

Clinical outcomes under hydroxyurea treatment in polycythemia vera: a systematic review and meta-analysis



Alberto Ferrari,¹ Alessandra Carobbio,¹ Arianna Masciulli,¹ Arianna Ghirardi,¹ Guido Finazzi,² Valerio De Stefano,³ Alessandro Maria Vannucchi⁴ and Tiziano Barbui¹

¹FROM Research Foundation, ASST Papa Giovanni XXIII, Bergamo; ²Hematology Division, Papa Giovanni XXIII Hospital, Bergamo; ³Institute of Hematology, Catholic University, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome and ⁴CRIMM-Center of Research and Innovation of Myeloproliferative Neoplasms, Azienda Ospedaliera Universitaria Careggi and Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Haematologica 2019
Volume 104(12):2391-2399

ABSTRACT

Hydroxyurea is the standard treatment in high-risk patients with polycythemia vera. However, estimates of its effect in terms of clinical outcomes (thrombosis, bleeding, hematologic transformations and mortality) are lacking. We performed a meta-analysis to determine the absolute risk of events in recent cases of patients under hydroxyurea treatment. We searched for relevant articles or abstracts in the following databases: Medline, EMBASE, clinicaltrials.gov, WHO International Clinical Trials Registry, LILACS. Sixteen studies published from 2008 to 2018 reporting number of events using World Health Organization diagnosis for polycythemia vera were selected. Through a random effect logistic model, incidences, study heterogeneity and confounder effects were estimated for each outcome at different follow ups. Overall, 3,236 patients were analyzed. While incidences of thrombosis and acute myeloid leukemia were stable over time, mortality and myelofibrosis varied depending on follow-up duration. Thrombosis rates were 1.9%, 3.6% and 6.8% persons/year at median ages 60, 70 and 80 years, respectively. Higher incidence of arterial events was predicted by previous cardiovascular complication. Leukemic transformation incidence was 0.4% persons/year. Incidence of transformation to myelofibrosis and mortality were significantly dependent on age and follow-up duration. For myelofibrosis, rates were 5.0 at five years and 33.7% at ten years; overall mortality was 12.6% and 56.2% at five and ten years, respectively. In conclusion, we provide reliable risk estimates for the main outcomes in polycythemia vera patients under hydroxyurea treatment. These findings can help design comparative clinical trials with new cytoreductive drugs and prove the feasibility of using critical end points for efficacy, such as major thrombosis.

Introduction

Polycythemia vera (PV) is a myeloproliferative neoplasm (MPN) characterized by clonal proliferation of the erythroid, myeloid, and megakaryocyte lineages. This disease is recognized for its distinct molecular profile (*JAKV* 617F mutation) and has a characteristic natural history marked by high frequency of thrombosis and a tendency to transform into acute myelogenous leukemia (AML) or myelofibrosis (MF). The first step in approaching an individual patient with PV is to identify the potential risk of developing major thrombotic or hemorrhagic complications. In patients under 60 years of age, carrying only reversible or controllable cardiovascular risk factors and without prior history of thrombosis, phlebotomy (PHL) or low-dose aspirin are recommended. Cytoreductive therapy with either hydroxyurea (HU), a

Correspondence:

TIZIANO BARBUI
tbarbui@asst-pg23.it

Received: March 7, 2019.

Accepted: May 20, 2019.

Pre-published: May 23, 2019.

doi:10.3324/haematol.2019.221234

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/104/12/2391

©2019 Ferrata Storti Foundation

Material published in *Haematologica* is covered by copyright. All rights are reserved to the Ferrata Storti Foundation. Use of published material is allowed under the following terms and conditions:

<https://creativecommons.org/licenses/by-nc/4.0/legalcode>.

Copies of published material are allowed for personal or internal use. Sharing published material for non-commercial purposes is subject to the following conditions:

<https://creativecommons.org/licenses/by-nc/4.0/legalcode>, sect. 3. Reproducing and sharing published material for commercial purposes is not allowed without permission in writing from the publisher.



ribonucleotide reductase inhibitor considered non-mutagenic, or interferon-alfa (IFN) are appropriate first-line drugs to prevent vascular complications in high-risk patients (age >60 years and/or prior thrombosis).¹

Hydroxyurea was recommended in the treatment of high-risk PV based on the results of the Polycythemia Vera Study Group (PVSG) protocol 08 in which this drug was found to be effective in reducing the rate of thrombotic events in 51 patients compared to historical controls treated with PHL alone.² Very few studies were designed to confirm these conclusions. Recently, a propensity score analysis of patients enrolled in the European Collaboration on Low-dose Aspirin in Polycythaemia Vera (ECLAP) trial documented superiority of HU in reducing thrombosis compared with well-matched control patients treated with PHL only.³ In three recent randomized controlled trials (RCT) in PV,^{4,6} HU was compared to IFN; unfortunately, the primary end point was not the reduction of vascular complications but included only hematologic response that cannot be considered a surrogate of vascular events.⁷ The only demonstration of an antithrombotic efficacy results from two RCT in essential thrombocythemia (ET) in which the drug was superior to chemotherapy-free and to anagrelide control arms.^{8,9} Therefore, the lack of a solid demonstration of thrombosis prevention or survival advantage in PV, and the concern that HU may increase the risk of leukemia led to this drug being under-used in clinical practice¹⁰ and to suggest that the first-line cytoreductive therapy in PV should be PHL only, irrespective of patient risk category.¹¹

However, even in the absence of a clear demonstration of benefit, there is a consensus among European LeukemiaNet (ELN) and National Comprehensive Cancer Network (NCCN) experts of HU use in high-risk cases and the drug is currently the first-line therapy in clinical practice. We have now several observational studies reporting single or multicenter experience regarding the risk-estimates of clinical events associated with HU. We, therefore, considered it useful to provide a summary of these results in order to help clinical decision-making and to offer estimates for a more realistic sample calculation in future comparative clinical trials. Responding to the unmet need for such knowledge requires a huge input of energy and expertise in order to retrieve and analyze data. Based on these premises, we carried out a literature review aimed at systematically assessing and performing a meta-analysis of the incidence rate and absolute risk of events in patients treated with HU.

