bjh research paper

Clinical evaluation of the European LeukaemiaNet criteria for clinicohaematological response and resistance/intolerance to hydroxycarbamide in essential thrombocythaemia

Juan-Carlos Hernández-Boluda,[1] Alberto Alvarez-Larrán,[2] Montse Gómez,[1] Anna Angona,[2] Paula Amat,[1] Beatriz Bellosillo,[3] Luz Martínez-Avilés,[3] Blanca Navarro,[1] Anabel Teruel,[1] Francisco Martínez-Ruiz[4] and Carles Besses[2]

[1]Haematology and Medical Oncology Department, Hospital Clínico Universitario, Valencia, [2]Haematology Department, and [3]Pathology Department, Hospital del Mar, Barcelona, and [4]Statistics Department, University of Valencia, Valencia, Spain

Received 4 August 2010; accepted for publication 2 September 2010
Correspondence: Dr Juan-Carlos Hernández-Boluda, Haematology Department, Hospital Clínico Universitario, Avda. Blasco Ibáñez 17, 46010 Valencia, Spain.
E-mail: hernandez_jca@gva.es

Summary

Standardized criteria of response to treatment and a unified definition of resistance/intolerance to hydroxycarbamide (HC) in essential thrombocythaemia (ET) have been proposed by the European LeukaemiaNet (ELN). We have retrospectively evaluated such criteria in 166 ET patients treated with HC for a median of 4·5 years. Overall, 134 patients achieved a complete clinicohaematological response (CR) and 25 a partial response. Thirty-three patients met at least one of the ELN criteria defining resistance ($n = 15$) or intolerance ($n = 21$) to HC. Fifteen cases developed anaemia with thrombocytosis, which was associated with a high incidence of myelofibrosis and death from any cause. Other definitions of resistance were less useful. Factors determining the thrombotic risk were a history of prior thrombosis and a baseline leucocyte count $>10 \times 10^9$/l. Of note, patients achieving a CR, even if sustained during the entire follow-up, did not benefit from a lower incidence of thrombosis or an improved survival. In conclusion, most ET patients respond to HC, but the achievement of response, as defined by the ELN, does not correlate with the patients' outcome. The best discriminating ELN criterion of resistance to HC was the detection of anaemia, which also identified a subgroup of patients with poor prognosis.

Keywords: hydroxycarbamide, essential thrombocythaemia, response criteria, resistance, intolerance.

Hydroxycarbamide (HC; previously termed hydroxycarbamide) is currently the treatment of choice for high-risk patients with essential thrombocythaemia (ET)(Cortelazzo *et al*, 1995; Harrison *et al*, 2005). It acts by slowing DNA synthesis and repair through inhibition of the ribonucleoside reductase, leading to a non-selective bone marrow suppression (Young *et al*, 1967). Side effects of HC are infrequent, and mostly include oral ulcers, nausea, diarrhoea, dermatological abnormalities (alopecia, leg ulcers, hyperpigmentation, actinic keratosis, nail changes)(Randi *et al*, 2005), and very rarely, fever (Braester & Quitt, 2000). There is controversy concerning the potential leukaemogenicity of this drug (Barbui, 2004). With regard to its efficacy, HC has been reported to provide an adequate control of the platelet count in most ET patients (Murphy *et al*, 1986; Lofvenberg & Wahlin, 1988; Cortelazzo *et al*, 1995; Finazzi *et al*, 2003; Harrison *et al*, 2005; Randi *et al*, 2005; Palandri *et al*, 2009; Carobbio *et al*, 2010), but the use of different target platelet levels precludes comparisons of the results across studies. With this background, a group of leading experts on behalf of the European LeukaemiaNet (ELN) recently published a set of standardized criteria of response to treatment in ET (Barosi *et al*, 2009), which should be of value to properly evaluate the outcomes obtained in different clinical trials. However, some ET patients fail to respond to HC, while others may develop unacceptable side effects requiring discontinuation of the drug. To address this, a unified definition of clinical resistance/intolerance to HC in ET has also been published (Barosi *et al*, 2007). However, there is limited information so far regarding the application of the abovementioned criteria in the clinical setting.

First published online 18 November 2010
doi:10.1111/j.1365-2141.2010.08430.x

Therefore, we evaluated the ELN criteria for clinicohaematological response and resistance/intolerance to HC in a series of 166 patients with ET from two institutions. Based on the results of the present study, some modifications to the published criteria might be suggested.

