bjh guidelines

A guideline for the diagnosis and management of polycythaemia vera. A British Society for Haematology Guideline

Mary Frances McMullin,[1] Claire N. Harrison,[2] Sahra Ali,[3] Catherine Cargo,[4] Frederick Chen,[5] Joanne Ewing,[6] Mamta Garg,[7] Anna Godfrey,[8] Steven Knapper S,[9] Donal P. McLornan,[2] Jyoti Nangalia,[10] Mallika Sekhar,[11] Frances Wadelin,[12] Adam J. Mead[13] and on behalf of the BSH Committee

[1]Centre for Medical Education, Queen's University, Belfast, [2]Guy's and St Thomas' NHS Foundation Trust, London, [3]Castle Hill Hospital, Hull and East Yorkshire Hospitals NHS Trust, Hull, [4]Leeds Teaching Hospitals NHS Trust, Leeds, [5]The Royal London Hospital, Bart's Health NHS Trust, London, [6]Birmingham Heart of England NHS Foundation Trust, Birmingham, [7]University Hospital of Leicester NHS Trust, Leicester (BSH representative), [8]Department of Haematology and Haematopathology and Oncology Diagnostic Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, [9]Cardiff University School of Medicine, Cardiff, [10]Wellcome Trust Sanger Institute, Cambridge, [11]Royal Free London NHS Foundation Trust, London, [12]Nottingham University Hospital, Nottingham, and [13]MRC Weatherall, Institute of Molecular Medicine, University of Oxford, Oxford, UK

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Methodology

This guideline was compiled according to the British Society for Haematology (BSH) process at b-s-h.org.uk. The Grading of Recommendation Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of the recommendations. The GRADE criteria can be found at http//:www.gradeworkinggroup.org.

Literature review details

The literature review was conducted on 2 March 2017. Databases searched include MEDLINE(OVID), Embase (OVID) and CENTRAL(The Cochrane library) using the search terms (and relevant MESH terms): polycythaemia vera, erythrocytosis, familial, high oxygen affinity haemoglobin, defects of oxygen sensing pathway, diagnosis, investigation, molecular, mutation, *JAK2*, *MPL*, *CALR*, bone marrow, red cell mass, erythropoietin, risk, management, treatment, cytoreduction, venesection, hydroxyurea, interferon, busulfan, pipobroman, radioactive phosphorus, aspirin, anagrelide, ruxolitinib, thrombosis, haemorrhage, pregnancy, pruritus, surgery and management. The search covered the period from 2005, the date of last version of the guideline (McMullin *et al*, 2005), to February week 3 2017. Exclusions included articles not in English, studies not in humans, single case reports and case series of under 5 cases. A total of 6062 articles were identified which, with exclusions and duplications, resulted in 1215 articles which were reviewed.

Review of manuscript

Review of the manuscript was performed by the BSH Guidelines Committee General Haematology Task Force, the BSH Guidelines Committee and the General Haematology Sounding Board of BSH. It was also placed on the members section of the BSH website for comment. A patient representative from MPN-Voice (www.mpnvoice.org.uk) participated in the guideline writing meeting. The guideline has been reviewed by MPN-Voice; this organisation does not necessarily approve or endorse the contents.

Introduction

The previous guideline was published in 2005 (McMullin *et al*, 2005) with an amendment in 2007 (McMullin *et al*, 2007) to update the diagnostic criteria following the discovery of the *JAK2* mutation in patients with polycythaemia vera (PV). Since that time, there has been a considerable amount of research in the area concerning diagnostics, risk stratification, new agents and reinvestigation of existing agents. It was therefore decided to evaluate the literature to formulate guidance on the diagnostic pathway for erythrocytosis, risk stratification of PV, management of PV (including specific situations) and the management of secondary erythrocytosis. Here we provide evidence-based guidance on diagnosis, risk stratification and management of PV. Our review of the evidence led us to some differences in diagnostic criteria and risk stratification than have been proposed by other

Correspondence: BSH Administrator, British Society for Haematology, 100 White lion Street, London N1, 9PF, UK.
E-mail: bshguidelines@b-s-h.org.uk

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international organisations. We discuss the reasons for this. An accompanying guideline looks at management of specific situations in PV and management of secondary erythrocytosis (McMullin *et al*, 2018).

The diagnostic pathway for investigation of an erythrocytosis

Patients with a persistently raised venous haematocrit (Hct) (males, >0·52; females, >0·48) should be investigated. As suggested in our previous guideline (McMullin *et al*, 2005) and confirmed in recent literature, Hct has been consistently shown to perform better in identifying patients with a raised red cell mass (RCM) than haemoglobin concentration (Alvarez-Larrán *et al*, 2012; Ancochea *et al*, 2014).

Patients should be investigated according to the proposed algorithm (Fig 1). Investigation requires knowledge of diagnostic criteria for both PV and potential secondary causes of erythrocytosis (Tables 1 and 2). A detailed history, examination and stage 1 investigations (listed below) should identify a potential cause in the majority of patients, although a proportion will require more extensive testing and, in some cases, a cause may not be found (idiopathic erythrocytosis). The potential for dual pathology should also be considered.

Initial assessment

Clinical history and examination

A detailed clinical history and examination are essential and, in the absence of a molecular marker of disease, will determine further investigations and management. Particular attention should be paid to the drug history (prescribed and recreational), smoking, alcohol consumption and body habitus. Systematic questioning should elicit symptoms related to other potential secondary causes of erythrocytosis (see Table 2). A proportion of patients, who have a clear secondary cause for their erythrocytosis, may not need any further investigations.

Stage 1 Investigations

Full blood count/blood film

The full blood count analysis will not only confirm a raised Hct but will also identify neutrophilia and thrombocytosis, which are common in *JAK*2 V617F-positive PV and part of the criteria for *JAK*2-negative PV (Table 1). As smokers have a significantly higher neutrophil count than non-smokers (Whitehead *et al*, 1995), neutrophilia is defined as $>12{\cdot}5 \times 10^9$/l in this patient group.

A blood film should be reviewed in all patients to look for any atypical features. In those with confirmed PV, abnormalities, such as circulating blasts, leucoerythroblastic features and monocytosis, would be indications for bone marrow assessment.

Renal and liver function

A number of renal and hepatic diseases can cause erythrocytosis. Serum calcium levels should also be determined to exclude a parathyroid adenoma/carcinoma, which rarely causes secondary erythrocytosis.