Methods

Inclusion criteria

The protocol of the original review was registered in PROSPERO (n. CRD42018117814¹²).

Inclusion criteria were:

- 1) studies in English language published in the period 2008-2018 using WHO diagnostic criteria for PV;
- 2) studies on adult (aged ≥ 18 years) non-pregnant patients;
- 3) RCT, prospective and retrospective cohort studies reporting frequency of outcomes of interests (thrombotic and/or hemorrhagic events and/or hematologic transformations in adult patients) stratified by HU therapy, as reported by authors;
- 4) studies with at least 20 participants.

The following studies were excluded: case reports, cross-sectional

studies, editorials, and narrative reviews. Studies aimed specifically at HU-resistant patients were excluded.

In the case of duplicate studies on the same sample, the most numerous, or most informative, or most recent study was taken into consideration. Studies not reporting follow-up duration were excluded.

Search strategy

We searched for articles or abstracts published between 2008 and 2018 in the following databases: Medline, EMBASE, clinicaltrials.gov, WHO International Clinical Trials Registry (for unpublished or ongoing trials), LILACS.

Terms used in research for primary end points were polycythemia vera and hydroxyurea/hydroxycarbamide and thrombosis and myelofibrosis. Research was focused on primary outcomes, although we also collected data on secondary outcomes (survival, leukemia, bleeding). Whenever possible, specific filters were used to exclude case reports, reviews, animal studies and studies on very young patients (aged < 18 years) or pregnant women. Conference abstracts and posters reporting relevant data were not excluded from consideration. Duplicate records were individually checked and merged using reference managing software.

Data extraction

The following data were extracted from selected studies: type of study, mean (or median) follow-up duration, number of HU treated patients in the study, incidence of myelofibrotic and/or leukemic transformations, number of patients with at least one incident or recurrent episode of thrombosis or one bleeding, mortality, median/mean age, gender of patients, number of patients with cardiovascular risk factors, number of patients with history of thrombosis, number of patients undergoing antiplatelet or anticoagulant therapy. Whenever possible, the number of patients with major arterial or venous thrombosis was also extracted.

Quality assessment

Quality assessment of eligible studies was performed independently by two reviewers (TB and AF) according to the Joanna Briggs Institute (JBI) critical appraisal tool for studies reporting prevalence data.¹³ The tool evaluates methodological quality of studies according to a 9-object scale accounting for representativeness of the sample, accuracy of reporting, adequacy of diagnostic criteria, and statistical analysis.

Statistical analysis

Incidence of each outcome was calculated and is reported as number of events per 100 persons/year. Forest plots show punctual estimates with exact binomial 95% confidence intervals for each study and globally. Persons/year were estimated by multiplying mean follow-up duration by number of HU-treated patients; when mean follow-up duration was not available, median duration was deemed to be a reasonable approximation.

In order to obtain global adjusted incidence estimates, a logistic Generalized Linear Mixed Model (GLMM) was used for meta-regression of outcomes on study-specific confounders. The model included follow-up duration and known risk factors for the outcome as fixed effects; the random component of the model included a random slope for follow-up duration in studies. The method assumes that probability of displaying the event at time zero is the same across the studies, but it increases as a function of follow-up duration at a study-specific rate under the effect of selected covariates. The advantage of this model is that it uses an exact binomial likelihood and error structure, and naturally accounts for heterogeneity in sample sizes.¹⁴⁻¹⁶ For meta-regression, missing data

about confounders were imputed to the sample size-weighted mean of the other studies. For reasons of interpretability and estimability of the model, predictor variables were all centered on their weighted mean. Intraclass Correlation Coefficients (ICC) were calculated conditional on fixed effects = 0 (i.e. the mean) and reported as heterogeneity measure.

To evaluate whether results could depend on model choice, a sensitivity analysis was conducted by fitting a negative-binomial regression on events count, with persons/year as exposure variable. As opposed to the GLMM, such a model assigns the same weight to each study regardless of sample size and assumes a constant yearly event rate with no upper boundary.

Results

Literature search and study characteristics

The study selection process is detailed in Figure 1.

The search on Medline and EMBASE retrieved a total 420 results; nine additional results were retrieved from different sources (clinicaltrials.gov, Cochrane Central Register of Controlled Trials, WHO International Clinical Trials Registry, references from relevant articles) for a total 429 results, which were reduced to 340 after removing duplicates. Abstract and full-text screening allowed for the exclusion of 291 articles, as they fell into the following categories: reviews, case reports, animal studies, patients aged <18 years or pregnant. Other studies were not considered as they had a total sample size < 20 patients,

and/or they did not report incidence data or follow-up duration.

Consequently, a total 49 studies were selected for methodological evaluation. Thirty-three were excluded. Eleven had unclear reporting of data (e.g. it was impossible to distinguish data due to HU-treated patients from those due to other cytoreductive treatments, or PV from other myeloproliferative neoplasms). Seven did not meet the number of 20 HU-treated patients as required by our study protocol. Seven studies referred to cases diagnosed outside the time window (2008-2018) and not with WHO 2008-2016 criteria. In one, follow-up data were missing. One was specifically aimed at HU-resistant patients. In case of multiple studies from the same author(s), we inquired whether they referred to overlapping populations, by questioning authors when necessary, and excluded duplicates (6 studies) from review. The final selection comprised 14 full text articles and two conference abstracts to be included in the meta-analysis.

Table 1 summarizes the main characteristics of the 16 eligible articles and abstracts. The selection included three reports on two RCT^{4,17,18} (one comparing HU and IFN therapy, and one comparing HU to ruxolitinib), one RCT in which HU was not a comparator,¹⁹ and 12 observational retrospective cohort studies.^{7,20-35} The great majority of the studies were conducted in Europe and some involved multiple countries; only one study in our selection³² was conducted in the US.

