Analysis of Risk Factors: The Rationale of the Guidelines of the Czech Hematological Society for Diagnosis and Treatment of Chronic Myeloproliferative Disorders with Thrombocythemia

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ABSTRACT

The rationale of the Czech Hematological Society guidelines for diagnosis and treatment of Philadelphia chromosome-negative myeloproliferative disorders with thrombocythemia (MPD-T) is reviewed. For diagnosis of MPD-T, the classification according to the World Health Organization or to the Rotterdam criteria is preferred because they distinguish true essential thrombocythemia from prefibrotic or early fibrotic idiopathic myelofibrosis and prepolycythemic polycythemia vera. The histopathology-based nosological distinction provided by these classifications yields valuable information on prognosis (including the risks of transition into secondary acute myeloid leukemia and myelofibrosis). Another serious complication in MPD-T is thrombosis (arterial or venous), the main risk factors of which are age, previous thrombosis, platelet counts 350 to 2200×10^9/L (peak at $\sim 900 \times 10^9$/L) and the presence of additional thrombophilic risk factors (hereditary thrombophilia, any hypercoagulable state, cardiovascular disease). The hemorrhagic risk starts increasing progressively at platelet counts $> 1000 \times 10^9$/L. Treatment should be stratified with respect to the thrombotic and hemorrhagic risks. In high-risk patients, thromboreductive therapy is warranted. All of the cytostatic drugs, including hydroxyurea, may be leukemogenic and should be given only to patients >60 years old, whereas anagrelide or interferon α are preferred in younger individuals. In low-risk patients, antiaggregation therapy is sufficient, unless the platelet count exceeds 1000×10^9/L, which

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is another indication for thromboreduction. Thrombapheresis is indicated in thrombocythemia $> 2000 \times 10^9$/L.

KEYWORDS: Myeloproliferative disease, essential thrombocythemia, polycythemia vera, idiopathic myelofibrosis, risk factors, thrombosis, treatment algorithm

In the beginning of the third millennium, a body of conflicting and unresolved issues regarding the diagnosis, prognostic stratification, and management of Philadelphia chromosome-negative (Ph-negative) myeloproliferative diseases with thrombocythemia (MPD-T) was pressing in clinical practice. The first concern was which diagnostic classification to use. Many centers used the diagnostic criteria defined by the Polycythemia Vera Study Group (PVSG)[1] for essential thrombocythemia (ET), even in therapeutic trials. However, the newly elaborated Rotterdam,[2,3] the pathological World Health Organization (WHO),[4–6] and the European Clinical and Pathological (ECP)[7–9] criteria for the diagnosis of ET, polycythemia vera (PV), and idiopathic myelofibrosis (IMF) appeared in sequence and produced a much better nosologic precision of the three MPD-Ts, and also provided prognostic and therapeutic implications.[10–12] It was disclosed that the majority of cases of ET according to the PVSG criteria were in fact unrecognized cases of prefibrotic or early fibrotic stages of IMF.[12] Another conflicting issue was that of the optimal treatment strategies, especially the issue of the indications for thromboreductive and antiaggregation therapies, and the question of whether any prognostic criteria could be helpful for making therapeutic decisions. The last major and unresolved issue concerned the toxicities of the thromboreductive drugs given: the potential leukemogenicity of the traditional cytostatic drug hydroxyurea (HU) and the possible advantages and disadvantages of the newer drugs used in management of MPD-T, such as anagrelide (ANG) or interferon α (IFN). The answers to these questions were unavailable, given the virtual absence of large prospective or randomized studies.

In 2002, two Czech centers, based on their experience with ANG, advocated similar attitudes toward the treatment strategies in ET or MPD-T at the Czech and Slovak Hematological Congress.[13,14] This precipitated formation of a Czech multi-institutional working group on MPD-T, which focused largely on the above-mentioned problems. The result of the joint effort was the formulation of consensus diagnostic and treatment guidelines (operationally called the Czech MPD-T guidelines), which were approved by the Czech Hematological Society of the J.E. Purkyně Czech Medical Society in 2005 and were published in a Czech journal the same year.[15,16] Herein, we explain the starting points and the rationale of these guidelines.

CONSIDERATIONS ON THE DIAGNOSTIC CRITERIA OF MPD-T

Diagnostic Relevance of the Rotterdam, WHO, and ECP Classifications

For several decades, ET has been diagnosed according to the PVSG criteria, the last revision of which was published in 1997.[1] PVSG has earlier elaborated criteria for diagnosis of PV, which are still generally accepted.[17,18] There are numerous schemes to diagnose overt IMF; the Italian guidelines published by Barosi et al[19] have gained quite a wide acceptance. The PVSG criteria[1] make ET a diagnosis by exclusion, and attempt to exclude cases with secondary (reactive) thrombocytosis and other MPDs, based on recognition of some specific positive criteria of the diseases to be excluded (Ph chromosome for chronic myeloid leukemia, the increased red cell mass or hematocrit for PV, excessive collagen fibrosis for IMF) and some nonspecific criteria to exclude secondary thrombocytosis (e.g., in inflammation or in iron store deficiency), but do not offer a single criterion of positive recognition for ET. In sharp contrast, the novel diagnostic criteria of MPDs, elaborated by Michiels et al[2] and the European Working Group on MPD as the Rotterdam, the Thrombocythemia Vera Study Group,[3] and the new ECP[7–9] criteria (as the extension of the pathological WHO[4–6] criteria) are based primarily on a positive recognition feature of each MPD subtype (i.e., on bone marrow histopathology). It has been shown by Thiele et al[10,11,20,21] that histopathology may distinguish between ET, PV (including its prepolycythemic stage), and IMF (including its prefibrotic and early fibrotic stages IMF-0 and IMF-1), and, in addition, it may even distinguish cases with secondary thrombocytosis or erythrocytosis.