Arterial oxygen saturation (SaO_2)/carboxyhaemoglobin

Identifying tissue hypoxia, a cause of secondary erythrocytosis, can be achieved most simply by using pulse oximetry in the clinic. An SaO_2 of <92% has been shown to be associated with an absolute erythrocytosis (Berlin, 1975). Clinicians should however be aware of three situations of hypoxic erythrocytosis where this testing is unreliable and will give a normal result. These are: carbon monoxide poisoning, high oxygen affinity haemoglobins and sleep apnoea syndrome. Those with suspected high oxygen affinity haemoglobins should undergo genetic testing as described below. In those with suspected sleep apnoea (heavy snoring with daytime somnolence or increased body mass index >30 kg/m^2), referral should be made to a respiratory or sleep physician.

Carboxyhaemoglobin (COHb) levels are significantly higher in smokers compared with non-smokers and cigarette consumption has been shown to be directly related to COHb levels (Castleden & Cole, 1975). Testing can therefore be performed at baseline where smoking is suspected.

Serum ferritin

Low serum ferritin levels are common in PV patients and iron deficiency can mask the presentation of PV, giving a misleadingly low Hct because iron deficiency limits erythropoiesis and hypochromic microcytosis develops.

Serum erythropoietin

Erythrocyte production is controlled by the hormone erythropoietin (EPO). Measurement of serum EPO can provide information on potential causes of erythrocytosis and help stratify further testing (see Fig 1). EPO levels are commonly high in hypoxic conditions or when erythrocytosis is secondary to exogenous administration or endogenous overproduction. In contrast EPO levels are typically low in PV, although their diagnostic utility in this setting is limited in the era of *JAK*2 mutation testing (Ancochea *et al*, 2014).

JAK2 *V617F mutational analysis*

The identification of *JAK*2 mutations in almost all PV patients has revolutionised the diagnosis of PV. The *JAK*2 V617F mutation can be found in over 95% of PV patients

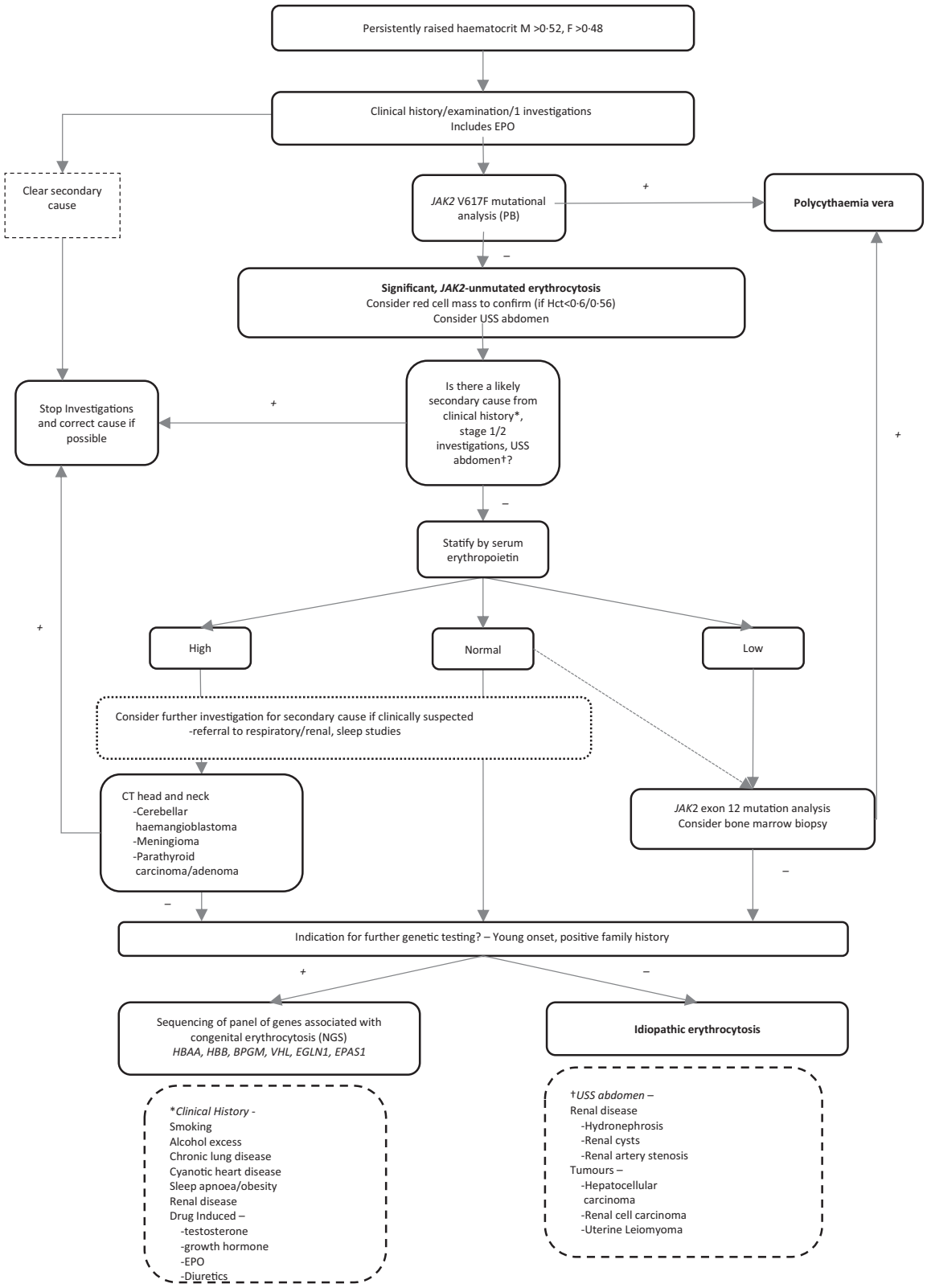

Figure 1. Algorithm for the investigation of an erythrocytosis. CT, computed tomography; EPO, erythropoietin; F, female; Hct, haematocrit; M, male; NGS, next generation sequencing; PB, peripheral blood; USS, ultrasound scan.

TABLE 1. Diagnostic criteria for erythrocytosis

Recommended diagnostic criteria for PV
JAK2-positive polycythaemia vera (requires both criteria)
A1 High haematocrit (>0·52 in men, >0·48 in women) OR raised red cell mass (>25% above predicted)
A2 Mutation in *JAK2*
***JAK2-negative polycythaemia vera (requires A1-A4 plus another A or two B* criteria)** *
A1 Raised red cell mass (>25% above predicted) OR haematocrit ≥0·60 in men, ≥0·56 in women
A2 Absence of mutation in *JAK2*
A3 No cause of secondary erythrocytosis
A4 Bone marrow histology consistent with polycythaemia vera
A5 Palpable splenomegaly
A6 Presence of an acquired genetic abnormality (excluding *BCR-ABL1*) in the haematopoietic cells
B1 Thrombocytosis (platelet count >450 × 10^9/l)
B2 Neutrophil leucocytosis (neutrophil count >10 × 10^9/l in non-smokers, ≥12·5 × 10^9/l in smokers)
B3 Radiological evidence of splenomegaly
B4 Low serum erythropoietin

*This is a very rare clinical entity.