Number of HU-treated patients ranged from 25 to 890

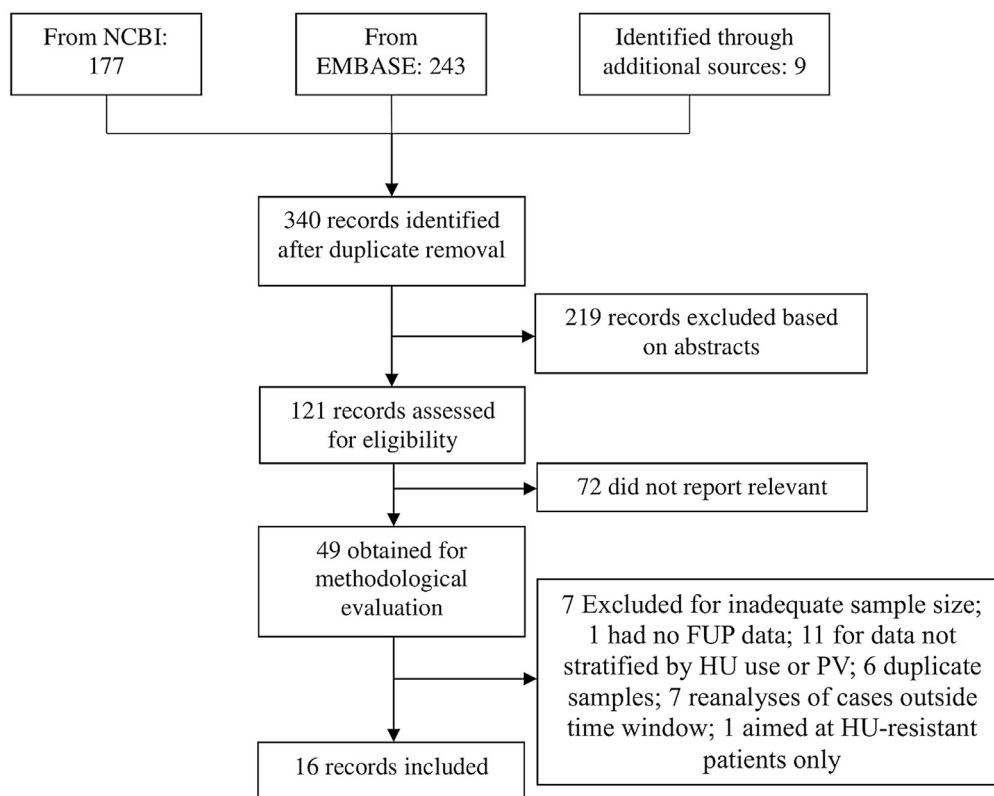


Figure 1. Study flowchart.

across studies; the final meta-analysis was conducted on a total of 3,236 patients in whom HU therapy was consistently administered. Follow-up duration ranged from 0.3 to 12.4 years.

Quality of studies was judged using the JBI critical appraisal tool for prevalence studies considering sample size, representativeness of the sample, sampling methods, objectively measured outcomes, and adequate information on follow-up duration and potential confounders.

Only two studies in our review, both by Alvarez-Larrán *et al.*,^{7,21} were specifically aimed at obtaining incidence estimates under HU treatment, and thus fully met these criteria. The other studies, not addressing the same specific question about outcomes of HU treatment, often missed some of the above information; the most frequent issue was lack of stratification by HU treatment. For six of these studies, original databases were readily available, allowing us to fully extract data about HU treatment, outcomes and potential confounders. We were unable to retrieve full information from two additional reports^{4,29} but, in spite of this, we were able to extract incidence of at least one of the outcomes of interest. In eight studies, we were able to univocally distinguish arterial and thrombotic events in 2,048 patients.^{7,19,23,26-28,31,33}

Overall, demographics were incomplete or not stratified by HU treatment (6 studies), cardiovascular risk factors were missing (10 studies), and history of thrombosis was not reported (6 studies), antithrombotic drug therapy was not mentioned in ten studies. However, in spite of missing data, in each of these studies we were able to retrieve the number of events for at least one outcome.

Two studies referred to the same population^{4,17} but reported different outcomes; therefore, we did not consider it as a duplicate for the aims of our analysis.

While most studies referred to events after first-line therapy, three focused on recurrent thromboses.

Hydroxyurea and risk of outcomes

Summary of events

Figure 2 shows forest plots of the study-specific and pooled yearly incidence of each outcome of interest as % person/years with 95% binomial Confidence Interval (CI). The incidence of outcomes shows remarkable variability across studies. In particular, with the exception of AML, for the other outcomes, 95% confidence intervals do not always overlap between studies.

A mixed effect logistic model was applied to the data in order to obtain incidence estimates adjusted for heterogeneity and study-specific confounders, including follow-up duration. Confounding effects that were verified in meta-regression were age (for all outcomes), percent of patients under antiplatelet/anticoagulant therapy (for mortality and thrombosis), percent of patients with history of thrombosis (mortality, thrombosis), percent of patients with cardiovascular risk factors (mortality, thrombosis). Overall, regression analysis of MF and AML was only adjusted for age. Results from logistic regression are detailed in *Online Supplementary Table S1*. Diagnostics of model fit were performed by visual inspection of observed *versus* fitted plots (*Online Supplementary Figure S1*).

Figure 3 shows probability of each outcome in follow up as predicted by regression models when all confounders are kept fixed at their weighted mean value, with estimated ICC and relative statistical tests of heterogeneity. Since all predictor variables were centered on the mean, predictions are to be interpreted as incidence in the presence of confounding factors equal to the (weighted) mean.

Table 1. Summary of study characteristics.