A similar attitude to histopathological evaluation of bone marrow biopsies was also reported by another German group from Hannover.[22] The older PVSG criteria for ET[1] recommended marrow biopsies as well. However, in comparison to the Rotterdam,[2,3] WHO,[4–6] or ECP[7–9] classifications, they emphasized neither the fine differences in morphology of the megakaryocytes and their spatial relationships, nor the varying bone marrow cellularity patterns, which collectively allow distinction among the respective nosologic entities within MPD-Ts. In other words, bone marrow histopathology, as interpreted by the PVSG group criteria for ET, could not positively identify true ET cases, and thus

the PVSG-defined ET included cases with prefibrotic and early fibrotic cases of IMF, as well as prepolycythemic latent stages of PV.[9,12] Indeed, Thiele and Kvasnicka[12] have shown that two thirds of the PVSG-defined ET cases are in fact cases of early IMF according to WHO classification; a smaller proportion of them (< 5%) may be even cases of prepolycythemic PV.[21]

Another important observation of the same authors[12] is that virtually all cases of unclassifiable MPD according to PVSG criteria were classified as early IMFs according to WHO criteria.[12] It should be emphasized that the new ECP/WHO criteria[4–9] enable the precise nosologic definitions of the divergent MPD entities from their onset. However, it is important to note that these novel ECP or WHO criteria have not yet reached general acceptance.[23] The results of German pathological schools in Cologne and Hannover have not been tested and validated by studies outside Germany, and the pathological WHO definitions are currently used mainly in Germany and Austria.

Prognostic Relevance of the New ECP/WHO Classifications

ET, early proliferative stages of IMF, and early prepolycythemic PV may not differ in their initial clinical presentation.[9] However, precise distinction between ET, IMF, and PV in their early stages on the basis of WHO[4–6] or ECP[7–9] classification is mandatory in view of their contrasting prognostic features.[11,12,24] The recent survival analysis from Cologne has shown a 8.9% shortening of life expectancy in patients with ET, which contrasts with 21.6%, 32.3%, and 37.5% in prefibrotic, early fibrotic, and overt stages of IMF (IMF-0, IMF-1, and IMF-2,3), respectively.[12] Patients with stage IMF-0 and IMF-1 have a projected median overall survival of 129 months and transit into overt IMF, whereby hematopoietic stem-cell transplantation should be considered in younger individuals with disease progression.[24] The diagnosis of MPD-T, when performed according to the Hannover or Cologne recommendations, yields important information on the biological behavior of the disease: it has been shown that in true ET, there is minimal tendency (if any) of evolution to IMF and virtually no cases transform into secondary acute myeloid leukemia (s-AML), which is contrary to the inherent propensity of IMF to stepwise increases of the grade of fibrosis, and to progress into s-AML.[12,22,25–27] We suggest that the major life-threatening complication of true ET is thrombosis, but not s-AML or myelofibrosis. Of note, the diagnosis of true ET is not consistent with more marked splenomegaly.[26,28]

It should be stressed that the above-mentioned survival estimates were made in patients, the majority of whom were treated with antiaggregants and with cytoreducing drugs. Therefore, they by no means reflect the true natural histories of ET or IMF, which are, in fact, largely unknown. The same applies to survival estimates in older studies of ET (the majority of which used the PVSG criteria for diagnosis). Life expectancy was slightly reduced in all studies, but was not very far from normal.[29–35] The Dutch studies described life expectancy in ET (treated with low-dose aspirin) as "normal"[33] or "close to normal,"[32] demonstrating a 15% loss in 10 years, which was similar to results from the study from Turin, in which a 4-fold higher risk of death compared with an age-matched healthy population was observed.[34]

Interestingly, the recent WHO[4–6] or ECP[2,7–9,36] criteria shift the prognostic relevance of diagnoses of ET and IMF (the former now being cleared from cases with relatively adverse outcomes, the latter being extended by addition of relatively more favorable cases). Therefore, seemingly paradoxically, by WHO or ECP definitions, both of the diagnoses have better overall prognosis than by the older criteria of PVSG[1] for ET or classic IMF, as defined by Barosi et al.[19]

Implementation of the New ECP/WHO Classifications in the Czech Guidelines

Given the above-mentioned prognostic potential of ECP or WHO diagnostic criteria, we believed there was no other choice than to adopt them into the Czech MPD-T guidelines. Therefore, we insist that bone marrow biopsy be performed as part of the diagnostic work-up, and we allow patients to be excluded from the requirement (and using the PVSG criteria) only if they are in very poor clinical condition, because the bone marrow biopsy procedure (when the patient commonly is under intensified analgosedation) might adversely affect their clinical state. The diagnostic biopsies ought to be performed in untreated patients because therapy (e.g., with HU, busulfan, IFN, or ANG) affects bone marrow histology.[37,38]