(James *et al*, 2005) and an exon 12 mutation in most remaining patients (Scott *et al*, 2007). Testing for *JAK2* V617F in peripheral blood is sensitive and bone marrow samples are not required to identify this (Takahashi *et al*, 2013). Testing for *JAK2* V617F is advised as a stage 1 investigation and should confirm the diagnosis the vast majority of PV patients. Separate guidance is available for assays used for detection of *JAK2* mutations (Bench *et al*, 2013).

Further investigations in *JAK2* V617F-negative erythrocytosis

Further investigations are warranted in those patients with a persistent, significant erythrocytosis if *JAK2* V617F studies are negative and a secondary cause is not immediately apparent (See Fig 1). Secondary causes must be considered because PV is rare in the absence of a *JAK2* V617F mutation.

Red cell mass studies

Patients with Hct >0·60 (males) or >0·56 (females) can be assumed to have an absolute erythrocytosis, but in others RCM studies can be helpful to confirm an absolute erythrocytosis. An RCM more than 25% above the mean predicted value is diagnostic of an absolute erythrocytosis (Pearson *et al*, 1995). Those with a raised Hct but an RCM within the normal range have an apparent erythrocytosis. A relative erythrocytosis, found in states of dehydration, can be confirmed when the RCM is within the normal range and plasma volume is below normal. Patients with a relative or apparent erythrocytosis require no further investigation. It is noted however, that due to the many drawbacks of this test including cost and labour, access to RCM studies is variable nationally.

Abdominal ultrasound

Radiological splenomegaly is a minor criterion for *JAK2* V617F-negative PV (Table 1) and ultrasound is the simplest method for detection. Abdominal ultrasound can also exclude secondary causes of erythrocytosis, particularly renal and hepatic pathology, including hepatocellular carcinoma.

Further testing can be stratified according to the EPO level measured during stage 1 investigations.

Normal or low EPO level

JAK2 *exon 12 analysis*

Compared with *JAK2* V617F, patients with exon 12 mutated-PV tend to be younger, with higher haemoglobin concentrations, lower white blood cell (WBC) and platelet counts, and an isolated increase in erythropoiesis without granulocytic or megakaryocytic morphological abnormalities (Scott *et al*, 2007; Passamonti *et al*, 2011). In contrast to *JAK2* V617F testing, a discrepancy between exon 12 mutant allele burden in bone marrow and peripheral blood has occasionally been described (Kjær *et al*, 2012).

Bone marrow biopsy

Bone marrow histology may be helpful in distinguishing PV from secondary erythrocytosis (Thiele *et al*, 2005). Bone marrow aspiration in PV typically reveals markedly increased erythropoiesis with moderate to marked increase in granulopoiesis and megakaryopoiesis; widely variable megakaryocyte size, including large forms with hyperlobated nuclei; and absent iron stores. The bone marrow trephine biopsy sections show hypercellularity, trilineage expansion of haemopoiesis (rarely preferentially erythroid) and normoblastic erythropoiesis. Granulocytic maturation may be left-shifted and disorderly; megakaryocytes show increased variation in size, often with a predominance of large forms with uneven or reduced nuclear lobulation, and megakaryocyte clusters are common. Reticulin is increased in a minority of patients [up to World Health Organization (WHO) grade 1 in most cases].

Table 2. Causes of erythrocytosis

Primary	Secondary
Congenital	Congenital
EPO receptor mutations	*Defects in oxygen sensing pathway*
	Chuvash erythrocytosis (*VHL* mutation)
	Mutations in *EGLN1 or EPAS1*
	Left shift of Hb-oxygen dissociation curve
	High oxygen-affinity haemoglobin
	2,3-BPG deficiency (*BPGM* mutations)
Acquired	Acquired
Polycythaemia vera	*Hypoxia driven*
	Central hypoxic process
	Chronic lung disease
	Right-to-left cardiopulmonary vascular shunts
	Carbon monoxide poisoning
	Smoker's erythrocytosis
	Hypoxic states (sleep apnoea/altitude)
	Local renal hypoxia
	Renal artery stenosis
	End-stage renal disease
	Hydronephrosis
	Renal cysts (polycystic kidney disease)
	Pathological EPO production
	Tumours
	Hepatocellular carcinoma
	Renal cell cancer
	Cerebellar haemangioblastoma
	Parathyroid carcinoma/adenoma
	Uterine leiomyoma
	Pheochromocytoma
	Meningioma
	Drug-associated
	Erythropoietin
	Use of androgen preparations
	Diuretics
	Alcohol excess
	Postrenal transplant erythrocytosis
Idiopathic erythrocytosis	

EPO, erythropoietin.

Presence of an acquired genetic abnormality is a major criterion for *JAK*2-negative PV and the presence of an abnormal karyotype can therefore support this diagnosis. An acquired *SH2B3* (*LNK*) mutation would also support the diagnosis. More recently, mutations in a number of other genes, most commonly *TET*2 and *DNMT*3A, have been reported in PV (Delic *et al*, 2016) These gene mutations are not, however, disease-specific and have also been reported in healthy individuals (Genovese *et al*, 2014; Jaiswal *et al*, 2014), limiting their application in the diagnostic setting, particularly when found in isolation.

High EPO level

A raised EPO level should lead to a thorough search for secondary causes of erythrocytosis (Table 2), which may require additional supplementary investigations (Fig 1).

Imaging

Further imaging (e.g. head and neck computed tomography) is indicated in this setting if a cause for the high EPO has not been identified. Imaging should aim to exclude rare tumours, such as a cerebellar haemangioblastoma, pheochromocytoma, meningioma or a parathyroid tumour, all of which can rarely cause erythrocytosis.

Gene sequencing for congenital erythrocytosis

A number of germline mutations in genes involved in oxygen sensing, erythropoiesis and oxygen transport have now been implicated in patients with otherwise unexplained erythrocytosis. These include mutations in the *EPO* receptor and genes in the oxygen sensing pathway (*VHL, EGLN1, EPAS1*), high oxygen affinity haemoglobinopathies caused by mutations in the globin genes *HBA1*, *HBA2, HBB*, and 2,3-bisphosphoglycerate deficiency as a result of *BPGM* mutations (Bento *et al*, 2014). These patients can present with low, normal or high EPO levels; high affinity haemoglobins and 2,3-BPG deficiency also cause a left shift of the oxygen dissociation curve. In the past, this group of mutations was detected by Sanger sequencing of individual genes, in an order directed by EPO levels and P50 analysis, but this approach is labour-intensive and time-consuming (Bento *et al*, 2014). More recently, next generation sequencing-based targeted panels have been developed to assess established and novel genes implicated in erythrocytosis (Camps *et al*, 2016), negating the need for P50 testing. A targeted panel should be performed in patients in whom congenital erythrocytosis is suspected, particularly young patients or those with a family history.