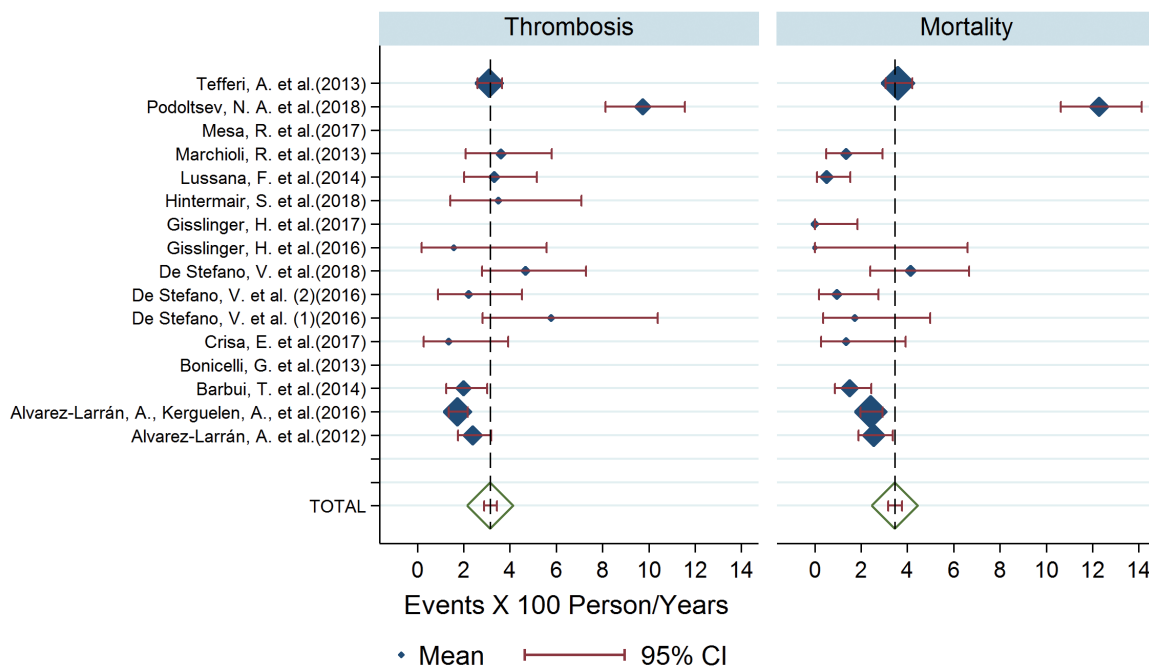
Study	N	FUP years	Median age (range)	Sex (M/F)	Mortality	MF	AML	Thrombosis	Bleeding	Study quality ²
Alvarez-Larrán, <i>et al.</i> (2012)	261	7.2	64 (16-88)	118/143	48	20	8	45	23	9/9
Alvarez-Larrán, Kerguelen, <i>et al.</i> (2016)	890	4.6	68 (18-95)	452/438	99	39	17	71	48	9/9
Barbui, <i>et al.</i> (2014)	137	7.7	60.5 (23-83)	69/68	16	12	3	21		8/9
Bonicelli, <i>et al.</i> (2013)	114	11					7			6/9
Crisa, <i>et al.</i> (2017)	35	6.3	55 (36-65)	23/12	3	3	2	3		8/9
De Stefano, <i>et al.</i> (2016a)	34	5.1	51.5 (19-80)	10/24	3	2	1	10	5	8/9
De Stefano, <i>et al.</i> (2016b)	45	7	71.5 (46-90)	24/21	3	6	1	7	1	8/9
De Stefano, <i>et al.</i> (2018)	104	3.7	73 (43-95)	46/58	16	2	2	18		8/9
Gisslinger, <i>et al.</i> (2016)	127	1	60 (21-81)	60/67	0	0	0	2		5/8 (1)
Gisslinger, <i>et al.</i> (2017)	73	2.7			0	0	2			5/8 (1)
Hintermair, <i>et al.</i> (2018)	25	8						7	2	8/9
Lussana, <i>et al.</i> (2014)	46	12.4	35.8 (22-40)	22/24	3	6	1	19	6	8/9
Marchioli, <i>et al.</i> (2013)	184	2.4	71 (44-87)	108/76	6	3	1	16	3	8/9
Mesa, <i>et al.</i> (2017)	56	0.3	66 (19-85)	34/22	1	0	0	2		6/7 (2)
Podoltsev, <i>et al.</i> (2018)	497	2.83	77		173			118		8/9
Tefferi, <i>et al.</i> (2013)	608	6.9	63.3 (19-95)	296/312	151	64	18	130		8/9
Total	3,236	.	68.4 ¹		522/3,097	157/2,600	63/2,714	469/2,552	88/1,485	

¹Weighted mean. ²Evaluation on 9 items according to JBI appraisal tool for prevalence studies. In parenthesis number of items for which evaluation was not applicable based on study design. MF: myelofibrosis; AML: acute myeloid leukemia; N: number; FUP: follow up; M: male; F: female; JBI: Joanna Briggs Institute.

