No correlation of intensity of phlebotomy regimen with risk of thrombosis in polycythemia vera: evidence from European Collaboration on Low-Dose Aspirin in Polycythemia Vera and Cytoreductive Therapy in Polycythemia Vera clinical trials

The natural history of polycythemia vera (PV) is marked by arterial and venous thromboembolism and evolution into myelofibrosis and/or acute myeloid leukaemia/myelodysplastic syndrome.

One of the major goals of treatment is to reduce the thrombotic events which account for 40% of causes of mortality. Several trials have investigated the outcomes of various therapeutic approaches and underscored the importance of therapeutic phlebotomy (TP). The first Polycythemia Vera Study Group (PVSG) prospective trial randomized 431 patients to receive either TP alone, or TP combined with chlorambucil or radioactive phosphate ($^{32}$P), and patients were then followed for 20 years. The median survival was 13, 11 and 9 years for patients randomly assigned to treatment with TP alone, radioactive phosphate, and chlorambucil plus TP as needed, respectively. The study also showed an increased incidence of thrombosis among the group treated with TP alone, especially during the first 3 years (23% compared to 16% in the $^{32}$P treatment arm). However, compared to patients given myelosuppressive therapy, patients who were treated with TP alone, had a lower incidence of hematological malignancies and solid tumors. The authors concluded that TP provides the best overall survival, but at the cost of increased risk of thrombosis during the first 3 years. One interpretation of these unexpected findings was attributed to the occurrence of thrombocytosis, a relatively frequent event after initiating TP. PVSG then conducted a trial in which phlebotomized patients were given high-dose aspirin and dipyridamole with the aim of reducing thrombosis, but the trial failed due to an increased incidence of gastrointestinal hemorrhage. In 2004, the antithrombotic role of aspirin was demonstrated in patients with PV who have no contraindications to such treatment. Currently, hydroxyurea (HU) is recommended in patients who are at high risk of thrombosis, progressive disease, or in those who cannot tolerate frequent therapeutic phlebotomies. Other therapeutic options include treatment with interferon-alpha, or ruxolitinib, as shown in 2 recent randomized clinical trials.

Furthermore, there is now evidence from the Cytoreductive Therapy in Polycythemia Vera (CYTO-PV) randomized clinical trial that therapeutic TP, aimed at keeping hematocrit (HCT) threshold <45%, represents the cornerstone in the therapeutic armamentarium of PV, either alone in low-risk PV or as a supplement to cytoreductive therapy in high risk patients. However, a recent retrospective study of a large cohort of PV patients raised the issue that in patients receiving HU, frequent TP (>3 per year) enhanced the risk of major thrombosis. This finding may be relevant for clinical practice and, if confirmed, may support the use of second-line therapy with JAK2 inhibitors or interferon-alpha to reduce the thrombotic risk associated with higher frequency of TP. Therefore, we reviewed the ECLAP and CYTO-PV database for information on the frequency of phlebotomies and vascular events.

In the ECLAP database, a subgroup of 793 patients (48%) out of 1638 included in the ECLAP study was treated with hydroxyurea for a median follow up of 28 months. In these patients, clinical outcomes, treatments, and laboratory values during the follow up were recorded at follow-up visits at 12, 24, 36, 48, and 60 months. For the purpose of this study, we calculated the total number of TP/time of follow up and the number of TP per year in HU treated patients, and looked at the correlation between frequency of TP and thrombosis. Three groups were created: NO TP (n=313, 39%), 1-2 TP per year (n=340, 43%), 3 or more TP per year (n=140, 18%). Time to first thrombotic event from HU start was calculated in each group, censoring patients with no event at last visit or at time of HU discontinuation, whichever occurred.
first. A total of 79 major thrombosis were observed during the follow up of these patients while receiving HU. As reported in Figure 1, the cumulative incidence of thrombosis was superimposable in the 3 groups prospectively evaluated. In the patients submitted up to 2 TP per year versus 3 or more TP per year, HCT median values were 44.73% (IQR: 41.05% - 47.00%) and 46.03% (IQR: 44.35% - 48.63%), respectively (P=0.102); however, this small difference did not impact the rate of incident thrombosis. Of note, in the patients requiring 3 or more TP per year, only 6 received more than 5 TP on an annual basis and, in these latter cases, the incidence of thrombosis was not different in comparison with those treated with less than 4 TP per year (Log-rank test, P=0.802).