Diagnostic criteria for polycythaemia vera

Presentation

Polycythaemia vera (PV) presents at a median age of 60 years with a slight male predominance. Patients can present with arterial or venous vascular occlusive events, microvascular disturbances or, occasionally, haemorrhage. Splenic pain and/or enlargement, pruritus, gout and constitutional symptoms, such as fatigue, may be present. Alternatively, asymptomatic patients may be identified incidentally following a full blood count. All patients who are newly diagnosed with PV should be discussed in a multidisciplinary team setting.

The recommended diagnostic criteria for *JAK*2-positive and the very rare *JAK*2-negative PV are given in full in Table 1.

Role of the bone marrow biopsy in *JAK*2 V617F-positive patients

Although the WHO classification considers histology to be useful in distinguishing PV from other myeloproliferative neoplasms (MPNs) (Arber *et al*, 2016), several studies have reported high rates of non-consensus or failure to reach a histological diagnosis in patients with PV (Koopmans *et al*, 2011; Madelung *et al*, 2013) (Alvarez-Larrán *et al*, 2014a) Given the uncertain utility of bone marrow histology in the diagnosis of uncomplicated PV, it is not mandatory in all patients, but should be considered if there are atypical features, such as marked splenomegaly or a history of splanchnic vein thrombosis, where it is necessary to establish if there is an occult MPN. The degree of baseline fibrosis can also be ascertained, which, as discussed below, may have a prognostic role. Bone marrow biopsy may nonetheless be useful in those patients likely to have a long disease history, as a baseline sample for comparison in the event of suspected disease transformation. Abnormal karyotype and other molecular abnormalities (e.g. *TET2* mutations) have been reported in PV and some may have prognostic value (Delic *et al*, 2016; Cerquozzi *et al*, 2017) but these tests are not routinely required at diagnosis.

Differentiation of *JAK*2 V617F-positive PV from other MPNs

In patients with a *JAK*2 V617F mutation, haemoglobin concentration and/or Hct are currently used as a surrogate for RCM to distinguish between PV and essential thrombocythaemia (ET) (Arber *et al*, 2016), but Hct has the better accuracy in predicting RCM (Alvarez-Larrán *et al*, 2012; Ancochea *et al*, 2014). Concerns have however been raised that distinguishing PV from ET based on blood count thresholds alone may fail to identify a subgroup of patients with "masked" PV, who may be better managed as PV rather than ET.

The definition of masked PV has been inconsistent across studies. When using a raised RCM to define an erythrocytosis, studies have shown that a Hct threshold of 0·52 in males will fail to identify approximately 20% of male patients with a raised RCM, whilst the threshold of 0·48 in women is more sensitive (Alvarez-Larrán *et al*, 2012). These ''masked'' PV patients were reported to have similar outcomes to those with "overt" PV when managed equivalently (Alvarez-Larrán *et al*, 2016).

By contrast, other studies have defined masked PV as those patients who did not meet the haemoglobin-based thresholds for PV but did meet the other WHO criteria, mainly bone marrow histology and *JAK2* status. Patients meeting this definition had poorer outcomes in terms of myelofibrotic or leukaemic transformation and survival, but no difference in rate of thrombosis. Hct thresholds of 0·48/0·49 (females/males) were subsequently proposed to discriminate ET from PV in the WHO 2016 revision (Arber *et al*, 2016).

The proposed BSH Hct-based thresholds have good specificity but will miss a minority of patients with a raised RCM. By lowering the Hct threshold it may be possible to identify patients with histology more typical of PV who may have certain adverse outcomes, but these findings have not yet been reproduced independently. It is unknown whether management of any of these patients using a strict Hct target benefits their vascular risk. However, in patients with a *JAK*2 V617F mutation and borderline Hct levels (especially males with Hct 0·48–0·52), the possibility of true erythrocytosis should be considered, especially if the patient is at high risk of vascular events. Options in this group include performing an RCM study to clarify the diagnosis or, pragmatically, managing the patient with a Hct target as for PV.

It should also be noted that Hct is a poor surrogate of RCM in patients who have had splanchnic vein thrombosis (Lamy *et al*, 1997), and these high-risk patients are best managed with standard blood count targets regardless of blood count parameters at diagnosis. Hct is also reduced by pregnancy and gestation-specific ranges should be used when considering the distinction between PV and ET in a patient presenting during pregnancy.

*JAK*2 V617F allele burden

Quantitative assessments of the *JAK*2 V617F allele burden in peripheral blood granulocytes have shown that this parameter tends to be higher in PV than ET. A higher mutant allele burden correlates with certain clinical features at presentation, including higher haemoglobin levels, higher WBC counts, lower platelet counts, lower mean cell volume (MCV), lower serum ferritin and EPO, more splenomegaly and more pruritus (Dupont *et al*, 2007; Tefferi *et al*, 2007; Vannucchi *et al*, 2007; Passamonti *et al*, 2010). However, there is no validated threshold at which *JAK*2 V617F allele burden can confirm or refute a diagnosis of PV and this investigation is not recommended routinely.

Low level *JAK*2 V617F allele burden

A low level *JAK*2 V617F mutation (allele burden <1–3%) should be interpreted in the context of clinical, haematological and other laboratory findings (Bench *et al*, 2013). If the result is reproducible and does not represent a false positive, this finding may provide support for a diagnosis of a PV in a patient with otherwise unexplained, significant erythrocytosis (Perricone *et al*, 2017). However, the *JAK*2 V617F mutation has been identified in normal individuals, often at a low allele burden, with a frequency that increases with age (Genovese *et al*, 2014; Jaiswal *et al*, 2014). Caution is therefore

warranted and comprehensive investigations should exclude an alternative secondary or congenital cause of erythrocytosis. The test should preferably be repeated within 3–6 months, and clinical assessment for other features of an MPN, e.g. splenomegaly, bone marrow histological features, and screening for an additional mutation in *JAK2* exon 12 may be helpful.