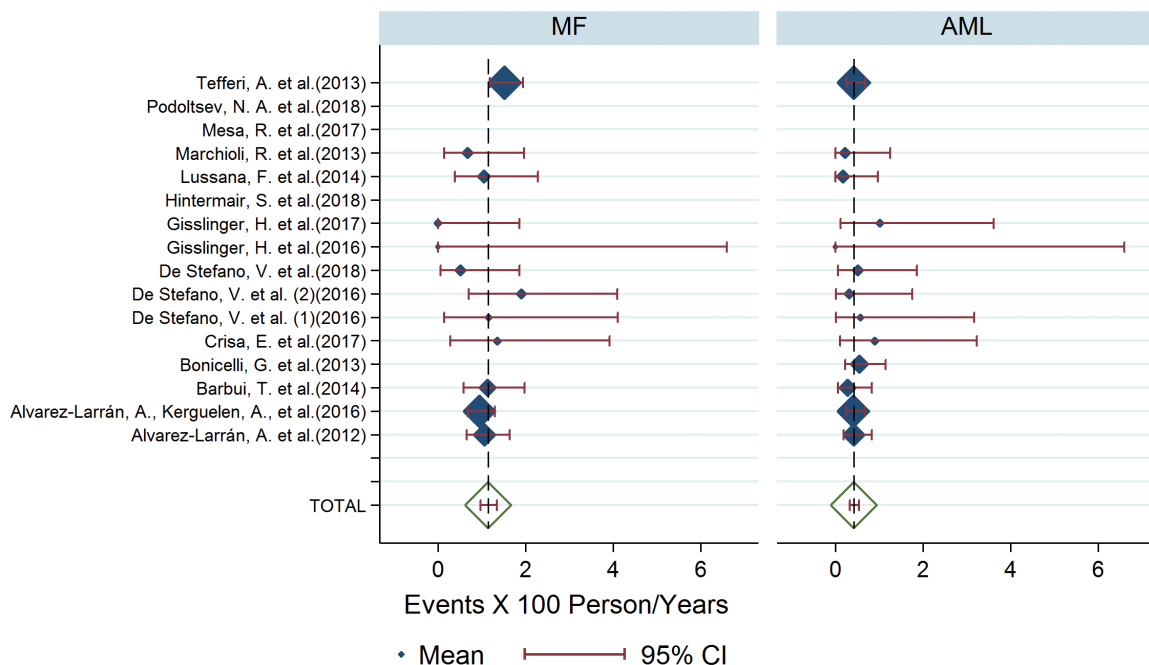
Event heterogeneity and timing

No evidence of excess heterogeneity was found in meta-regression for MF ($P=0.281$) or AML ($P=1.000$) once adjusted for potential confounders, as opposed to mortality and thrombosis, where a small but non-zero amount of heterogeneity was observed despite adjustment. The

distribution of events during follow up as carried out by meta-regression highlighted a significant effect of age on probability of MF and thrombosis (and obviously on mortality), but not of AML (Figure 2 and *Online Supplementary Table S1*). This effect is particularly strong for thrombosis. Remarkably, history of thrombosis was not a significant



Graphs by outcome_f



Graphs by outcome_b

Figure 2. Forest plot of outcomes incidences. The incidence is not graphed for Mesa et al. since its very large Confidence Interval could not fit in the plot, but is accounted for in global estimates. Size of markers annotates study sample size. MF: myelofibrosis; AML: acute myeloid leukemia.

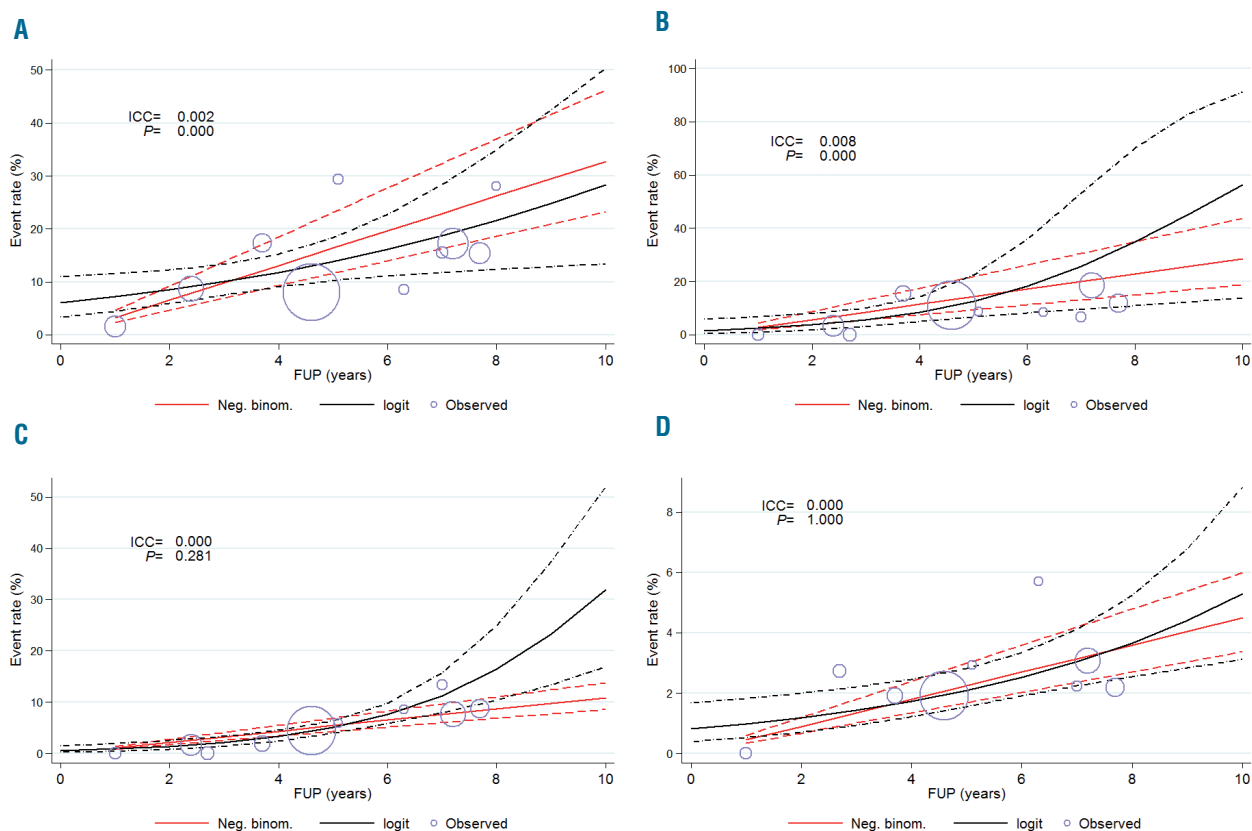


Figure 3. Outcomes incidence during follow up according to logistic Generalized Linear Mixed Model (GLMM) and comparison with negative-binomial model. Dashed lines are 95% Confidence Interval (CI), observed frequencies are plotted in hollow circles of size proportional to sample size in person/years. ICC (Intracluster Correlation Coefficients) and *P*-values of Likelihood Ratio Tests of random slopes are reported. Thrombosis (A). Mortality (B). Myelofibrosis (C). Acute myeloid leukemia (D).

predictor of thrombosis risk in meta-regression.

