocardial infarction, peripheral arterial thrombosis and venous thromboembolism. All fatal and major events were objectively documented and validated by an ad hoc committee of expert clinicians blinded to patients' treatment assignment. Haematological evolution to myelofibrosis or AL, and overall mortality were also evaluated. Standard statistical methods were used for analysis.

Clinical course of patients

Thirty-five percent of the 1638 enrolled patients had been newly diagnosed or diagnosed in the two years before registration, whereas in 27% and 38% of cases the diagnosis of PV had been made between 2 and 5 years and more than 5 years prior to registration respectively. Median age at diagnosis and at registration was 60 and 65 years respectively. Thrombotic events before registration were documented in 633 (38.4%) cases. The median duration of follow-up from registration was 2.8 years (range 0–5.3), and the median time elapsed from diagnosis was 6.3 years (range 1–18). Overall mortality during follow-up was 3.5 deaths/100 persons per year. As compared with the general Italian population standardised for age and sex, the excess in mortality of PV patients is 2.1 times. Cardiovascular events, haematological transformation (mainly AL) and major bleeding are responsible for 41%, 13% and 4% of deaths, respectively.

During follow-up, nonfatal major thromboses were observed in 122 patients (7.4%), of which 87 were arteri-

al (53 cerebral ischaemia, 14 acute myocardial infarction and 20 peripheral arterial thrombosis) and 50 (3%) venous. Progression to MF occurred in 38 patients (2.3%), with an incidence rate of approximately 1% patient-year. Transformation to AL during 2.7 years follow-up was registered in 22 cases (1.3%) with a median time lapse from diagnosis of 6.3 years [5].

Risk stratification

In the ECLAP study, the incidence of cardiovascular complications is higher in patients aged more than 65 years (5.0% patient-year, hazard ratio 2.0, 95% confidence interval [CI] 1.22–3.29, $p<0.006$), or with a history of thrombosis (4.93% patient-year, hazard ratio 1.96, 95% CI 1.29–2.97, $p=0.0017$) than in younger subjects with no history of thrombosis (2.5% patient-year, reference category). Patients with both a history of thrombosis and age more than 65 years have the highest risk of cardiovascular events during follow-up (10.9% patient-year, hazard ratio 4.35, 95% CI 2.95–6.41, $p<0.0001$) [5]. These data confirm previous findings that increasing age and a history of thrombosis are the two most important prognostic factors for the development of vascular complications [3]. Other significant predictors of survival and cardiovascular morbidity are smoking, diabetes mellitus and congestive heart failure.

Therefore, patients with PV should be stratified into a different risk category on the basis of their probability of developing thrombotic complications. Young age and no prior thrombosis define a low-risk category, whereas age above 65 years or prior thrombosis define a "high-risk" category. This classification forms the rationale for a risk-adapted therapy [2].

The aspirin trial

The efficacy and safety of low-dose aspirin (100 mg daily) has been formally assessed in a nested double-blind, placebo-controlled, randomised clinical trial carried out in the frame of the ECLAP project [8]. Five hundred and eighteen patients (32% of the total ECLAP study population) without a clear indication or contraindication to aspirin were enrolled. Median age at recruitment was 61 years and 59% of patients were men. Previous cardiovascular events were reported in only 10% of cases, so that this trial included mainly an asymptomatic, low-risk population. Median follow-up was 2.8 years. Aspirin significantly lowered the risk of a primary combined end-point including cardiovascular death, nonfatal myocardial infarction, nonfatal stroke and major venous thromboembolism (relative risk 0.4 [95% CI 0.18–0.91], $p=0.0277$). Total and cardiovascular mortality were also reduced by 46% and 59%, respectively. Major bleeding episodes were only slightly increased by aspirin (relative risk 1.6, 95% CI 0.27–9.71). Thus, the results of this trial eliminate the concern raised by the PVSG about the benefit/risk ratio of aspirin in PV.

In other studies, aspirin at different doses (30–500 mg/day) was found to control microvascular symptoms, such as erythromelalgia, and transient neurological and ocular disturbances including dysarthria, hemiparesis, scintillating scotomas, amaurosis fugax, migraine and seizures [9].