Patients and methods

Between December 1986 and July 2009, 166 adult patients diagnosed with ET at two Spanish hospitals were treated with HC. Diagnosis of ET was made in accordance with either the Polycythaemia Vera Study Group (Murphy *et al*, 1986) or the World Health Organization criteria (Tefferi *et al*, 2007). Bone marrow biopsy was performed at diagnosis in all patients and the silver stain was scored on a 0–3 scale, with grade 0 corresponding to absence of fibrosis, grade 1 to a minimally increased reticulin network and grade 2 to an increased reticulin network (Thiele *et al*, 2005). The main clinical characteristics of the series are summarized in Table I. The median interval between diagnosis of ET and HC start was 3·6 months. Reasons for initiating HC were: age older than 60 years with ($n = 41$) or without ($n = 105$) history of thrombohaemorrhagic complications, prior thrombosis ($n = 14$), prior bleeding ($n = 1$), microvascular symptoms refractory to antiplatelet therapy ($n = 1$), and extreme thrombocytosis ($>1500 \times 10^9$/l)($n = 4$). Patients were scheduled an initial HC dose of approximately 15 mg/kg per day, usually 1000 mg/d, which was subsequently adjusted to maintain the platelet count $<400 \times 10^9$/l, without significant cytopenias. Concomitant use of antiplatelet or anticoagulant therapy was given to 127 and seven patients, respectively, whereas other cytoreductive drugs were used at any time during follow-up in 38 patients, including anagrelide ($n = 29$), interferon alpha ($n = 4$), busulphan ($n = 6$), melphalan ($n = 4$), radiophosphorus ($n = 4$), and chlorambucil ($n = 1$).

Table I. Main clinico-haematological characteristics at diagnosis in 166 patients with essential thrombocythaemia treated with HC.

Age, years*	71 (18–88)
Male/female	59/107
Cardiovascular risk factors, *n* (%)†	122 (73)
Major thrombosis prior HC start, *n* (%)	47 (28)
Major haemorrhage prior HC start, *n* (%)	16 (10)
Splenomegaly, *n* (%)	13/164 (8)
Haemoglobin, g/l*	141 (82–169)
Leucocytes, $\times 10^9$/l*	9·0 (4·4–24·0)
$>10 \times 10^9$/l, *n* (%)	76 (46)
Platelets, $\times 10^9$/l*	773 (257–2226)
$>1000 \times 10^9$/l, *n* (%)	31 (19)
High LDH serum levels, *n* (%)	58/156 (37)
JAK2 V617F mutation, *n* (%)	84/144 (58)
Grade 1–2 marrow fibrosis, *n* (%)	52/139 (37)

HC, hydroxycarbamide; LDH, lactate dehydrogenase.
*Median (range).
†Diabetes, hypertension, smoking, hypercholesterolaemia.

Response to HC treatment was categorized using the recently published ELN criteria (Barosi *et al*, 2009). Accordingly, a complete clinicohaematological response (CR) was defined as normalization of the platelet count ($<400 \times 10^9$/l) in the absence of disease-related symptoms, with a normal spleen size and a leucocyte count $\leq 10 \times 10^9$/l. A partial response (PR) was defined as a reduction of the platelet count by 50% from baseline or a platelet count of $\leq 600 \times 10^9$/l, in patients not fulfilling the criteria for CR. Any response that did not satisfy PR criteria was classified as a non response. Loss of response was recognized when a responder no longer met the criteria for response in two consecutive measurements separated by at least 1 month.

The ELN definitions of resistance/intolerance to HC required the fulfilment of at least one of the following criteria: platelet count $>600 \times 10^9$/l after 3 months of at least 2 g/d of HC (2·5 g/d in patients with a body weight over 80 kg); platelet count $>400 \times 10^9$/l combined with leucocyte count $<2{\cdot}5 \times 10^9$/l or haemoglobin (Hb) <100 g/l at any dose of HC; presence of leg ulcers or other unacceptable mucocutaneous manifestations at any dose of HC; HC-related fever (Barosi *et al*, 2007).

The study was approved by the Research Ethics Committee of the Hospital Clínico of Valencia and the Hospital del Mar of Barcelona, Spain.

Statistical analysis

The following clinical characteristics at ET diagnosis were evaluated for their potential relationship with the response to HC, overall survival and thrombosis-free survival: age, gender, concomitant cardiovascular risk factors, history of prior thrombosis, splenomegaly, Hb concentration, leucocyte and platelet counts, serum lactate dehydrogenase (LDH) level, *JAK2* mutational status, and presence of bone marrow reticulin fibrosis. In addition to these baseline characteristics, the ELN response categories, and the development of thrombosis or myelofibrosis during follow-up were evaluated in the Cox models as time-dependent covariates. Survival and time-to-event curves were estimated using the Kaplan-Meier method. In all analyses, Cox regression models were first fitted for each of the parameters studied. The proportional hazard assumption was checked using both graphic and analytic methods. For variables not fulfilling the proportional hazards assumption, a temporal interaction in an extended Cox regression model was considered. Factors attaining a significant level ($P < 0{\cdot}1$) at univariate analysis were included in a Cox proportional hazards model for assessing the independent effect of each covariate controlled for the others. Finally, clinically meaningful factors without statistical significance at the univariate level were individually entered in the final model, in order to assure that no significant changes in the final estimates were produced. In all tests, P values $\leq 0{\cdot}05$ were considered as

statistically significant. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 15.0 (SPSS, Chicago, IL, USA).