Adopting WHO[4–6] or ECP[7–9] criteria also brings about several obstacles. The major one is that although we are now equipped with a new classification, both our clinical experience and the literature of the past decades is largely based on the older classification. We have to be aware that the majority of cases with ascribed diagnosis of ET were actually cases of early IMF.[12] Therefore, most of the previous reports would need reinterpretation on the basis of ECP or WHO classifications. Because it is not realistic to expect this to happen, we advocate the term MPD-T for patients in whom the distinction between early IMF and ET is not clearcut with regard to the ECP/WHO systems. Another difficulty with adoption of the ECP and WHO criteria lies in the expertise required to perform histopathological examination. The new classifications were developed by the highly experienced pathologists from Cologne who have reviewed at least hundreds or rather thousands of

biopsies. However, pathologists with less expertise may have difficulty trying to reproduce their results. Thus, we have to insist on a second reading of the bone marrow specimens, which has to be performed in larger centers that have personnel with adequate experience. We are aware that it may take several years of training to achieve fully reproducible results. On the other hand, we deem that the possible benefits of introducing ECP/WHO criteria are worth the effort required.

ANALYSIS OF RISK FACTORS IN MPD-T

First, it should be noted that the analysis of the risk factors in the literature, at least of the last two decades, applies to patients receiving some kind of treatment. Therefore, it is extremely difficult to ascertain the natural history of the diseases, and especially so against the background of the novel ECP/WHO classifications, which have been developed in an era when leaving the patients without treatment would be considered unethical (given that efficient therapies were already at hand). Because the natural history of the diseases are not known, all of the assumptions about the risk factors are influenced by the treatments given.

The Paradoxical Risks of Both Thrombotic and Bleeding Complications as a Function of the Platelet Count and Duration of Thrombocythemia

The hallmark of ET is the paradoxical predisposition to hemorrhage and thrombosis, which was referred to by Dameshek as "double jeopardy."[39] In 1970, Dawson and Ogston[40] realized that PV patients suffering from thrombotic and hemorrhagic complications had higher platelet counts than patients without these complications, although the hematocrit values were the same in the two groups. The importance of the platelet counts was confirmed in a larger study of 101 consecutive patients with various MPDs by Barbui et al[41] in 1983. In the late 1990s, a meta-analysis of 809 patients with MPD-T from 11 studies was performed by Griesshammer et al[42]; Michiels et al[36,43,44] further elucidated the relations of the risks of both thrombosis and hemorrhage to platelet counts in the same cohort in detail. Whereas the risk of bleeding becomes imminent at the platelet count of 1000×10^9/L and increases with the increasing platelet count (Fig. 1), thrombotic complications may occur in patients with platelet counts of 350 to 2200×10^9/L, and are more likely at 500 to 1900 (peaking at 900) $\times 10^9$/L platelets (Fig. 1).[36,43,44] It is clear that the paradoxical combination of both risks (i.e., thrombosis and hemorrhage) is imminent at platelet counts of $\sim$1000 to 2200×10^9/L.[36,43,44] There was a very low incidence ($< 5\%$) of deep vein thrombosis among the patients analyzed.

The majority of thromboses were microcirculatory disturbances (erythromelalgia or acrocyanosis in 30 to 40% of patients) and arterial thromboses (strokes, transient ischemic attacks [TIAs], myocardial infarctions, peripheral arterial occlusions in $\sim$20 to 25% of patients).[42,44] In contrast, in a much smaller Czech single-institution study of 43 consecutive MPD-T patients,[45] the majority of thromboses seen were venous (in 16% of all patients); the arterial thromboses occurred less frequently (in 7% of patients). In the majority of patients, these events represented the first symptom of the disease. Of note, the major venous and arterial events occurred more frequently in patients with inherited thrombophilia. Importantly, there are also well-documented cases of thrombosis even at normal platelet counts.[46,47]

The study of Cortelazzo et al[48] in a cohort of 100 historical patients has shown that the thrombotic risk is a function of the MPD-T patient's exposure time to elevated platelets. This is an important phenomenon and we suspect it is neglected frequently in current treatment strategies. The importance of the elevated platelet counts has been confirmed in therapeutic trials with agents reducing their number or function: in another study reported by Cortelazzo et al,[49] it was shown in a randomized trial in 114 high-risk MPD-T patients that reducing platelet counts using HU resulted in prevention of thrombosis, even on a background of antiaggregation therapy by acetylsalicylic acid (ASA) or ticlopidine administered in nearly 70% of patients in both the HU-treated and the HU-untreated arms of the study.

A French retrospective study of ET patients has also shown that control of platelet counts by means of therapy with radiolabeled phosphorus (^{32}P), busulfan, or HU can prevent large vessel thrombosis.[31] The other line of evidence to support the importance of the elevated platelet counts in MPD-T is the efficacy of therapy blocking their function (i.e., antiaggregation therapy). It has been shown that low-dose ASA (40 to 100 mg daily) may prevent recurrence of thrombosis in MPD-T.[32,33] Low-dose ASA lowered the incidence of thrombosis in PV patients (however, only a minority of them had elevated platelets) in the large randomized European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) study of 518 patients.[50]