Current treatment recommendations (Fig. 1)

Based on the PVSG seminal RCT [3], phlebotomy is recommended in all patients with PV, and should represent the only cytoreductive treatment in patients at low risk for vascular complications. The target HCT of 0.45 in men and 0.42 in women was suggested by this study group, although not supported by solid data. This recommendation is made by Pearson and Wetherley-Mein [10], who showed in univariate analysis a correlation between thrombosis and HCT when this is greater than 45%. In the ECLAP study, despite the recommendation of maintaining the HCT values at less than 45%, only 48% of patients have HCT values below this threshold, while 39% and 13% have values between 45% and 50% and greater than 50% respectively. Thus, an appropriate controlled study to establish the real HCT target in PV is needed.

Given the results of the ECLAP trial [8], low-dose aspirin (100 mg daily) is recommended in all PV patients without history of major bleeding, gastric intolerance or extreme values of thrombocytosis. However, a recent study shows that previous gastrointestinal bleeding is not an absolute contraindication to the prophylactic use of aspirin,

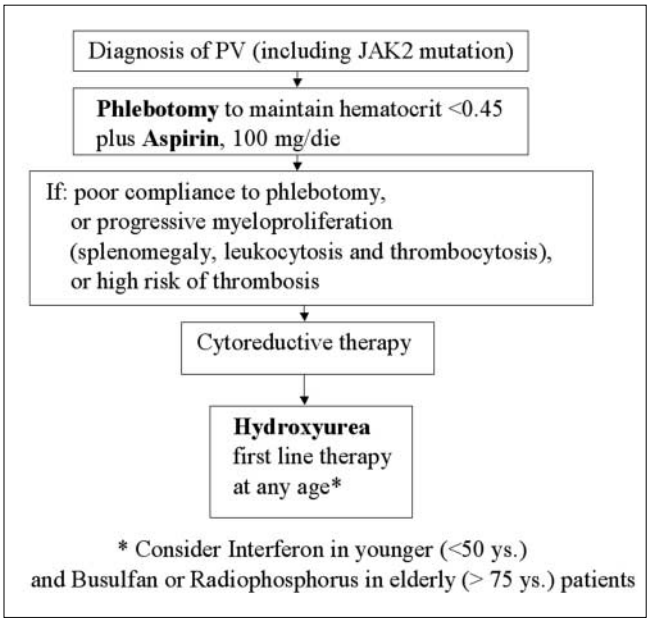

Fig. 1 Flow-chart of recommended treatment for patients with polycythaemia vera (PV)

as the use of proton pump inhibitors may overcome the risk of gastric bleeding due to aspirin [11].

Hydroxyurea

HU is highly effective in patients with myeloproliferative disorders at high risk of thrombosis, and should be considered as first-line therapy; however, concerns regarding its leukaemogenic potential should be carefully considered [12–14].

To date there are no randomised studies powered to assess the relative risk of malignant transformation in myeloproliferative disease (MPD) patients given HU. These disorders have an inherent tendency to evolve into AL, even in the absence of specific therapy. Thus, studies that enrolled patients in need of therapy automatically selected patients with more active disease and a higher propensity to malignant transformation. Furthermore, leukaemic transformation occurs after a lead time of several years, and only long-term studies with a large number of patients are suitable to assess this issue [15].

The 1638 patients prospectively enrolled in the ECLAP study, with a median disease duration of 6.3 years, represent an appropriate population to reach this goal. In a recent analysis of the leukaemogenic risk in these patients, HU alone does not enhance the risk of leukaemia in comparison with patients treated with phlebotomy only (hazard ratio 0.86, 95% CI 0.26–2.88; p=0.8) [16]. Over the same time frame, the risk is significantly increased by exposure to radiophosphorus, busulphan or pipobroman (hazard ratio 5.46, 95% CI 1.84–16.25; p=0.002). The use of HU in patients already treated with alkylating agents or radiophosphorus also enhances the leukaemic risk (hazard ratio 7.58, 95% CI 1.85–31; p=0.0048,) and this is seen also in ET [16, 17].

Thus, the bulk of evidence does not support a leukaemogenic risk for HU, but the debate on whether AL is part of the natural history of PV or is a consequence of therapy is still matter of discussion. It is wise to adopt a cautionary principle, and to consider carefully the use of this agent in young subjects and in those previously treated with other myelosuppressive agents or carrying cytogenetic abnormalities.

Interferon alpha (IFN-α)

The use of IFN-α in PV was pioneered by Silver [18]. IFN-α suppresses the proliferation of haematopoietic progenitors, has a direct inhibiting effect on bone marrow fibroblast progenitor cells and antagonises the action of platelet-derived growth factor, transforming growth factor-beta and other cytokines, which may be involved in the development of myelofibrosis [19].