Results

Response to HC therapy

Patients received HC for a median time of 4·5 years (range, 0·1–21). Overall, 134 patients achieved a CR and 25 a PR, after a median of 3·3 months on therapy. The estimated cumulative rates of CR at 6, 12 and 24 months were 57%, 70% and 76% respectively. Median daily dose to attain the best response was 1000 mg (range, 500–2500), and the median daily maintenance dose for responders was 850 mg (range, 250–2500). Factors associated with the response to HC are outlined in Table II. Of note, the presence of the *JAK2* V617F mutation at baseline correlated with higher rates of CR [Hazard Ratio (HR) 1·5, 95% confidence interval (CI): 1·0–2·2, $P = 0·03$] (Fig 1) and lower dosages of HC to maintain the responses ($P = 0·02$). During follow-up, 51 patients lost the CR after a median of 19 months (range, 1–99). Thus, 83 patients (50% of the total series) had a sustained CR during the entire follow-up period. The only clinical factor that predicted for a higher risk of losing the response was a platelet count $>1000 \times 10^9$/l at ET diagnosis (HR 2·4, 95% CI: 1·3–4·4, $P = 0·006$).

At the last visit, 109 patients were alive and still on HC therapy. Thirty patients had withdrawn HC due to toxicity ($n = 14$), lack of efficacy ($n = 5$), occurrence of a second malignancy ($n = 2$) or other reasons ($n = 9$).

Application of the ELN definitions of resistance/intolerance to HC

A total of 33 patients (20% of the total cohort) met at least one of the ELN criteria defining resistance ($n = 15$) or intolerance ($n = 21$) to HC (Table III). Of note, no patient had a platelet count $>600 \times 10^9$/l after 3 months on HC at ≥2 g/d. At the 3-month time point, six patients were receiving HC at 2 g/d or more, but one of them was in CR and the remaining five had

Table II. Analysis of factors associated with the clinicohaematological response to hydroxycarbamide in 166 patients with essential thrombocythaemia.

		Univariate		Multivariate	
Factor	No. CR (%)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)
Age					
≤70 years	61/79 (77%)	0·001	(baseline)	0·003	(baseline)
>70 years	73/87 (84%)		1·8 (1·2–2·5)		1·8 (1·2–2·7)
Gender					
Female	84/107 (78·5%)	0·6	(baseline)		
Male	50/59 (85%)		1·1 (0·8–1·5)		
Splenomegaly					
No	125/151 (83%)	0·08	(baseline)	0·05	(baseline)
Yes	7/13 (54%)		0·5 (0·2–1·1)		0·4 (0·2–1·0)
Hb level					
≤140 g/l	64/80 (80%)	0·7	(baseline)		
>140 g/l	69/85 (81%)		1·1 (0·8–1·5)		
Leucocyte count					
$\leq 10 \times 10^9$/l	82/96 (85%)	0·002	(baseline)	0·003	(baseline)
$>10 \times 10^9$/l	51/69 (74%)		0·6 (0·4–0·8)		0·6 (0·4–0·8)
Platelet count					
$\leq 1000 \times 10^9$/l	111/135 (82%)	0·6	(baseline)		
$>1000 \times 10^9$/l	23/31 (74%)		0·9 (0·6–1·4)		
Serum LDH levels					
Normal	83/98 (85%)	0·9	(baseline)		
High	45/58 (78%)		1·0 (0·7–1·5)		
JAK2 V617F mutation					
No	44/60 (73%)	0·03	(baseline)	0·01	(baseline)
Yes	69/84 (82%)		1·5 (1·0–2·2)		1·7 (1·1–2·5)
Marrow fibrosis					
No	69/87 (79%)	0·4	(baseline)		
Yes	44/52 (85%)		1·2 (0·8–1·7)		

CI, confidence interval; CR, complete clinicohaematological response; LDH, lactate dehydrogenase.

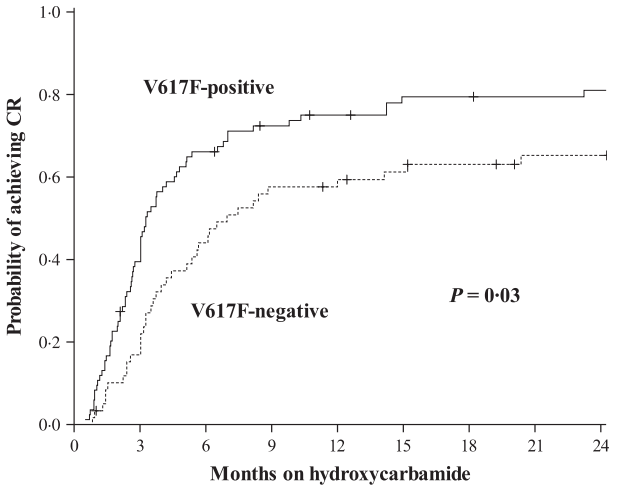

Fig 1. Probability of achieving a complete clinicohaematological response to hydroxycarbamide in 166 ET patients according to *JAK2* mutational status at baseline.