The bleeding diathesis in MPD-T is probably not due to impaired platelet function[41] (although platelet dysfunction in terms of both increased and more frequently decreased function was commonly reported[30,31,51,52]), but rather due to an acquired von Willebrand disease caused by proteolytic reduction of large von Willebrand factor (VWF) multimers. There is an inverse relationship between VWF levels and platelet counts. The VWF large multimer deficiency appears at platelet counts of 1000 to 1500×10^9/L and increases

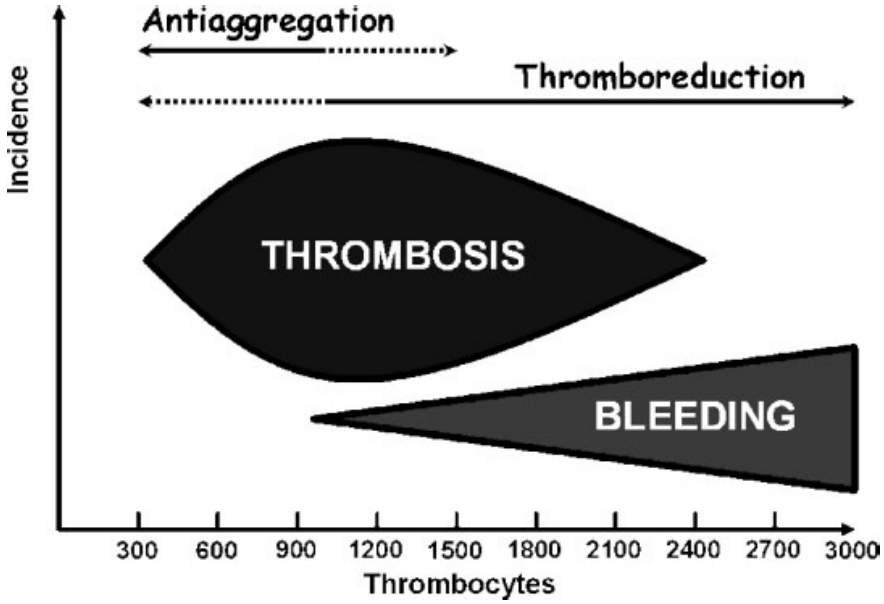

Figure 1 Relative incidence of thrombosis and hemorrhage in essential thrombocythemia as a function of platelet count. It is based on a meta-analysis of 809 patients from 11 studies. Note the evident paradox of both of the complications at platelet counts 1000 to 2200×10^9/L. The main therapeutic recommendations (i.e., thromboreduction and antiaggregation strategies) are depicted. (From Michiels JJ, Kutti J, Stark P, et al. Diagnosis, pathogenesis and treatment of the myeloproliferative disorders essential thrombocythemia, polycythemia vera and essential megakaryocytic granulocytic metaplasia and myelofibrosis. Neth J Med 1999;54:46–62.)

thereafter.[44,53–55] Naturally, the tendency to bleeding may be worsened by administration of ASA.

Age and Previous Thrombotic Event as Major Risk Factors

The above-mentioned study of Cortelazzo et al[48] from Bergamo has identified other important risk factors of thrombosis in MPD-T: a previous thrombotic event and age older than 60 years. The former risk factor was later confirmed by the Dutch and Spanish MPD-T studies,[56,57] comprising 68 and 148 patients, respectively. The latter factor (age) was confirmed by the Spanish and by another Italian study from Padova,[57,58] but not by the Dutch study.[56] In the study from Turin,[34] at the age cut-off of 55 years, no significant differences in life expectancy were found. In three articles,[33,34,48] neither diabetes, hyperlipidemia, hypertension, nor smoking influenced the incidence of thrombosis. In contrast, in the Spanish study,[57] hypercholesterolemia was a thrombotic risk factor in multivariate analysis. In the Padova study, it was shown that sex stratified by age might also play a role in the incidence of thrombosis.[58] We stress that the evaluated risks in all of these studies apply to treated patients. Thus the Dutch group might have come to the conclusion that age is not a risk factor because their patients received ASA and some of them received thromboreducing drugs in addition.[56] Efficient treatments always temper the differences in prognosis.

Increased Risk of Thromboembolism in MPD-T with Hereditary Thrombophilia

An increasing body of evidence points to the importance of the hereditary thrombophilic factors, which aggravate the risks of thrombosis arising from MPD-T itself. These factors comprise antithrombin, protein C and S deficiencies, as well as the mutation of the prothrombin gene G → A 20210 and the Leiden mutation of factor V in heterozygous or homozygous forms.[45,59–63] In general, in hereditary thrombophilia, more venous thromboses are seen. Even the relatively more benign thrombophilic state, such as carriership of factor V Leiden, increased the risk of thromboembolism (but not of arterial thrombosis) in a cohort of 178 patients with PV and 126 patients with MPD-T.[62] A similar observation in 214 patients with PV and MPD-T, tested for the prothrombin gene mutation, was presented by Gisslinger et al.[63] The Czech study[45] has demonstrated significantly more thromboses (mainly venous thromboses) in patients with an additional, mostly inherited thrombophilic state in MPD-T. Only recently has the presence of these thrombophilias been seriously taken into account in therapeutic considerations, as reviewed by Kessler.[64] Their presence or absence is considered in certain patient subsets in the recently published practice guidelines of the Italian Society of Hematology.[65] The Czech MPD-T guidelines dictate that appropriate coagulation and molecular tests be performed for inherited thrombophilia for the risk-stratification of MPD-T patients.