A logistic model allows for incidence rates to change over time. To confirm that our results do not heavily depend on this assumption, we carried out a sensitivity analysis comparing the logistic GLMM to a negative binomial regression. In a negative binomial regression, yearly incidence is assumed constant over time. Results from the two models were fundamentally in agreement for thrombosis and AML outcomes, whereas for MF and overall mortality, they started diverging after five years of follow up. This indicates that, for practical purposes, thrombosis incidence rate can be assumed to be constant over time, at least up to a 10-year observation period.

Thrombosis incidence

Adjusted estimates for annual incidence of thrombosis are reported in Table 2, globally and stratified by median age and previous thrombosis. Average incidence rate was 3.3% persons/year, ranging from 1.9% at 60 years of age with no history of thrombosis to 6.8% at a median age of 80 years. Estimates increase with median age and are higher in presence of history of thrombosis, but the latter difference is not statistically significant. On the other hand, in a sub-analysis on arterial and venous thrombotic events, previous thrombosis was a highly significant ($P < 0.001$) predictor of incidence of arterial thrombosis, but not of venous.

Hematologic transformations and mortality

Interestingly, incidence of MF and overall mortality increases steeply after five years of follow up according to the logistic GLMM. Estimates of myelofibrosis risk at a median age of 68 years are 0.9%, 5.0% and 33.7% at 1, 5 and 10 years respectively, whereas mortality under the same conditions was 2.4%, 12.6% and 56.2%, but these estimates increase or decrease with age at the start of follow up. Specifically, the odds of MF transformation increase on average 6% (95%CI: 1-11%) for each year of age, while those of mortality increase by 21% (95%CI: 9-33%).

Acute myeloid leukemia evolution, on the other hand, showed a stable incidence over time. According to the negative binomial model, the annual rate of AML transformation was 0.4%, although the logistic model suggests a slight tendency to increase after around eight years.

Bleeding

The number of major bleedings was considered too small for reliable inference. Based on 88 events over 1,485 patients, pooled incidence of bleeding was 1% per year, independently of follow-up duration or antithrombotic therapy, as shown by meta-regression. This estimate was quite consistent, since no evidence of study heterogeneity was found for this outcome, but the small sample size may have limited accurate detection of these effects.

Table 2. Thrombosis incidence by age and history of thrombosis.

	Risk	Average		60 years			Age 70 years			80 years		
		Risk	95% CI	Risk	95% CI	Risk	95% CI	Risk	95% CI	Risk	95% CI	
Average	3.3%	2.2	4.4	1.9%	0.7	3.2	3.6%	2.4	4.8	6.8%	2.6	11.1
No previous thrombosis	3.0%	1.3	4.6	1.8%	0.3	3.2	3.3%	1.5	5.0	6.1%	2.0	10.2
Previous thrombosis	4.5%	1.1	7.9	2.7%	0.6	4.7	5.0%	1.0	8.9	9.3%	0.0	19.7

Second cancer and side effects

The number of second cancers was too small and between-study heterogeneity too high to allow for reliable inference on this outcome. Based on 59 events on 755 patients, pooled incidence of second cancer was 1.7% persons/year (95%CI: 1.3-2.2%), mainly comprising non-melanoma skin cancer.

Only two studies in our selection reported HU-associated adverse events, which does not allow reliable estimates to be made.

Discussion

We systematically collected literature on the benefit-risk profile of HU treatment in patients diagnosed with PV published in the 2008-2018 period. Out of 429 records, we selected 16 reports which allowed retrieval of incidence of specific clinical outcomes in these patients: namely major thrombosis, bleeding, evolution into MF and/or AML, mortality.

Concerning thrombosis, in previous studies, the incidence of thrombosis in high-risk PV patients candidates to cytoreductive treatment was estimated from large patient cohorts including both patients under HU and patients not receiving cytoreduction or taking drugs other than HU,^{34,35} so that the effect of HU was not clearly evidenced. Overall incidence of thrombosis in our population was approximately 3% per year, obtained by pooling together event rates from each study. This estimate does not account for heterogeneity across studies, yet a meta-regression analysis accounting for study-specific confounders, such as median age, antithrombotic therapy, CV risk factors and history of thrombosis, provides a slightly lower estimate (2.8%). This rate does not seem to change over follow-up time, as shown by a comparison between a logistic and a negative binomial model, and depends on age. Based on 2,552 patients and 469 events, estimates of thrombosis incidence rate in patients with a median age of 60, 70 and 80 years under HU treatment are 1.6%, 3.6% and 6.8%, respectively.

Contrary to the commonly held view, we did not find a statistically significant effect of history of thrombosis on incidence of new vascular events. However, this is not surprising in meta-regression analysis, since it is prone to the "ecological bias", i.e. the loss of information that follows from dealing with aggregate data.³⁶ This mirrors the effect of increasing age on the thrombotic risk of the general population observed either for arterial or thrombotic events.^{37,38} However, we highlight the fact that the residual incidence of thrombosis in HU-treated PV patients is still elevated, corresponding to approximately 3-fold higher than that estimated in the general population.³⁷ It is, there-

fore, advisable to promote new pharmacological strategies and to consider our reported thrombosis rate as a benchmark for future comparative studies.

With regard to hematologic transformations, we observed that annual incidence of AML is fairly constant and the cumulative 10-year incidence is approximately 4% (0.4% patients/year).