Recommendations

- **In patients with persistent, significant and unexplained erythrocytosis, testing for *JAK2* V617F is recommended, using a peripheral blood sample and an assay sufficiently sensitive to detect a mutant allele burden as low as 1–3%. (GRADE 1B)**

Risk stratification in PV

The principal aims of risk stratification in PV are a) to select patients at higher risk of thrombosis for consideration of cytoreductive therapy and b) to provide the most accurate information to patients on the risks and implications of a diagnosis of PV.

Thrombosis and bleeding risk

At diagnosis, in the largest prospective study to date, the European Collaboration on Low-Dose Aspirin in Polycythaemia Vera (ECLAP), age ≥65 years and a prior history of thrombosis were found to be the most important predictors of cardiovascular events (Marchioli *et al*, 2005). A baseline WBC count of >15·0 $\times$ 10^9/l is a significant predictor of thrombosis, particularly an increased risk of myocardial infarction (Landolfi *et al*, 2007); however, prognostic models including leucocytosis have not been prospectively validated. Cardiovascular risk factors (smoking, diabetes mellitus, arterial hypertension, hypercholesterolaemia) also contribute to thrombotic risk in PV (Barbui *et al*, 2017) (Landolfi *et al*, 2007) (Gangat *et al*, 2007). Once treatment is initiated, cardiovascular events occur more frequently in patients with less stringent Hct control (Marchioli *et al*, 2013) and when the WBC count remains elevated >11 $\times$ 10^9/l (Barbui *et al*, 2015). A relationship between thrombocytosis (either at diagnosis or follow-up) and thrombotic risk has not been established in PV (Di Nisio *et al*, 2007), but extreme thrombocytosis (≥1500 $\times$ 10^9/l) is associated with increased risk of bleeding due to acquired von Willebrand disease and should be considered an indication for initiation of cytoreductive therapy (Budde & van Genderen, 1997).

Survival and transformation risk

The impact of age, degree of leucocytosis and prior history of venous thrombosis on long term prognosis are well-established. In the ECLAP study, age >65 years was associated with inferior survival and age >70 years was associated with increased incidence of leukaemia/myelodysplasia (Marchioli *et al*, 2005). In the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) study, age>61 years was associated with inferior overall and leukaemia-free survival (Tefferi *et al*, 2013). Longer disease duration has been associated with increased risk of myelofibrotic transformation (Marchioli *et al*, 2005). Leucocytosis with WBC count ≥15 $\times$ 10^9/l is associated with an inferior leukaemia-free survival. A prior history of venous thrombosis also impacts negatively on overall survival. The IWG-MRT Prognostic Score uses these three parameters (age, WBC count and thrombotic history) to delineate distinct risk groups for overall survival (Tefferi *et al*, 2013), but this prognostic score has not been independently validated in prospective studies.

Several other clinical and laboratory variables have been reported to influence overall survival and/or risk of disease transformation. The presence of splenomegaly in PV patients has been associated with shorter overall survival and increased risk of transformation to both myelofibrosis (MF) and acute myeloid leukaemia (AML) (Abdulkarim *et al*, 2011). The presence of an abnormal karyotype adversely impacts overall and leukaemia-free survival (Tefferi *et al*, 2013). A raised lactate dehydrogenase (LDH) level and the presence of reticulin fibrosis at diagnosis predict a higher rate of transformation to MF but not to AML (Alvarez-Larrán *et al*, 2009) (Barbui *et al*, 2012). Prospective analysis indicates that a *JAK2* mutant allele burden of >50% is also associated with increased risk of MF (but not of AML or thrombosis) (Passamonti *et al*, 2010) but the clinical utility of this measurement is not yet well-established. Although *JAK2* exon12-mutated disease has a subtly different clinical phenotype to *JAK2 V617F*-driven PV (higher haemoglobin concentration, lower WBC count), there appears to be no difference in long-term prognosis (Passamonti *et al*, 2011). Targeted gene sequencing is a rapidly advancing area; approximately 15% of PV patients have mutations of ≥1 of *ASXL1*, *SRSF2* and *IDH2* and these patients have a reduced rate of overall survival in univariate analysis (Tefferi *et al*, 2016).

Recommendations: risk stratification

- **Age and thrombotic history should be used to define risk groups for thrombosis in polycythaemia vera (PV) (GRADE 1A).**
- **'High risk': age ≥65 years and/or prior PV-associated arterial or venous thrombosis (GRADE 1A)**
- **'Low risk': age <65 years and no PV-associated thrombotic history (GRADE 1A)**
- **Some 'low risk patients' may be to be considered at higher risk in the presence of cardiovascular risk factors, elevated white blood cell (WBC) count, extreme**

thrombocytosis or haematocrit (Hct) uncontrolled with venesection (GRADE 1B)

- **A number of variables including age, prior thrombosis, the presence of splenomegaly, serum lactate dehydrogenase (LDH) level, degree of reticulin staining, presence of an abnormal karyotype and *JAK2* mutant allele burden may be utilised when counselling the patient on longer term prognosis including overall survival and disease transformation risk (GRADE 2B).**
- **Deep sequencing for 'high risk mutations' e.g. *ASXL1, SRSF2, IDH1/2* is not yet 'standard of care' but may be considered in selected cases where their presence may influence management. (GRADE 2B).**

Management of polycythaemia vera

Patients with PV may present with thrombosis or cardiovascular disease. Disease-related PV symptoms, such as microvascular disturbance, pruritus (which may be excruciating), migraine-type headache and fatigue, may also be presenting features which can significantly impact on quality of life (Harrison *et al*, 2017). However, patients may be asymptomatic at presentation.

The goals of treatment are to reduce complications and therefore improve survival. (Table 3). Mortality is chiefly related to thromboembolic events and the principal aim of therapy is to reduce this risk. Targeted assessment and management of cardiovascular risk factors, such as hypertension, hypercholesterolaemia, diabetes mellitus and smoking, is essential. Reduction in symptom burden is also a valid target for treatment. There is evidence that patients with inadequately controlled PV, as determined by hydroxycarbamide (HC) use, splenomegaly and venesection requirements, have a significantly higher symptom score measured by the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) (Geyer *et al*, 2016). Frequent requirement for concurrent venesection may indicate the need for dose alteration and or change of treatment.

Haematocrit target

The target for Hct control in PV was originally based on data from assessment of numbers of vascular events at different Hct levels and it was estimated that a target below 0·45 should be maintained (Pearson & Wetherley-Mein, 1978). This target has now been validated in a randomised clinical trial by the Cytoreductive Therapy in PV (CYTO-PV) investigators who assessed the impact of stringent Hct reduction to <0·45 compared with a more liberal target range of 0·45–0·50 (Marchioli *et al*, 2013). Patients with a Hct target of < 0·45 had a significantly lower rate of cardiovascular death and major thrombosis than those with a target of 0·45–0·50 (Marchioli *et al*, 2013). It was noted that the median WBC count was significantly lower in the low Hct group, which may have been related to variation in the use of cytoreductive therapy between the groups. The impact of this parameter on the difference in outcome between the groups has been debated (McMullin *et al*, 2013).