Table III. Application of the European LeukaemiaNet criteria of resistance/intolerance to hydroxycarbamide in 166 patients with essential thrombocythaemia.

Criteria	No. of patients	Time to occurrence*
Platelet count >600 × 10^9/l after 3 months with ≥2 g/d of HC	0	–
Platelet count >400 × 10^9/l and leucocytes <2·5 × 10^9/l at any dose	3	97 (27–168)
Platelet count >400 × 10^9/l and Hb <100 g/l at any dose	15	45 (4–171)
Leg ulcers or other unacceptable mucocutaneous toxicity	20	31 (1–115)
HC-related fever	1	32
Any of the above criteria, no. (% of total patients)	33 (20)	

HC, hydroxycarbamide; Hb, haemoglobin.
*Median time from HC start, months (range).

platelet counts between 400 and 600 × 10^9/l. On the other hand, five patients had a platelet count >600 × 10^9/l after 3 months of treatment, but all of them were receiving HC at doses ranging from 1000 mg to 1750 mg/d. Despite the lack of an adequate control of the thrombocytosis in such patients, no further dose escalation of HC was prescribed due to the presence of side effects. Three patients with a platelet count >400 × 10^9/l developed leucopenia of <2·5 × 10^9/l while on HC at a daily dose of 1000 mg, 1000 mg, and 1500 mg respectively. Interestingly, all three had previously met the criterion of resistance to HC due to a decrease in the Hb level to <100 g/l together with a platelet count >400 × 10^9/l. In fact, the latter criterion was the one more frequently encountered in the present series, occurring in a total of 15 cases. In two of these, a diagnosis of myelofibrosis preceded the detection of the anaemia by 6 and 11 months respectively, whereas in five cases the anaemia antedated the myelofibrotic transformation by 1, 2, 2, 10, and 32 months respectively. Eleven patients developed leg ulcers at various HC doses, ranging from 500 to 2500 mg/d (median, 1000 mg/d). This complication led to a permanent discontinuation of the drug in eight cases, with three patients being able to resume HC upon resolution of the ulcer with no later recurrence. Other unacceptable mucocutaneous manifestations occurred in nine patients. Haematological and mucocutaneous complications were unrelated, with only two patients presenting both types of toxicities. Finally, one instance of fever probably related to HC was documented.

Clinical outcome of the patients

With a median follow-up from ET diagnosis of 7 years (range, 0·5–23), 38 patients (23%) had died, resulting in a survival probability of 65% at 10 years from HC start. Causes of death included cardiovascular complications (n = 12), infections (n = 8), leukaemic transformation (n = 5), an unrelated malignancy (n = 5), haemorrhage (n = 1), or were unknown (n = 7). Factors independently associated with survival are shown in Table IV. As can be seen, the risk of death from any cause was increased by 6·2-fold (95% CI: 2·3–16·7, P < 0·001) in patients who met any of the ELN criteria of resistance to HC. Thus, death was registered in 11 of the 15 resistant patients and in 27 of the 151 non-resistant patients, corresponding to a survival probability at 10 years of 26% and 79% respectively (P < 0·001) (Fig 2). Anaemia was, in all instances, the first finding qualifying for resistance to HC, with the median subsequent survival of patients with anaemia being only 2·4 years (range, 0·01–4·9). When compared with the others, resistant patients were more likely to display hyperproliferative features at ET diagnosis, such as higher levels of leucocytes (P = 0·05), platelets (P = 0·004) and serum LDH (P = 0·02).

With a follow-up of 1034 person/years, 25 patients experienced thrombotic events, at a median of 40 months (range, 4–207) from HC start, with a thrombotic incidence rate of 2·4% patients/year and a cumulative incidence at 10 years of 21%. A total of 20 arterial and five venous thromboses were recorded. At the time of thrombosis, the platelet count was normal in 8 of 20 patients (40%) with available data. In multivariate analysis, the clinical factors that correlated with a higher risk of thrombosis after HC start were a history of prior thrombosis (HR 3·8, 95% CI: 1·6–8·8, P = 0·002) and a leucocyte count >10 × 10^9/l at ET diagnosis (HR 3·1, 95% CI: 1·2–7·6, P = 0·016) (Table IV). By contrast, we did not find any relationship between cardiovascular risk factors and the occurrence of arterial or venous thrombosis during HC treatment. Moreover, the same was true when the patients were stratified according to their clinical features (e.g. age, sex, history of thrombosis). Notably, there was no statistical difference in the cumulative incidence of thrombosis between

Table IV. Multivariate analysis of risk factors for survival and thrombotic complications in 166 patients with essential thrombocythaemia treated with hydroxycarbamide.