In contrast, annual incidence of evolution into MF, as predicted by meta-regression, increases steeply after five years of follow up. Therefore, in the 0-5/5-10 years of observation periods, the average annual rate of MF evolution was 1.0% and 5.7%, respectively. Mortality followed a similar pattern as MF, although the divergence between the two meta-regression models was much less remarkable, with an overlap in 95%CI. We retrieved an incidence of second cancer of 1.7% patients per year. However, this may not be a reliable estimate given the limited number of events and the very large between-study heterogeneity for this outcome.

The first major strength of our work is the remarkable sample size we were able to put together, which allowed us to obtain robust estimates for the most relevant outcomes in PV. However, a possible limitation of our analysis is that most reports did not specifically address our study questions, and consequently the relative estimates are based on raw frequency data extracted from descriptive tables or text. Furthermore, we cannot exclude bias in reporting events in individual studies, since most of these were not specifically designed to answer our primary questions. On the other hand, the fact that the studies did not address our question makes publication bias in favor of certain results very unlikely.

A second strength of our approach is that we managed to greatly reduce the issue of study heterogeneity by using adequate statistical methods, namely a logistic GLMM. In this way we mitigated any possible distortion. Furthermore, by adjusting for study-specific co-variables, we were able to account for the effect of the most relevant confounders, which for some outcomes (namely MF and AML) allowed us to reduce heterogeneity to negligible values. Interestingly, for most studies, we were able to extract data on study-specific confounders stratified by treatment; this was to be expected to greatly reduce the effect of "ecological bias", which is a common issue in meta-analysis of aggregated data. Another limitation is that while our methods supposedly reduce "ecological bias", it is probably impossible to entirely remove its effect in a meta-regression on aggregate data. Some known predictors of clinical outcomes, such as history of thrombosis (which is a well-known risk factor for recurrences) turned out to be not significant in meta-regression. This may suggest that, under HU treatment, history of thrombosis is no longer a risk factor for recurrences; but it

may also be a byproduct of using aggregate data as predictors, with subsequent loss of information on individual patients.³⁶

A third strength is that by extracting data on follow-up duration and integrating them in the analysis, we were able to model the time-dependent evolution of outcome risk, thus overcoming a common bias in meta-analysis of binary outcomes, i.e. lack of temporal information. A potential source of bias in this respect is our decision to use median follow-up time when the mean was not available, which can lead to biased risk estimates when the actual distribution of follow-up times in the study is very skewed. However, using the median as an estimator of mean has been shown to be reliable in most cases.³⁹

In conclusion, this meta-analysis provides reliable risk estimates for thrombosis, hemorrhage, evolution to MF and AML, and mortality in PV patients under standard treat-

ment with HU. This can be a valid point of reference for the clinician. It can support the information given to the patient and counseling, and can also help calculate sample size in future comparative clinical trials by providing a reference value. We also prove the feasibility of clinical trials adopting critical efficacy end points such as frequency of cardiovascular events in selected populations. Lastly, we underline the value of a cheap, old and safe molecule as a reliable and accessible resource for those settings where there is a need to reconcile economic sustainability with the right to a qualitative-quantitative life advantage.

Acknowledgments

We wish to thank Franca Boschini (Ospedale Papa Giovanni XIII, Bergamo, Italy), for help with database searches and Gianni Tognoni (FROM research foundation, Ospedale Papa Giovanni XIII, Bergamo, Italy), for useful discussion of the results.