Additional Thrombophilic Risk Factors Described in MPD-T

In addition to the above-mentioned risk factors of thrombosis, some additional parameters were studied and were determined to increase the thrombotic risk in MPD-T patients. They include the presence of antiphospholipid immunoglobulin M antibodies[66]; increased expression of selectins P and E, thrombospondin, and vascular endothelial growth factor; immunohistologically detected decreased expression of the thrombopoietin receptor c-mpl, and in one of several studies, also clonality proven by the human androgen receptor assay (HUMARA).[67–70] Clonal mutations of the *JAK2* tyrosine kinase gene do not seem to play an important prognostic role, at least on the background of PVSG-defined ET.[71] The levels of homocysteine were identified as an additional risk factor in one study,[61] but not in others.[45,72] The Czech MPD-T guidelines take into account the presence of antiphospholipid antibodies.

Other Risks of Thrombosis Known in the General Population: Hypercoagulable States

Many divergent stimuli may lead to a hypercoagulable state, which in turn may lead to thrombosis. Among these, the physiological hypercoagulable state—pregnancy—is of specific concern. In gravidity in the setting of MPD-T, there is an imminent risk of fetal loss (due to placental thrombosis and infarction), as well as a risk of thromboembolism and bleeding on the maternal side.[73–75] Hypercoagulable states are frequent in various pathological conditions, including widespread cancer and infection, major trauma, burns, surgery, and implantation of central catheters.[75–77] Finally, increased levels of some coagulation factors (F), such as FVIII, VWF, fibrinogen (and dysfibrinogenemia), as well as decreased FXII levels, may cause thrombosis in the general population.[78,79] Although, to our knowledge, the majority of these issues have not ever been studied in the setting of MPD-T, it is only logical that in MPD-T these factors must increase the risk of thrombosis to a higher extent, in analogy to the already recognized risk of pregnancy.[73] Our recommendation is that these factors be taken into account in the risk stratification of MPD-T patients.

The Risk Categories of Patients

Most of the published treatment algorithms work with three risk categories of patients: low, intermediate, and high risk.[9,23,56,65,80] However, reviewing the source literature, we have found no firm substantiation for the intermediate-risk category (usually defined as platelet counts from 1000 to 1500×10^9/L and no previous thrombohemorrhagic event, but possibly some additional vascular risk). In view of the fact that the major therapeutic decision is simply either to administer or not administer thromboreductive therapy, we found this category unnecessary. A similar speculation has appeared in the recent review by Harrison.[23]

THE RISK OF TRANSITION OF MPD-T INTO s-AML

Ph-negative MPD-Ts are a heterogenous group of diseases with a divergent prognosis. The overall prognosis is largely dependent on the natural tendency of the respective MPD-T to transform into s-AML (a state analogous to blastic crisis in chronic myelogenous leukemia [CML]). PVSG-defined ET transforms into s-AML only rarely (in ~1–10% of cases), perhaps depending on the therapy administered.[9,29–31,81–83] However, in such cases, a missed diagnosis of IMF according to ECP/WHO criteria should be ruled out,[9] given that ET diagnosed with special attention to megakaryocytic appearance in biopsies does not transform into s-AML.[22] We are not aware of a study that has evaluated progression of ET in s-AML in untreated patients, and the chance to obtain this information on the natural history of ET in the future is virtually nil, given that it would be unethical to perform studies that offer no treatment.

Conversely, the tendency to progress into s-AML is considerably higher in IMF: 5 to 20%.[9,28,83,84] It is unclear whether IMF must inevitably lead to s-AML in the same manner as in the chronic phase of CML; it could be speculated that progression into s-AML may be the final stage of the biological development of IMF. It might be that the relatively low percentages (~10%) of leukemic transformation given in the older literature[85] may result from the fact that the patients died of other IMF complications before s-AML could develop. The propensity of PV to progress into s-AML is intermediate in comparison with that of ET and IMF.[9,83] There are two possible scenarios of transformation of PV into s-AML. The first is direct transition; the other is that postpolycythemic (spent phase) IMF develops, and only then does the blastic phase of the disease develop in the form of s-AML.[9,83,85,86]

Therapy-Related Increase of Risk of Progression into s-AML

As noted, the disease-defined natural risk to progress into s-AML in the published studies is always shadowed by the fact that the vast majority of patients in proliferative stages of MPD-T receives therapy by cytostatic drugs, which themselves may induce s-AML or secondary myelodysplastic syndrome (s-MDS). There is no doubt that many of these drugs are indeed leukemogenic.

No controversy in this respect exists about ^{32}P or alkylating agents, including chlorambucil or melphalan.[87–89] However, the leukemogenic potential of the most frequently used cytoreductive drug at present, HU, is still a matter of ongoing controversy.