Published reports concern small consecutive series of patients in whom haematological response and side effects were evaluated. One review analyses the cumulative experience with IFN-α in 279 patients from 16 studies [20]. Overall responses are 50% for reduction of HCT to less than 0.45% without concomitant phlebotomies, 77% for reduction in spleen size and 75% for reduction of pruritus. Results from single-institution studies with long-term follow-up are similar [21, 22].

In a recent review article, Silver updates his experience on the long-term use (median 13 years) of IFN-α in 55 patients with PV [23]. Complete responses, defined by phlebotomy free, HCT less than 45% and platelet number below 600 000/ml, are reached in the great majority of cases after 1–2 years of treatment, and the maintenance dose can be decreased in half of the patients. Noteworthy is the absence of thrombohaemorrhagic events during this long follow-up.

The main problem with IFN-α therapy, apart from its costs and parenteral route of administration, is the incidence of side effects. Fever and flu-like symptoms are experienced by most patients and usually require treatment with paracetamol. Signs of chronic IFN-α toxicity, such as weakness, myalgia, weight and hair loss, severe depression, and gastrointestinal and cardiovascular symptoms, make it necessary to discontinue the drug in about one third of patients [20]. Overall, the role of IFN-α in PV therapy requires controlled clinical trials evaluating long-term clinical end-points.

The JAK2 mutation and future clinical trials

The recent description of a point mutation (V617F) in the JAK2 kinase of a large proportion of patients with MPD has fundamentally changed our understanding of the molecular aetiology of the disorders [24–30]. This change is observed in 70%–95% of PV patients, as well as in 50%–70% of ET and 35%–50% of IMF patients. The large variation in the reported frequencies is most likely due to both technical differences in allele detection (DNA sequencing *vs*. allele-specific PCR) and to relatively small sample size. The majority of patients carry a heterozygous mutation, retaining one wild type JAK2 allele. About 30% of patients, however, have experienced mitotic recombination and loss of heterozygosity at chromosome 9p, leading to duplications of the mutant JAK2 allele. Hence these patients are homozygous for the mutation. The mutation at amino acid 617 is located in the JH2 pseudokinase domain, and renders the enzyme constitutively active. In cell culture and mouse models, transduction of Jak2V617F leads to cytokine hypersensitivity and erythrocytosis. These data demonstrate that this mutation plays a pivotal role in causing the PV phenotype, and generates great interest in determining if patients are sensitive to small molecular agents specific to the pseudokinase domain of JAK2.

Clinical trials are currently exploring whether *Imatinib* may have a role in PV. Therapy with this inhibitor of several protein-tyrosine kinases, including ABL, PDGFR, KIT and FMS tyrosine kinase, has been proposed. Silver et al. report responses in 13 of 23 (57%) PV patients at doses up

to 800 mg day [31]. Effects are predominantly on erythroid progenitors with decrease in spleen size and elimination of need for phlebotomy. Control of thrombocytosis is less effective, necessitating additional therapy in some patients. The relation of Imatinib treatment and JAK2 mutation is explored by Jones et al. in 9 PV patients for whom pre-treatment samples were available [32]. There are two cases that achieved complete haematologic remission and a 2–3-fold reduction in the percentage of V617F alleles. According to these authors, the observed clinical benefit may be a consequence of KIT rather than JAK2 inhibition.

The same authors report that therapy with *IFN-α* (1 MU 3 times weekly to 3 MU daily) in 7 PV patients induces a lower percentage of V617F alleles compared to a control group of patients treated by phlebotomy and HU [32]. Recently, in a multicentre phase II trial of *Peg-IFN-α2a* in 27 PV patients, Kiladjian et al. show a decrease of JAK2 mutant expression in 24 cases (89%). In one patient mutant JAK2 was no longer detectable after 12 months of therapy [33].

The recently established MPD Research Consortium (MPD-RC) is going to test the hypothesis of therapeutic intervention with these novel agents in controlled randomised phase III clinical trials enrolling high risk patients with PV. The aim is to compare changes in key biomarkers including JAK2 mutation with specific clinical end-points.

Conclusions

RCTs from PVSG and from ECLAP provide evidence-based guidelines for the management of patients with PV. New clinical trials should be aimed at reducing fatal and nonfatal events of our patients, and ought to be planned according to the added benefit that could be expected from new study intervention as compared to standard treatment. It is likely that the new discoveries of biomarkers and genetic abnormalities in MPD will allow the substitution of clinical with nonclinical end-points. As PV is a relatively rare disease, and the related complications evolve over several years, future trials including surrogate end-points and specifically JAK2 mutation will be welcome.

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