	Survival				Thrombosis			
	Univariate		Multivariate		Univariate		Multivariate	
Presenting features	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)
Age								
≤70 years	<0·001	(baseline)	<0·001	(baseline)	0·8	(baseline)		
>70 years		4·3 (2·1–8·9)		7·1 (2·7–18·7)		0·9 (0·4–2·0)		
Gender								
Female	0·5	(baseline)			0·2	(baseline)		
Male		1·2 (0·6–2·4)				1·7 (0·8–3·9)		
Vascular risk factors*								
No	0·8	(baseline)			0·6	(baseline)		
Yes		1·1 (0·5–2·3)				1·3 (0·5–3·2)		
Prior thrombosis								
No	0·3	(baseline)			0·005	(baseline)	0·002	(baseline)
Yes		1·5 (0·7–3·0)				3·2 (1·4–7·0)		3·8 (1·6–8·8)
Splenomegaly								
No	0·2	(baseline)			0·04	(baseline)	0·11	
Yes		1·8 (0·7–4·3)				2·9 (1·0–8·0)		
Hb level								
≤140 g/l	0·8	(baseline)			0·7	(baseline)		
>140 g/l		1·1 (0·6–2·0)				1·2 (0·5–2·6)		
Leucocyte count								
$\leq 10 \times 10^9$/l	0·05	(baseline)	0·7		0·02	(baseline)	0·016	(baseline)
$>10 \times 10^9$/l		1·9 (1·0–3·6)				2·6 (1·2–5·8)		3·1 (1·2–7·6)
Platelet count								
$\leq 1000 \times 10^9$/l	0·11	(baseline)			0·5	(baseline)		
$>1000 \times 10^9$/l		1·8 (0·9–3·7)				1·4 (0·6–3·6)		
Serum LDH level								
Normal	0·003	(baseline)	0·13		0·08	(baseline)	0·2	
High		2·8 (1·4–5·5)				2·1 (0·9–4·9)		
Marrow fibrosis								
No	0·2	(baseline)			0·3	(baseline)		
Yes		1·8 (0·8–4·1)				1·8 (0·6–5·2)		
JAK2 mutation								
No	0·4	(baseline)			0·5	(baseline)		
Yes		1·4 (0·6–3·2)				1·4 (0·5–3·9)		
Evolutive variables†								
CR with HC therapy								
No	0·2	(baseline)			0·5	(baseline)		
Yes		1·7 (0·8–3·7)				0·7 (0·3–1·8)		
Resistance to HC								
No	<0·001	(baseline)	<0·001	(baseline)	0·5	(baseline)		
Yes		10·4 (5·0–21·5)		6·2 (2·3–16·7)		2·0 (0·3–15·3)		
Intolerance to HC								
No	0·2	(baseline)			NE			
Yes		1·9 (0·8–4·6)						
Thrombotic events								
No	<0·001	(baseline)	0·008	(baseline)	NE			
Yes		4·2 (2·1–8·3)		2·8 (1·3–5·8)				
Myelofibrosis								
No	<0·001	(baseline)	0·003	(baseline)	NE			
Yes		5·9 (2·4–14·0)		7·5 (1·9–29·2)				

CI, confidence interval; Hb, haemoglobin; HC, hydroxycarbamide; CR, complete clinicohaematological response; LDH, lactate dehydrogenase; NE, not evaluated.

*Diabetes, hypertension, smoking, hypercholesterolaemia.

†These factors were considered as time-dependent covariates in the Cox regression analysis.

patients who remained in CR throughout the entire follow-up and the others (HR 0·9, 95% CI: 0·4–2·1, $P = 0·8$) (Fig 3).

Transformation to myelofibrosis was observed in 11 patients at a median of 64 months (range, 23–160) from the initiation of HC, with a cumulative risk at 10 years of 13%. In all cases, a review of the marrow biopsies performed at ET diagnosis allowed exclusion of the 'prefibrotic' form of myelofibrosis (Tefferi *et al*, 2007). However, patients displaying reticulin accumulation (grade 1–2) in their initial marrow biopsy had a trend to a higher risk of myelofibrotic transformation (HR 4·3, 95% CI: 0·9–21·2, $P = 0·08$). Of note, a remarkable incidence of myelofibrosis was observed in patients fulfilling the ELN criteria for resistance, as this complication was recorded in 7 of 15 such cases, whereas only four cases were documented in the remaining patients ($P < 0·001$). Lastly, five patients developed acute leukaemia at a median of 74 months (range, 53–118·5) from HC start, with an estimated actuarial risk at 10 years of 8·5%. Three of these patients were not exposed to cytotoxic drugs other than HC, one had previously been given busulphan, whereas the remaining patient had received radiophosphorus and busulphan following HC withdrawal due to intolerance. Thus, the cumulative incidence of leukaemia among patients exposed to HC alone was of 4% at 10 years. A trend towards a higher leukaemic risk was observed in the subgroup of patients ($n = 13$) who were sequentially exposed to several cytotoxic drugs (HR 5·0, 95% CI: 0·8–30·5, $P = 0·08$).