IN VITRO DATA ON LEUKEMOGENICITY OF HU

The leukemogenic potential of HU has been studied in various in vitro models. We are aware that it is sometimes difficult to translate these results into clinical practice. However, they should serve as a warning to HU enthusiasts. The molecular effects of HU are explained eloquently in a review by Yarbro,[90] which was more recently cited by Hong and Erusalimsky.[91] Although according to one publication, HU did not induce the sister chromatid exchange (in contrast to alkylating agents),[92] another study could demonstrate this phenomenon. However, it was grossly dependent on the experimental system chosen.[93] Importantly, HU is an inhibitor of ribonucleotide-diphosphate reductase[90,94] and is mutagenic in bacteria[95]; prevents DNA repair and increases genetic instability and genotoxicity.[94,96,97] Insufficient DNA repair is one of the most important leukemogenic mechanisms. When this drug is administered to younger individuals, both the patient and the physician have to be aware of its teratogenic effects and of possible spermiogenesis-reducing effects, as seen in experimental animals.[91,98]

IN VIVO DATA ON LEUKEMOGENICITY OF HU

Clinical research aimed at determining the leukemogenic potential of HU is difficult, given the long period of time between diagnosis of MPD and its transition into s-AML. The warning that HU might be leukemogenic was given by several authors studying various forms of MPD.[86,99–102] None of the cited observations on MPD-T used histopathological evaluation according to the ECP/WHO criteria. There might have been varying proportions of patients with true ET, IMF stage 0 or 1, along with PV (i.e., diseases with a variable tendency of progression into s-AML). With the knowledge that the majority of cases within the PVSG group-defined ET were in fact early IMFs[12] (with the highest propensity to leukemic transformation among all MPD-Ts), valuable information on this issue might be rather inferred from studies of PV patients, which have a lower propensity of transformation into s-AML.[83]

In a large cohort of 292 patients with PV, a French randomized study has revealed induction of s-AML or s-MDS following pipobroman or HU therapy in 10% of patients after 13 years from diagnosis; in addition, there was a higher tendency to transition into IMF in the arm with HU therapy than with pipobroman.[86] The incidence of s-AML rapidly escalated between 10 and 16 years of follow-up. Another French group has shown a relatively high incidence of s-AML/s-MDS (4.5% of 357 patients studied), frequently associated with deletion of 17p and loss of the *TP53* gene in largely unbiopsied ET patients treated with HU.[103] Two other similar cases of deletions of 17p with onset of s-AML in PV patients were reported from Slovakia.[104]

The most relevant information on leukemogenicity of HU is expected to come from prospective or randomized studies of patients diagnosed according to the ECP/WHO guidelines. One such study (the ANAHYDRET trial conducted by AOP Company; Vienna, Austria) is in progress in ET, but the median follow-up is currently too short to draw any conclusions. The Italian-based international prospective study ECLAP in 1638 PV patients has shown that exposure to ^{32}P, busulfan, and pipobroman, but not to HU alone, had an independent role in producing an excess risk for progression to s-AML compared with treatment with phlebotomy or IFN.[105] Altogether, 23 cases of AML according to WHO have been diagnosed, with a median time of 8.4 years from diagnosis of PV; of 793 patients receiving HU monotherapy at registration, six developed AML. The ECLAP study has registered patients with variably long histories of PV (from 0 to nearly 40 years; median, 3.5 years according to the one publication,[105] and 4.9 years according to the other publication,[106] both of which were issued in April 2005). Notably, the only information about the given therapies (that were exclusively up to the decisions of the participating centers) was related to the date of accrual of the patients; no data are provided on the duration of the respective available treatments, so that theoretically, the exposition of the patients to HU might have ranged from 1 day to nearly 40 years. The patients had a follow-up of 2.7 to 2.8 years (medians are given in the two articles[105,106]) from the time of their enrollment. There are no data provided about the therapy administered during the follow-up period. If we would assume that the patients that were described as being treated by HU monotherapy (at the time of accrual) had been treated by HU since the date of diagnosis until the very end of follow-up (which need not be the case; they might have received HU for 1 day only), the cumulative exposure time would be 6.3 to 7.6 years. This period is still insufficient in view of the data of the French PV study group, which has reported excess s-AML/s-MDS only after 10 to 16 years from diagnosis.[86] It is clear that the design of the ECLAP study offered a very limited chance to address the issue of leukemogenicity of HU, although it was able to demonstrate this treatment sequel of the even more leukemogenic drugs, such as ^{32}P. A consensus already exists that sequential use of busulfan and HU is associated with a higher incidence of s-AML.[107,108] To conclude on this issue, we suggest that leukemogenicity of HU has never been ruled out with certainty.

TREATMENT OF MPD-T

Although the complex treatment strategies in the main types of MPDs differ significantly in the full-blown cases (based on the differences of laboratory findings, and varying clinical manifestations and prognoses), we have to cope with the phenomenon of thrombocythemia, if present, in each of them, especially in the early stages of the respective diseases. From this point, the therapeutic strategies currently are analogous, as also the risks of thrombocythemia are analogous (but perhaps not equal), whether in patients with ET, IMF, PV, or eventually, CML. It remains to be seen in the future whether we will have to look to the specific features of some MPD nosological entities, or cases within the defined entity (e.g., leukocytosis or leukocyte activation), which may activate in turn the hemostatic system and endothelium cells.[109,110] Leukocytosis may be on average slightly higher in early stages of IMF than in ET, although relatively more cases of thrombosis were documented in ET (and even more in PV) than in IMF.[11]

There are two ways to combat the elevated platelet counts in MPD-T. The first possibility is to lower the counts by means of thromboreductive therapy; the other is to weaken their function by means of antiaggregation therapy. Briefly, among the cytoreductive drugs, the cytostatic and potentially leukemogenic drug HU[111] is used most frequently. Two newer drugs are definitely nonleukemogenic: the immunomodulating drug with antiproliferative potential (recombinant IFN,[112] including its pegylated long-acting form, PEG-IFN),[113] and the imidazoquinazoline derivative, ANG, which is the only agent that affects the megakaryocytic lineage selectively.[114,115] In cases with a grossly elevated number of platelets, thrombapheresis may be practiced successfully,[39] although the effect is only transient. Within the antiaggregants, low-dose ASA (40 to 100 mg daily) plays a dominant role in ET,[32,33] similar to its role in PV, whereby the antithrombotic efficacy has been proven in a randomized, double-blind, placebo-controlled ECLAP trial.[50] Especially the digestive tract-friendly formulations may be of value for long-term management. Only in patients with intolerance or contraindication to ASA, such as ticlopidine, clopidogrel, or indobufen, should other drugs be tried with caution.