The European LeukaemiaNet (ELN) has, by consensus, recommended response criteria for PV. There is, however, little evidence that stringent achievement of these contributes to improved outcomes apart from the Hct target. These are a valuable set of measures to assess treatment outcome with consistency across clinical trials but are not as useful in clinical practice (Barosi *et al*, 2015).

Platelet and leucocyte target

There is considerable published evidence of an association between increased WBC count and thrombosis risk in PV (Barbui *et al*, 2009; Caramazza *et al*, 2009) ((De Stefano *et al*, 2010; Barbui *et al*, 2015) (Cerquozzi *et al*, 2017). In contrast, one prospective study did not find such an association (Passamonti *et al*, 2010). An analysis of long-term outcome of patients enrolled into the ECLAP study demonstrated that in patients with WBC counts $>15 \times 10^9$/l there was increased incidence of thrombosis in comparison with those with WBC counts $<10 \times 10^9$/l, largely related to an increase in myocardial infarction (Landolfi *et al*, 2007). In a retrospective study of PV to determine whether blood counts influenced the complication rate and survival, older age and elevated LDH level at diagnosis were found to be risk factors for vascular complications. When the vascular complication occurred, 41% of the patients with a complication had elevated WBC counts compared with 20% of patients without a complication (Enblom-Larsson *et al*, 2017).The CYTO-PV study treatment arms, which showed a lower thrombotic risk in those intensively managed to Hct <0·45, showed a comparatively lower WBC count, which may have contributed to the lower rate of thrombotic events (Marchioli *et al*, 2013) (McMullin *et al*, 2013). There is no evidence from randomised trials to determine whether treatment targeted at reducing leucocyte count impacts on overall

TABLE 3. Treatment goals

- Reduce thrombosis and haemorrhage risk
- Minimise complications and symptomatology
- Minimise risk of transformation to myelofibrosis and acute leukaemia
- Manage specific situations such as pregnancy and surgery
- Achieve good haematocrit control to <0·45

outcome and therefore no recommendation to target WBC counts as a treatment goal can be made. Indeed, no evidence for improved survival or lower thrombosis risk was seen in patients achieving complete or partial response according to ELN criteria in an analysis of PV patients treated with HC, whereas a better prognosis was seen when there was a WBC and platelet response (Alvarez-Larrán *et al*, 2012). There is evidence that, at extremes of platelet count, there is a risk for bleeding and haemorrhage which may necessitate cytoreductive treatment in those with high counts.

Allele burden reduction

There is currently no indication to monitor allele burden sequentially outside the clinical trial setting. Whilst many studies have used allele burden reduction to assess impact of treatment, there is currently no clear clinical impact of this as a target. Allele burden over 50% may correlate with progression to MF (Passamonti *et al*, 2010) but there is no evidence that this alters outcome and no evidence that lowering allele burden alters outcome.

Bone marrow response

There is no indication that serial monitoring of bone marrow morphology or fibrosis grade is of value but this should be undertaken if there is suspected progression from blood counts or symptomatology.

Venesection

Randomised trial data supports that venesection of 200–500 ml blood at intervals suitable for patient size/tolerability should be used to achieve and maintain a Hct of <0·45 (Marchioli *et al*, 2013). In low risk patients this is usually adequate to maintain target Hct. Where frequent venesection is needed to achieve this target then an alternative approach using a cytoreductive agent may need to be considered. High levels of venesection requirement have been reported to have an association with higher thrombosis risk in patients on HC, specifically in those patients requiring 3 or more venesections per year (Alvarez-Larrán *et al*, 2017).

No study has explicitly defined a gender difference in Hct target. A different target Hct in males and females is not recommended.

Iron deficiency may result from venesection. Whilst generally this is asymptomatic, restless legs, concentration problems, impaired cognitive function, dizziness, fatigue, headaches and inactivity and other symptoms may warrant a different treatment approach. Iron administration must be undertaken with extreme caution and with close supervision and monitoring of blood counts. Severe symptoms may warrant an alternative approach, such as cytoreductive therapy.

Aspirin

The value of low-dose aspirin in patients with PV was demonstrated in the ECLAP study. In this double blind, placebo-controlled randomised trial, those randomised to aspirin 100 mg daily had significantly fewer vascular events at 3 years compared to placebo. There was a 60% decrease in the risk of the combined primary end-point, which was of thrombotic events and death from cardiovascular causes. Major bleeding events were not significantly increased (Landolfi *et al*, 2004).

Cytoreductive therapy

High-risk patients should be considered for cytoreductive therapy. Low-risk patients who may benefit from cytoreduction include those with progressive splenomegaly, progressive leucocytosis (e.g. WBC count $>15 \times 10^9$/l) thrombocytosis (e.g. platelet count$>1500 \times 10^9$/l) and poor tolerance of venesection.

Hydroxycarbamide

Hydroxycarbamide is a cytoreductive agent, a non-alkylating antimetabolite which acts through inhibition of ribonucleotide reductase thereby regulating the rate of DNA synthesis. HC has a dose-dependent effect and needs to be individually titrated to achieve optimal count control. The efficacy of HC in controlling blood counts and preventing thrombosis has been extrapolated from the evidence in ET (Cortelazzo *et al*, 1995) and HC has been used in the management of PV. A recent retrospective study of PV demonstrated that patients treated with HC experienced significantly fewer vascular complications (11%) than patients treated with venesection only, with a survival advantage for patients treated with HC when adjusted for variables supporting the use of this agent in first-line treatment (Enblom-Larsson *et al*, 2017).

Side effects

HC is generally well tolerated. Macrocytosis is expected and myelosuppression is seen in some patients. Mucocutaneous side effects occur, including ulceration in perimalleolar areas, oral aphthous ulceration, actinic keratosis, squamous cell cancer and other skin lesions. Gastrointestinal side effects have been reported.