In the Italian randomized CYTO-PV trial, we assessed the benefit/risk profile of cytoreductive therapy with TP or HU aimed at maintaining HCT < 45% versus maintaining HCT in the range 45%–50% in 365 patients meeting

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<th>Table 1. Hazard Ratio (HR) of time to thrombotic events, and 95% confidence intervals (CI), estimated by a multivariable Cox proportional hazard model fitted in the whole cohort (column A) and among patients treated with hydroxyurea alone or in combination to phlebotomies (column B).</th>
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<tr>
<td><strong>N° of TP</strong></td>
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<td>Low-HCT arm</td>
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<td>High-risk category**</td>
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HR: Hazard Ratio; 95% CI:95% Confidence Interval. *The number of phlebotomies (TP) during follow up was included in the model as a time-dependent variable. **Patients were in the high-risk category if they were aged more than 65 years and/or if they had previous thrombosis.
the 2008-WHO criteria of PV. For all patients, exposure to (and dose of) hydroxyurea, as well as exposure to (and number of) phlebotomies, were available every 6 months from randomization until the end of follow up. The arm maintained at HCT target less than 45%, had a significant 4 times lower rate of cardiovascular death and major thrombosis than the arm with a hematocrit target of 45 to 50%. According to the therapy administered during the follow-up period, 3 categories were created: stable patients not requiring TP or HU (n=36), patients on TP alone (n=92), and patients on HU alone or in combination with TP (n=237). As shown in Figure 2, the frequency of TP during follow up in the 365 patients (cohort A) was significantly higher in the low-HCT arm (P<0.000) than in the high-HCT arm. The same trend was documented when analysis was restricted to patients under HU treatment alone or in combination with TP (cohort B) (P<0.000). In contrast, the dose of HU was not statistically different in the two arms of the study. Thus, more TP were needed to keep the threshold HCT<45% and, in this way, 3-fold fewer thrombotic events were registered.

The effect of frequent TP on the risk of thrombosis was estimated by a multivariable Cox proportional hazard model, adjusted for HCT arm and high-risk category (age 65 years or older and/or previous thrombosis) and fitted in cohort A and in cohort B (Table 1). Since the number of TP changed over time, a TP was treated as a time-dependent covariate (updated in the model every six months), to assess whether the increase in the number of phlebotomies during the follow up was associated with the probability of having a thrombotic event. In the whole cohort (A), for every additional phlebotomy performed, no increased risk of thrombosis was seen (HR=0.92, 95% CI 0.67-1.26). Conversely, patients in the low-HCT arm (with higher median frequency of TP), had a 62% lower risk of thrombosis (HR=0.38, 95% CI 0.17-0.87) if compared with those in high-HCT arm. The same results were obtained in cohort B, in which the HR of low-HCT arm was 0.37 (95% CI 0.13 - 1.04), not reaching the full statistical significance due to the smaller number of available events.

In a separate analysis of the two arms of the trial, the results were similar to those of the whole cohort. In particular, for every additional phlebotomy, the risk of thrombosis did not change either in the low-HCT arm (HR=1.18, 95% CI 0.81-1.73) or in the high-HCT arm (HR=0.71, 95% CI 0.42-1.22). This was also seen in the subgroup of patients receiving HU (in the low-HCT arm: HR=0.72, 95% CI 0.26-1.96 and in the high-HCT arm: HR=0.99, 95% CI 0.61-1.64), confirming the lack of association between the requirement of additional phlebotomies and the risk of thrombotic events.

These results, based on a more powerful analysis of PV patients treated with HU in the settings of controlled prospective trials, do not confirm those recently reported by Alvarez-Larran et al., who showed a correlation between the number of TP and a higher incidence of thrombosis in HU treated patients in an observational cohort. It should be underlined that the median value of HCT in the group treated with 3 or more phlebotomies (46.03%) of the ECLAP study was lower than the median value of hematocrit in the high-HCT arm of the Cyto-PV trial (always above 47.5%), and that this latter value was comparable to the group treated with 3 or more phlebotomies of the Spanish cohort in which higher risk of thrombosis was reported. Clearly, the increased number of phlebotomies is obviously related to the need to reach the target hematocrit level. Thus, it could be argued that the higher risk of thrombosis might be related to an uncontrolled hematocrit value, rather than to the use of phlebotomies per se.

In conclusion, our results indicate that the frequency of phlebotomies in PV patients on HU does not represent a risk factor for future thrombosis in PV patients and do not support the need to shift from HU plus TP to second-line drugs, indirectly reinforcing the fact that a low HCT is the key variable to reduce thrombotic risk in PV patients.

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