To date, there is only one major comparative study of the cytoreductive drugs published. HU and ANG were randomly administered in high-risk ET defined by PVSG criteria in the large British Medical Research Council PT1 trial in 809 patients, ASA was used in addition in both arms.[80] This study has come to a series of conclusions: more arterial thromboses (not major events, but exclusively cerebral TIAs) and fewer venous thromboses were documented in the ANG arm, as well as more serious bleeding events and more transitions into myelofibrosis.

However, this study has many drawbacks that substantially hamper the huge effort taken. First, half of the patients were pretreated with ASA and one third of the patients were pretreated with cytoreductive drugs (HU, busulfan, a minority even with ^{32}P). Second and most prominent, the median age was older than 61 years in both arms (in contrast, the Czech MPD-T guidelines do not recommend ANG for first-line treatment in patients older than 60 because of the possibility of cardiovascular problems and cardiotoxicity[91]). Third, patients were diagnosed according to PVSG criteria, so that the majority of them might have had early-stage IMF or early-stage PV rather than ET according to the ECP/WHO criteria.[4–9] This may have affected the nosological precision of evaluating the rates of transition into myelofibrosis and s-AML. However, it must be realized that the PT1 study was designed before the time when the ECP/WHO criteria were published.

Fourth, the dosage of ANG was suboptimal, starting only with 1 mg daily, with possible dose escalations of 0.5 mg in weekly intervals, so that patients requiring higher dosage (e.g., 4 mg daily) for effective platelet control received it only in the 7th week of treatment. (The starting dose in the original US-based Anagrelide Study Group trial was 2 mg daily.[114]) Indeed, there were higher platelet counts reported within the first 6 months of therapy in the ANG arm (and no difference following 9 months). We know well from the Cortelazzo et al study[48] that the risk of thrombosis depends on platelet counts and on the time of exposition to elevated platelet counts.

Fifth, the number of hemorrhagic events was understandably high if patients with platelet counts as high as $> 1000 \times 10^9$/L might have received ASA, although this was not encouraged (it was not reported how many of the patients actually received ASA in that situation; moreover, half of them were pretreated by ASA). In the Czech MPD-T guidelines, ASA is contraindicated (with minor exceptions) in elderly patients with $> 1000 \times 10^9$/L platelets.

Sixth, there is no analysis of the thrombotic and hemorrhagic events with respect to the platelet counts at which they occurred, although this is a critical feature of the events.

Seventh, patients with a previous history of venous thromboembolism (7% and 5% in HU and ANG arms, respectively) did not receive anticoagulation therapy (as would be preferred by the Czech MPD-T guidelines) but rather received ASA.

Eighth, there were excessive death rates in both arms within a median of follow-up of 3.25 years: 58 of 809 (7.1%) patients died, the majority of whom died due to MPD-T complications or progression. At our institutions in Prague and Brno, we have seen only two deaths attributable to MPD-T or sequels of its therapy in 135 registered patients (1.5%), with a median of

4 years of follow-up and treated also with cytoreductive and antiaggregation agents (unpublished data). Perhaps prognostically a very unfavorable subset of patients was selected to enter the PT1 trial. Each of the 138 participating centers delivered on average six patients only (i.e., 1.2 patients per year in the 5-year period of accrual). This may reflect either selection or less experience in the field. The analysis of the Anagrelide Study Group trial has noticed inferior results in smaller centers with less experience of the physicians with ANG.[116]

Ninth and most important, the interpretation of the results and their superficial generalization, "HU plus low-dose ASA is superior to ANG plus low-dose ASA for patients with high risk ET," is inadequate. It appears that the interpretation that HU + ASA may be the optimal treatment for elderly patients would be more accurate in describing the presented results. By no means should the study results be applied to the younger subset of patients, in whom ANG is actually indicated according to the Czech MPD-T guidelines, as well as according to others.[9,65,115,117] It may be of concern how quickly (within 4 months of publication) the conclusions of the PT1 study were adopted as an undisputed evidence in the recent reviews.[23,118,119] In their editorial remarks, Barbui and Finazzi have characterized the PT1 study as "well-designed and well-conducted."[120]