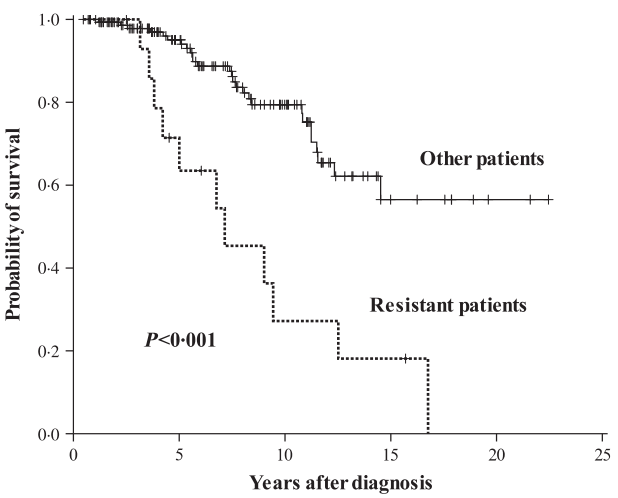

Fig 2. Probability of survival in 166 ET patients according to whether or not they met the European LeukaemiaNet criteria for resistance to hydroxycarbamide during treatment.

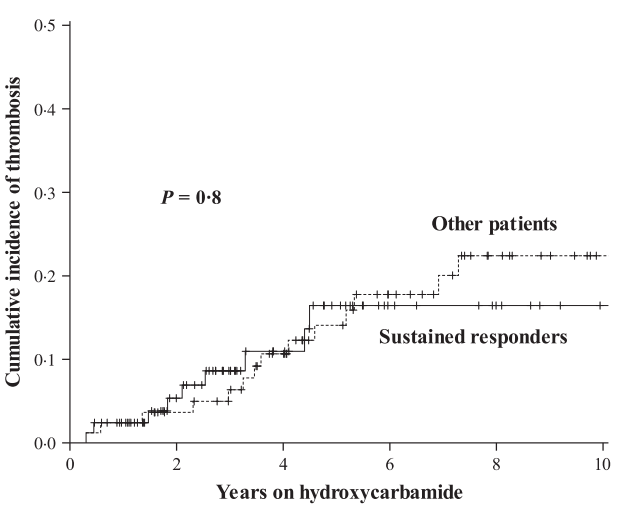

Fig 3. Cumulative incidence of thrombosis during follow-up in 166 ET patients according to the response to hydroxycarbamide treatment.

Discussion

Definitions of response to treatment (Barosi *et al*, 2009) and resistance/intolerance to HC in ET (Barosi *et al*, 2007) have been recently proposed on behalf of the ELN. Notably, they resulted from a consensus process among experts and were not evidence-based, and therefore need to be validated in the clinical setting. For this reason, we have retrospectively applied the proposed criteria to all patients treated with HC in our institutions, focusing on the potential correlation between the ELN response categories and the clinically meaningful outcomes, namely, the overall survival and the thrombosis-free survival.

In our series, most ET patients were able to achieve a CR with HC therapy (70% at 1 year), a figure that is considerably higher than the one reported in a recent study (25% at 1 year) applying the same criteria to a large cohort of ET patients (Carobbio *et al*, 2010). This discrepancy most likely reflects the different therapeutic endpoints used in both studies, with a target platelet level of less than 400×10^9/l in the present series as opposed to one of less than 600×10^9/l in the Italian series. These data support the well-recognized effectiveness of HC in controlling the platelet count in ET (Cortelazzo *et al*, 1995; Harrison *et al*, 2005; Randi *et al*, 2005; Palandri *et al*, 2009). Of interest, the presence of the *JAK2* V617F mutation was associated with a higher probability of attaining CR, as well as with lower dosages of HC to sustain the responses, in line with previous evidence suggesting a greater sensitivity to the drug in these cases (Campbell *et al*, 2005).

To our knowledge, the present study is the first one to report on the frequency of ET patients fulfilling the ELN criteria for resistance/intolerance to HC (Barosi *et al*, 2007). Overall, 20% of the total series met such criteria. With regard to the definitions of resistance, the most discriminating criterion was the detection of anaemia along with thrombocytosis, which was observed in 9% of cases, while other criteria were less useful. In fact, no patient had a platelet count above 600×10^9/l after 3 months on HC at 2 g/d or more. There were occasional patients who had platelet counts above such threshold at that time point, but the presence of minor side effects precluded the escalation of the HC dose above 1750 mg. Consequently, the latter patients could not be categorized as resistant or intolerant to HC according to the

ELN definitions. Moreover, all patients developing leucopenia ($<2{\cdot}5 \times 10^9$/l) on HC therapy had previously experienced anaemia (Hb < 100 g/l), and therefore this criterion failed to identify any additional resistant patients. These findings suggest that the current ELN criteria for resistance to HC might be improved with some modifications. Thus, we propose a definition of resistance to HC based on the presence of a platelet count $>600 \times 10^9$/l after 3 months of at least 2 g/d of HC or at a lower dose if concomitant side effects preclude a dose increase, and/or on the detection of anaemia (Hb < 100 g/l) together with thrombocytosis ($>400 \times 10^9$/l) at any time during treatment.