Leukaemogenic risk and risk of secondary malignancy

There has been much debate and concern over the potential leukaemogenic risk of treatments used for PV and also the

potential for secondary malignancies. The natural history of PV is that a proportion of patients will experience progression to acute leukaemia and MF. There is currently no conclusive evidence that this risk is exacerbated by the use of HC alone. A recent large study European ET trial, the EXELS study, compared anagrelide-treated patients versus those treated with other cytoreductive therapies and found there was a higher incidence of leukaemia and increased incidence of other cancers in those treated with other cytoreductive therapies, including HC alone (Besses *et al*, 2013) (Birgegård *et al*, 2018). A Swedish population-based study with a nationwide MPN cohort identified those who developed AML/myelodysplastic syndrome (MDS) and matched controls, and performed a retrospective case record analysis. Whilst the risk of AML/MDS was increased in patients exposed to high doses of ^{32}P and alkylators or 2 or more cytoreductive agents, this was not seen in those patients treated exclusively with HC (Björkholm *et al*, 2011). A long-term assessment from the ECLAP study showed no increased MDS/AML in those treated with HC alone (Finazzi *et al*, 2005) and a retrospective analysis showed no association between HC or busulfan and AML (Tefferi *et al*, 2013).

A higher incidence of second malignancies has been seen in a small cohort of patients treated with HC compared to interferon (IFN) alone (Hansen *et al*, 2017), particularly non-melanoma skin cancers, and this has been seen in population-based studies with increased risk of non-melanotic skin cancer in patients treated with HC, especially in older patients of male sex (Gómez *et al*, 2016). Another study looked at treatment characteristics of a large number of patients with ET, diagnosed and followed during a 30-year period. The different therapies administered, comprising HC and alkylating agents, did not appear to have any impact on the development of secondary malignancy with a similar rate of secondary malignancies in untreated patients. Male gender and age >60 years were the only factors that were correlated with higher risk (Santoro *et al*, 2017).

HC is recommended as a first line cytoreductive treatment option for all patients for whom this is required. The risk benefit profiles need to be discussed with patients. HC is not safe in pregnancy and it is recommended that it be stopped 3 months prior to intended conception. Adequate contraception should be used by patients receiving this medication.

Hydroxycarbamide intolerance and resistance

There has been an attempt to define the criteria to suggest failure of HC as first line therapy for PV. The ELN have by consensus suggested a unified definition of resistance to intolerance or HC. This classification identifies a group of patients who have a poorer prognosis who may require or benefit from a change of treatment (Barosi *et al*, 2010) (Table 4). In retrospective studies resistance is associated with worse survival, with development of anaemia or cytopenias identifying a group with poorer outcome (Alvarez-Larrán *et al*, 2012, 2017).

Interferons

Numerous single centre studies have observed that IFN-α can be successfully used to normalise blood counts, reduce splenomegaly and prevent thrombosis in PV (Silver, 2006) It is also effective in many patients in reducing pruritus (Taylor *et al*, 1996). This agent is of particular interest due to its anti-clonal activity as demonstrated by molecular (as assessed by mutation burden of *JAK*2 V617F) and histological remissions (Larsen *et al*, 2009) (Stauffer Larsen *et al*, 2013). No leukaemogenic effect has been identified. However, side effects often limit use and most commonly include flu-like symptoms and mood changes. In a minority of patients, endocrine and autoimmune disorders also occur, warranting regular monitoring of thyroid function and additional investigations where indicated. Treatment with IFN-α is usually continuous but occasionally it can be stopped for prolonged periods of time.

Longer acting pegylated IFN-α-*2a* (PEG-α-2a) requires less frequent administration and is generally better tolerated. Two Phase II studies of PEG-α-2a demonstrated complete responses of 70–95% as well as complete molecular remissions of 14–24%, with treatment discontinuation due to side-effects observed in only 8–10% of patients (Kiladjian *et al*, 2006) (Quintás-Cardama *et al*, 2009) Comparable results have been noted in single-centre studies (Crisà *et al*, 2017; Gowin *et al*, 2017) A Phase III study of PEG-α-2a versus HC as first-line treatment for high-risk PV is underway and interim analysis shows no significant advantage for PEG-α-2a over HC (Mascarenhas *et al*, 2016).

PEG-α-2b has also been assessed in two studies that included PV patients and, whilst it has been shown to be effective in controlling disease, clinical use has been limited by high discontinuation rates due to side effects (Samuelsson *et al*, 2006) (Jabbour *et al*, 2007). Recently, interim analysis from a phase III study of proline-PEG-α-2b (Ropeginterferon) has demonstrated complete haematological responses in 71% of PV patients, sustained reductions in mutation burden of *JAK*2 V617F, good tolerability and confirmed non-inferiority (or no significant advantage) to HC as first line treatment for patients with high risk PV (Gisslinger *et al*, 2017).

Ruxolitinib

Following early studies of the JAK1/2 inhibitor, ruxolitinib, in MF, this agent was tested in PV and ET, in patients resistant or intolerant to HC. The majority of PV patients became phlebotomy-independent and had an improvement in symptoms and splenomegaly (>50%) (Verstovsek *et al*, 2014).

TABLE 4. European LeukaemiaNet criteria for hydroxycarbamide intolerance and resistance

1. Need for phlebotomy to keep haematocrit <0·45 after 3 months of at least 2 g/day of hydroxycarbamide OR
2. Uncontrolled myeloproliferation, i.e. platelet count >400 × 10^9/l AND white blood cell count >10 × 10^9/l after 3 months of at least 2 g/day of hydroxycarbamide OR
3. Failure to reduce massive* splenomegaly by more than 50% as measured by palpation OR failure to completely relieve symptoms related to splenomegaly, after 3 months of at least 2 g/day of hydroxycarbamide OR
4. Absolute neutrophil count <1·0 × 10^9/l OR platelet count <100 × 10^9/l OR haemoglobin <100 g/l at the lowest dose of hydroxycarbamide required to achieve a complete or partial clinico-haematological response OR
5. Presence of leg ulcers or other unacceptable hydroxycarbamide -related non-haematological toxicities, such as mucocutaneous manifestations, gastrointestinal symptoms, pneumonitis or fever at any dose of hydroxycarbamide.

Reproduced from: Barosi, G., Birgegard, G., Finazzi, G., Griesshammer, M., Harrison, C., Hasselbalch, H., Kiladijan, J., Lengfelder, E., Mesa, R., Mc Mullin, M.F., Passamonti, F., Reilly, J.T., Vannucchi, A.M. and Barbui, T. (2010), A unified definition of clinical resistance and intolerance to hydroxycarbamide in polycythaemia vera and primary myelofibrosis: results of a European LeukemiaNet (ELN) consensus process. *British Journal of Haematology*, **148**: 961–963. © 2010 John Wiley & Sons, Inc.

*Organ extending by more than 10 cm from the costal region.