References

- Barbui T, Tefferi A, Vannucchi AM, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. *Leukemia*. 2018;32(5):1057-1069.
- Fruchtman SM, Mack K, Kaplan ME, Peterson P, Berk PD, Wasserman LR. From efficacy to safety: a Polycythemia Vera Study group report on hydroxyurea in patients with polycythemia vera. *Semin Hematol*. 1997;34(1):17-23.
- Barbui T, Vannucchi AM, Finazzi G, et al. A reappraisal of the benefit-risk profile of hydroxyurea in polycythemia vera: A propensity-matched study. *Am J Hematol*. 2017;92(11):1131-1136.
- Gisslinger H, Klade C, Georgiev P, et al. Ropoginterferon Alfa-2b Induces High Rates of Clinical, Hematological and Molecular Responses in Polycythemia Vera: Two-Year Results from the First Prospective Randomized Controlled Trial. *Blood*. 2017;130(Suppl 1):320.
- Knudsen TA, Hansen DL, Ocias LF, et al. Long-Term Efficacy and Safety of Recombinant Interferon Alpha-2 Vs. Hydroxyurea in Polycythemia Vera: Preliminary Results from the Three-Year Analysis of the Daliah Trial-a Randomized Controlled Phase III Clinical Trial. *Blood*. 2018;132(Suppl 1):580.
- Mascarenhas JO, Prchal JT, Rambaldi A, et al. Interim Analysis of the Myeloproliferative Disorders Research Consortium (MPD-RC) 112 Global Phase III Trial of Front Line Pegylated Interferon Alpha-2a Vs. Hydroxyurea in High Risk Polycythemia Vera and Essential Thrombocythemia. *Blood*. 2016;128(22):479.
- Alvarez-Larrán A, Pereira A, Cervantes F, et al. Assessment and prognostic value of the European LeukemiaNet criteria for clinico-hematologic response, resistance, and intolerance to hydroxyurea in polycythemia vera. *Blood*. 2012;119(6):1363-1369.
- Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. *N Engl J Med*. 1995;332(17):1132-1136.
- Harrison CN, Campbell PJ, Buck G, et al. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *N Engl J Med*. 2005;353(1):33-45.
- Parasuraman S, Yu J, Paranaagama D, et al. Cytoreductive treatment patterns among US veterans with polycythemia vera. *BMC Cancer*. 2018;18(1):528.
- Spivak JL. Myeloproliferative neoplasms. *N Engl J Med*. 2017;376(22):2168-2181.
- Barbui T, Ferrari A, Finazzi G, De Stefano, Valerio Vannucchi AM. Benefits and harms of hydroxyurea therapy in polycythemia vera. PROSPERO: International prospective register of systematic reviews. http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018117814 (2018).
- Institute JB. Methodology for JBI Scoping Reviews. Joanna Briggs Institute Reviewers' Manual: 2015 Edition/Supplement. South Aust Aust Joanna Briggs Inst. [Epub ahead of print].
- Seide SE, Rover C, Friede T. Likelihood-based random-effects meta-analysis with few studies: empirical and simulation studies. *BMC Med Res Methodol*. 2019;19(1):16.
- Hamza TH, van Houwelingen HC, Stijnen T. The binomial distribution of meta-analysis was preferred to model within-study variability. *J Clin Epidemiol*. 2008;61(1):41-51.
- Tu YK. Use of generalized linear mixed models for network meta-analysis. *Med Decis Mak*. 2014;34(7):911-918.
- Gisslinger H, Klade C, Georgiev P, et al. Final Results from PROUD-PV a Randomized Controlled Phase 3 Trial Comparing Ropoginterferon Alfa-2b to Hydroxyurea in Polycythemia Vera Patients. *Blood*. 2016;128(22):475.
- Mesa R, Vannucchi AM, Yacoub A, et al. The efficacy and safety of continued hydroxycarbamide therapy versus switching to ruxolitinib in patients with polycythemia vera: a randomized, double-blind, double-dummy, symptom study (RELIEF). *Br J Haematol*. 2017;176(1):76-85.
- Marchioli R, Finazzi G, Specchia G, et al. Cardiovascular events and intensity of treatment in polycythemia vera. *N Engl J Med*. 2013;368(1):22-33.
- Alvarez-Larrán A, Angona A, Ancochea A, et al. Masked polycythemia vera: presenting features, response to treatment and clinical outcomes. *Eur J Haematol*. 2016;96(1): 83-89.
- Alvarez-Larrán A, Kerguelen A, Hernández-Boluda JC, et al. Frequency and prognostic value of resistance/intolerance to hydroxycarbamide in 890 patients with polycythemia vera. *Br J Haematol*. 2016;172(5): 786-793.
- Bai J, Xue Y, Ye L, et al. Risk factors of long-term incidences of thrombosis, myelofibrosis and evolution into malignance in polycythemia vera A single center experience from China. *Int J Hematol*. 2008;88(5):530-535.
- Barbui T, Thiele J, Gisslinger H, et al. Masked polycythemia vera (mPV): results of an international study. *Am J Hematol*. 2014;89(1):52-54.
- Bonicelli G, Abdulkarim K, Mounier M, et al. Leucocytosis and thrombosis at diagnosis are associated with poor survival in polycythemia vera: a population-based study of 327 patients. *Br J Haematol*. 2013;160(2):251-254.
- Crisa E, Cerrano M, Beggiato E, et al. Can pegylated interferon improve the outcome of polycythemia vera patients? *J Hematol Oncol*. 2017;10(1):15.
- De Stefano V, Carobbio A, Di Lazzaro V, et al. Benefit-risk profile of cytoreductive drugs along with antiplatelet and antithrombotic therapy after transient ischemic attack or ischemic stroke in myeloproliferative neoplasms. *Blood Cancer J*. 2018;8(3):25.
- De Stefano V, Ruggeri M, Cervantes F, et al. High rate of recurrent venous thromboembolism in patients with myeloproliferative neoplasms and effect of prophylaxis with vitamin K antagonists. *Leukemia*. 2016;30(10):2032-2038.
- De Stefano V, Vannucchi AM, Ruggeri M, et al. Splanchnic vein thrombosis in myeloproliferative neoplasms: risk factors for recurrences in a cohort of 181 patients. *Blood Cancer J*. 2016;6(11):e493.
- Hintermair S, Zwickl-Traxler E, Pecherstorfer M, et al. Evaluation of vascular events in patients with myeloproliferative syndromes and mutations of either the

- januskinase-2 or calreticulin gene at the university hospital Krems from 2008 to 2015. *Oncotarget*. 2018;9(9):8450-8462.
30. Linardi CCG, Pracchia LF, Buccheri V. Diagnosis and treatment of polycythemia vera: Brazilian experience from a single institution. *Sao Paulo Med J*. 2008;126(1):52-57.
 31. Lussana F, Carobbio A, Randi ML, et al. A lower intensity of treatment may underlie the increased risk of thrombosis in young patients with masked polycythaemia vera. *Br J Haematol*. 2014;167(4):541-546.
 32. Podoltsev NA, Zhu M, Zeidan AM, et al. The impact of phlebotomy and hydroxyurea on survival and risk of thrombosis among older patients with polycythemia vera. *Blood Adv*. 2018;2(20):2681-2690.
 33. Tefferi A, Rumi E, Finazzi G, et al. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. *Leukemia*. 2013;27(9):1874-1881.
 34. Marchioli R, Finazzi G, Landolfi R, et al. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol*. 2005;23(10):2224-2232.
 35. Barbui T, Vannucchi AM, Carobbio A, et al. Patterns of presentation and thrombosis outcome in patients with polycythemia vera strictly defined by WHO-criteria and stratified by calendar period of diagnosis. *Am J Hematol*. 2015;90(5):434-437.
 36. Lambert PC, Sutton AJ, Abrams KR, Jones DR. A comparison of summary patient-level covariates in meta-regression with individual patient data meta-analysis. *J Clin Epidemiol*. 2002;55(1):86-94.
 37. Raskob GE, Angchaisuksiri P, Blanco AN. Thrombosis: a major contributor to the global disease burden. *Arter Thromb Vasc Biol*. 2014;34(11):2363-2371.
 38. Wendelboe AM, Raskob GE. Global burden of thrombosis: epidemiologic aspects. *Circ Res*. 2016;118(9):1340-1347.
 39. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol*. 2005;5:13.