Severe drug-related mucocutaneous complications were observed in 12% of cases, and usually led to HC withdrawal. This figure is similar to the one reported in the United Kingdom Medical Research Council Primary Thrombocythaemia 1 Study, where dermatological events were registered in 11% of patients treated with HC (Harrison *et al*, 2005). Unfortunately, we failed to identify any clinical factor that could reliably predict the appearance of these complications. Of note, mucocutaneous and haematological toxicities were mostly unrelated, with very few patients presenting both types of side effects. In line with previous studies (Braester & Quitt, 2000; Randi *et al*, 2005), the occurrence of HC-related fever was very rare.

On the whole, the most striking finding of the present study was the poor survival registered in the subgroup of patients qualifying for resistance to HC. Thus, such patients had a six-fold higher risk of death by any cause than the others, and the median subsequent survival from the onset of anaemia, which was always the first factor defining resistance, was only 2·4 years. This increased mortality could be attributed in part, but not exclusively, to the high incidence of myelofibrosis registered in this subgroup. Therefore, the detection of anaemia along with thrombocytosis during treatment should probably be considered a marker of disease progression, unless proved otherwise. Of interest, patients destined to develop resistance to HC had a distinctive clinical profile at ET diagnosis, characterized by marked hyperproliferative features. By contrast, their baseline Hb level was similar to that of the other cases.

Finally, a lack of correlation between the response to HC and the thrombotic risk was observed. Indeed, patients who maintained CR status at all times during follow-up (50% of total series) did not benefit from a lower incidence of thrombosis as compared with the others. These results challenge the notion that any particular threshold for the platelet count should be used as an endpoint of treatment in ET. In this sense, other authors have suggested that the leucocyte count could constitute a better surrogate endpoint for therapy (Carobbio *et al*, 2010), in view of the prognostic impact of this factor on both the thrombotic risk (Wolanskyj *et al*, 2006; Carobbio *et al*, 2008) and the survival (Gangat *et al*, 2007; Girodon *et al*, 2010). In fact, a detrimental effect on the thrombotic risk of a high leucocyte count at ET diagnosis was also observed in the present series. However, it should be noted that no clear benefit of controlling the leucocytosis was evident in the present study, since patients with sustained CR kept, by definition, a leucocyte count below 10×10^9/l at all times during follow-up, and yet this was not translated into less vascular complications. Therefore, prospective randomized trials are warranted to properly evaluate whether different parameters could eventually constitute better surrogate markers for the long-term treatment success.

In conclusion, treatment with HC can induce complete clinicohaematological responses in most ET patients, but such responses do not seem to correlate with the risk of thrombosis nor with the overall survival. The best discriminating ELN criterion of resistance to HC is based on the detection of anaemia. Moreover, this criterion is particularly useful since it also identifies a subset of ET patients with a poor prognosis. These results should be taken into account in the design of future studies addressing the value of new treatment modalities for ET.

Acknowledgements

The present study was supported by the grants PS09/02324 and EC07/90791 from the Fondo de Investigaciones Sanitarias, Spanish Ministry of Health, and IMPULSA/2010 from the Conselleria de Sanitat, Generalitat Valenciana, Spain. The first author would like to thank Professor Francisco Cervantes for his continuous mentorship and for kindly reviewing the paper.

Conflict of interest disclosures

All authors declare no competing financial interests.

References

Barbui, T. (2004) The leukemia controversy in myeloproliferative disorders: is it a natural progression of disease, a secondary sequela of therapy, or a combination of both. *Seminars in Hematology*, **41,** 15–17.

Barosi, G., Besses, C., Birgegard, G., Briere, J., Cervantes, F., Finazzi, G., Gisslinger, H., Griesshammer, M., Gugliotta, L., Harrison, C., Hasselbalch, H., Lengfelder, E., Reilly, J.T., Michiels, J.J. & Barbui, T. (2007) A unified definition of clinical resistance/intolerance to hydroxyurea in essential thrombocythemia: results of a consensus process by an international working group. *Leukemia*, **21,** 277–280.

Barosi, G., Birgegard, G., Finazzi, G., Griesshammer, M., Harrison, C., Hasselbalch, H.C., Kiladjian, J.J., Lengfelder, E., McMullin, M.F., Passamonti, F., Reilly, J.T., Vannucchi, A.M. & Barbui, T. (2009) Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet consensus conference. *Blood*, **113,** 4829–4833.