Following this, a phase III trial RESPONSE evaluated the efficacy and safety of ruxolitinib in a specific subgroup of PV patients who were both refractory to, or intolerant of, HC and who required ongoing phlebotomy and had splenomegaly (Vannucchi, 2015). Patients were randomized between ruxolitinib and best available therapy (BAT), which could include any therapy, and crossover was permitted. Patients on ruxolitinib achieved good Hct control and spleen response, although only 21% of patients achieved both. Improvements in disease-related symptoms were described. Anaemia and thrombocytopenia were the main haematological adverse events. Herpes zoster infection was described in patients on ruxolitinib. Non-melanoma skin cancer was reported in both arms. Thromboembolic events were more frequent in the BAT arm but this was not a pre-determined outcome. Data published from this study also suggested that molecular responses can occur, perhaps to the same extent as with IFN (Pieri *et al*, 2015; Vannucchi *et al*, 2017) and that even profoundly iron deficient patients can normalise their iron parameters with ruxolitinib therapy (Verstovsek *et al*, 2017). Disease transformation occurred and there is no information to suggest that ruxolitinib therapy impacts these events.

However, the RESPONSE study had some inherent bias: first, the population of the study is highly selected as they had to be venesection-dependent. Second, patients on the BAT arm were allowed to receive HC and many did so, reflecting the lack of therapy options in this setting. Finally, as patients received other treatments before ruxolitinib and crossed between the treatment arms it is difficult to establish if the events, such as skin cancer, or disease transformation could be an effect of the ruxolitinib or occur as an accumulative effect of other cytoreductive therapies.

Following RESPONSE, a second randomized open label phase 3b study (RESPONSE-2) was developed to determinate the efficacy of ruxolitinib versus BAT in a similar population of PV patients as RESPONSE but the patients were not required to have splenomegaly (Passamonti *et al*, 2017). Here, ruxolitinib showed good responses in controlling Hct and PV-related symptoms. However, the follow-up for the study is short, the majority of patients had received HC previously and HC was part of BAT options.

RELIEF was a randomized study focusing on PV-related symptoms for patients on a stable dose of HC (Mesa *et al*, 2017) with crossover to ruxolitinib allowed after week 16. The primary endpoint, the percentage of patients with ≥50% reduction in symptoms, was seen in significantly more patients in the ruxolitinib arm. A statistically significant reduction in itching was also noted in the ruxolitinib arm. This study was perhaps underpowered, but showed that ruxolitinib improves symptoms in patients with controlled PV.

The evidence from these trials suggests that ruxolitinib has a role in the treatment of HC-resistant or intolerant PV.

Other treatments

There are several cytotoxic agents that are effective in controlling blood counts but have been associated with increased rates of leukaemic transformation. Busulfan, a cell cycle non-specific alkylating agent, has such an association. However, retrospective studies show that it is an effective therapy for MPNs (Begna *et al*, 2016) although an actuarial probability of leukaemia transformation of 10% at 3 years was reported in one study (Alvarez-Larrán *et al*, 2014b). Busulfan is useful in treating PV in those with limited life expectancy. It can be given in dosing regimens of 2–4 mg/day until counts are controlled, but patients need to be seen frequently to check for neutropenia or thrombocytopenia so that treatment can be interrupted. An alternative regimen is pulsed single 25–50 mg doses at intervals of approximately 6 weeks.

^{32}P is has a leukaemogenic potential but a single intravenous dose can be effective for long term control. One retrospective study showed its efficacy with remission rates of 90% (Lawless *et al*, 2016). Doses can be repeated if the response is lost but the leukaemogenic risk increases with the cumulative exposure. ^{32}P is a suitable treatment for those with limited life expectancy who are self-caring (so that there is no risk for carers).

Pipobroman, a bromide derivative of piperazine similar to alkylating agents, has been compared to HC in randomised trials and shown to be effective but has continuing leukaemogenic potential (Kiladjian *et al*, 2011) and therefore should only be used in those with limited life expectancy.

Anagrelide, a megakaryocyte differentiation inhibitor, is licensed as second line therapy in ET. Retrospective reports of anagrelide used in combination with HC in PV have shown that it is effective at lowering the platelet count and it may be useful in combination when an elevated platelet count is an issue (Ahn *et al*, 2013).

A number of other agents have been used for cytoreduction in PV with varying efficacy. A small study investigated the use of imatinib. The complete response rate was 30% with frequent side effects. This has not been studied further and is not recommended for the treatment of PV (Silver *et al*, 2012).

Histone-deacetylase inhibitors (HDACi) inhibit proliferation of cells with a *JAK2* V617F mutation. Two HDACi have been tested in PV in phase 2 trials: vorinostat in a trial of PV and ET achieved a response rate of 35% but with a very high drop-out rate because of adverse events (Andersen *et al*, 2013). Givinostat was assessed in a phase 2 study of PV unresponsive to HC monotherapy with response rates in the order of 50% and with high rates of improvement in pruritus (Finazzi *et al*, 2013). HDACi need to be assessed further in trials before they can be recommended for clinical use.

Following the evaluation of all recent evidence, it is recommended that all patients, including those stratified as low-risk, should be venesected to a Hct target of 0·45 and given low dose aspirin if there are no specific contraindications. High-risk patients should, in addition, be treated with cytoreductive therapy. However, low-risk patients with any of the criteria listed below, may also need to be considered for cytoreductive therapy.

Recommendations: management options for ALL PV including low-risk patients

- **Target haematocrit of <0·45 in all patients (GRADE 1A)**
- **Low dose aspirin (75–100 mg) in all patients (GRADE 1A)**
- **Targeted intervention to reduce cardiovascular risk factors**

Consider cytoreductive therapy in low-risk patients with:

- **History of treated arterial hypertension, ischaemic heart disease or diabetes mellitus**
- **Persistent leucocytosis (e.g. WBC count >15 × 10^9/l)**
- **Uncontrolled haematocrit (or poor tolerability of venesection)**
- **Extreme/progressive thrombocytosis (e.g. ≥ 1500 × 10^9/l) and/or haemorrhagic symptoms**
- **Progressive/symptomatic splenomegaly**
- **Uncontrolled or progressive disease-related symptoms, e.g. weight loss, sweats**
- **(GRADE 1B)**

Recommendations: Management options in high-risk patients

- **First Line: hydroxycarbamide (HC) or interferon (preferably pegylated interferon)**
- **Second line: in patients treated with HC as first line, interferon as second line treatment, or, where treated with interferon as first line, recommend HC as second line treatment**
- **Consider pegylated interferon as second line in those patients who have had non-pegylated interferon first line and could not tolerate it**
- **Ruxolitinib second/third line in HC resistant or intolerant patients**
  (GRADE 1A)

Third-line or further treatment options

- **Busulfan or ^{32}P or pipobroman in those with limited life expectancy (GRADE 1B).**
- **Anagrelide in combination with HC may be helpful in those where platelet control is difficult (GRADE 2C)**

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