Braester, A. & Quitt, M. (2000) Hydroxyurea as a cause of drug fever. *Acta Haematologica*, **104,** 50–51.

Campbell, P.J., Scott, L.M., Buck, G., Wheatley, K., East, C.L., Marsden, J.T., Duffy, A., Boyd, E.M., Bench, A.J., Scott, M.A., Vassiliou, G.S., Milligan, D.W., Smith, S.R., Erber, W.N., Bareford, D., Wil-

kins, B.S., Reilly, J.T., Harrison, C.N. & Green, A.R. (2005) Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet*, **366,** 1945–1953.

Carobbio, A., Antonioli, E., Guglielmelli, P., Vannucchi, A.M., Delaini, F., Guerini, V., Finazzi, G., Rambaldi, A. & Barbui, T. (2008) Leukocytosis and risk stratification assessment in essential thrombocythemia. *Journal of Clinical Oncology*, **26,** 2732–2736.

Carobbio, A., Finazzi, G., Antonioli, E., Vannucchi, A.M., Barosi, G., Ruggeri, M., Rodeghiero, F., Delaini, F., Rambaldi, A. & Barbui, T. (2010) Hydroxyurea in essential thrombocytemia: rate and clinical relevance of responses by European LeukemiaNet criteria. *Blood*, **116,** 1051–1055.

Cortelazzo, S., Finazzi, G., Ruggeri, M., Vestri, O., Galli, M., Rodeghiero, F. & Barbui, T. (1995) Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. *New England Journal of Medicine*, **332,** 1132–1136.

Finazzi, G., Ruggeri, M., Rodeghiero, F. & Barbui, T. (2003) Efficacy and safety of long-term use of hydroxyurea in young patients with essential thrombocythemia and a high risk of thrombosis. *Blood*, **101,** 3749.

Gangat, N., Wolanskyj, A.P., McClure, R.F., Li, C.Y., Schwager, S., Wu, W. & Tefferi, A. (2007) Risk stratification for survival and leukemic transformation in essential thrombocythemia: a single institutional study of 605 patients. *Leukemia*, **21,** 270–276.

Girodon, F., Dutrillaux, F., Broseus, J., Mounier, M., Goussot, V., Bardonnaud, P., Chretien, M.L. & Maynadie, M. (2010) Leukocytosis is associated with poor survival but not with increased risk of thrombosis in essential thrombocythemia: a population-based study of 311 patients. *Leukemia*, **24,** 900–903.

Harrison, C.N., Campbell, P.J., Buck, G., Wheatley, K., East, C.L., Bareford, D., Wilkins, B.S., van der Walt, J.D., Reilly, J.T., Grigg, A.P., Revell, P., Woodcock, B.E. & Green, A.R. (2005) Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *New England Journal of Medicine*, **353,** 33–45.

Lofvenberg, E. & Wahlin, A. (1988) Management of polycythaemia vera, essential thrombocythaemia and myelofibrosis with hydroxyurea. *European Journal of Haematology*, **41,** 375–381.

Murphy, S., Iland, H., Rosenthal, D. & Laszlo, J. (1986) Essential thrombocythemia: an interim report from the Polycythemia Vera Study Group. *Seminars in Hematology*, **23,** 177–182.

Palandri, F., Catani, L., Testoni, N., Ottaviani, E., Polverelli, N., Fiacchini, M., De Vivo, A., Salmi, F., Lucchesi, A., Baccarani, M. & Vianelli, N. (2009) Long-term follow-up of 386 consecutive patients with essential thrombocythemia: safety of cytoreductive therapy. *American Journal of Hematology*, **84,** 215–220.

Randi, M.L., Ruzzon, E., Luzzatto, G., Tezza, F., Girolami, A. & Fabris, F. (2005) Safety profile of hydroxyurea in the treatment of patients with Philadelphia-negative chronic myeloproliferative disorders. *Haematologica*, **90,** 261–262.

Tefferi, A., Thiele, J., Orazi, A., Kvasnicka, H.M., Barbui, T., Hanson, C.A., Barosi, G., Verstovsek, S., Birgegard, G., Mesa, R., Reilly, J.T., Gisslinger, H., Vannucchi, A.M., Cervantes, F., Finazzi, G., Hoffman, R., Gilliland, D.G., Bloomfield, C.D. & Vardiman, J.W. (2007) Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood*, **110,** 1092–1097.

Thiele, J., Kvasnicka, H.M., Facchetti, F., Franco, V., van der Walt, J. & Orazi, A. (2005) European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica*, **90,** 1128–1132.

Wolanskyj, A.P., Schwager, S.M., McClure, R.F., Larson, D.R. & Tefferi, A. (2006) Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. *Mayo Clinic Proceedings*, **81,** 159–166.

Young, C.W., Schochetman, G. & Karnofsky, D.A. (1967) Hydroxyurea-induced inhibition of deoxyribonucleotide synthesis: studies in intact cells. *Cancer Research*, **27,** 526–534.