Nevertheless, the PT1 high-risk study has brought interesting observations, and although the study had to be closed (because of an excessive number of events), more in-depth analyses of the obtained data certainly would be fruitful. The relative abundance of transition into myelofibrosis in the ANG + ASA arm deserves attention (although Thiele et al[121] reported no effect of therapies on progression of IMF cases, and on the other hand, HU was suspected to induce more myelofibrosis than pipobroman in the French PV trial[86]). The PT1 study confirms the result of a previous observational study[122] that the combination of ANG + ASA may precipitate minor bleeding events. However, neither of the two studies[80,122] dealt with the exact platelet counts at which the bleeding events had occurred, and both of them used the slow-escalation dosing of ANG, resulting in a more prolonged patient exposure to elevated platelet counts. The other randomized study comparing HU and ANG in ET, ANAHYDRET, is in progress, but it is too early in the study to address leukemogenicity of HU in the years to come. However, the advantage of this study lies in the use of the WHO diagnostic criteria,[117] and the study is devoid of the major pitfalls of the PT1 trial mentioned previously. No results comparing IFN either to HU or ANG have been published, and the Dutch Hematology-Oncology Association (HOVON) study (comparing PEG-IFN with HU)[123] had to be closed prematurely as well.

The Italian Society of Hematology, in collaboration with other Italian societies, was the first to publish their national practice guidelines for therapy of ET in 2004.[65] The guidelines serve as an example of risk-adapted treatment of ET, taking into account the thrombophilic risk factors in certain defined subsets of patients. Importantly, these guidelines also define the therapeutic goal of thromboreductive therapy (achieving $< 400 \times 10^9$/L of platelets). They do not report on the diagnostic procedures. These guidelines advocate ANG or IFN to high-risk patients younger than age 60, and HU to patients older than age 60 years, with respect to the cautionary principle based on "low-grade evidence" that HU may be leukemogenic.

The Czech Guidelines for Diagnosis and Treatment of MPD-T

Given that the Czech group (from seven clinical/academic institutions) basically agreed with the Italian attitude (the risk-adapted principle) of their guidelines, but had some alternative views, they formulated their own consensus recommendations in a series of meetings and discussions. (One institution disagreed and is not on the list of authors.) The recommendations were approved by the Czech Hematological Society and published in a Czech journal in 2005.[15] Herein, we only summarize the main principles and starting points[16] (discussed in the preceding paragraphs).

1. Nosological diagnosis of MPD-T according to the ECP or WHO criteria[4–9] is strongly recommended. We emphasize that trephine biopsy must be performed before treatment is administered. Due to the high level of expertise needed, we insist that the majority of histopathological evaluation be performed in major centers, and second-opinion reading should be routine. Only in patients already pretreated with cytoreductive drugs and when no diagnostic biopsy was performed, and in elderly patients and those in poor clinical condition is the diagnosis according to the PVSG criteria[1] acceptable.
2. The aim of management of MPD-T is to overcome the possible fatal complications and to prevent or alleviate the clinical symptoms. The most important goal is to prevent thrombosis and thromboembolism as the main cause of morbidity and mortality.
3. Treatment must be adapted to the individual patient's risk of thrombosis and major bleeding. Bleeding can be prevented easily by keeping the platelet counts below 1000×10^9/L (or below 1500×10^9/L in the patients younger than 40 years) by using cytoreductive drugs and avoiding antiaggregants at these high counts. The following criteria are recognized as major risks of thrombosis and embolism:
   (a) Age older than 60 years

Table 1 Primary Treatment Algorithm for MPD-T, Based on Individual Risk Estimates

Platelet Count ($\times 10^9$/L)	18–60 Years Asymptomatic/ Negative History of T-E Events, Thrombophilia Negative	18–60 years Symptomatic/ Positive History of T-E events, Thrombophilia Positive	> 60 Years
400–1000	(0) *or* ASA	**IFN *or* ANG + ASA**	(HU*) + ASA
600–1000 progressive†	IFN *or* ANG + ASA	**IFN *or* ANG + ASA**	HU + ASA
1000–1500	IFN *or* ANG *or* ASA‡	**IFN *or* ANG (+ ASA‡)**	HU (+ ASA‡)
1500–2000	**(HU →) IFN *or* ANG**	**HU → IFN *or* ANG**	HU
> 2000	**HU (± TAF) → IFN *or* ANG**	**HU (± TAF) → IFN *or* ANG**	HU
> 2000 + major bleeding	**HU + TAF → IFN *or* ANG**	**HU + TAF → IFN *or* ANG**	TAF + HU
	Standard risk	High risk	

*ASA allowed in very young patients or in older patients with cardiological indication for ASA.
†Progressive thrombocythemia with increments of platelet counts $>200 \times 10^9$/L in 2 months.
‡HU must be given to patients with an additional thrombophilic state; in others it is optional.
MPD-T, myeloproliferative disorder with thrombocythemia; T-E, thromboembolism; ASA, acetylsalicylic acid; IFN, interferon α; ANG, anagrelide; HU, hydroxyurea; TAF, thrombapheresis; (), optional.

(b) Previous thrombotic event
(c) Platelet count ($350–2200 \times 10^9$/L with a peak at 900×10^9/L), as inferred from the metaanalysis made by Michiels et al[44] (Fig. 1), and from the finding that cytoreductive treatment prevents thrombotic complications[49]
(d) Additional risk factors include inherited thrombophilia (protein C and S deficiencies, the Leiden mutation of FV, mutation of the prothrombin gene G → A 20210, antithrombin deficiency). Very high levels of FII and FVIII, as well as low levels of FXII may be taken into account if (optionally) tested. Further recognized risk factors include antiphospholipid syndrome; clinically serious forms of atherosclerosis of the coronary, cerebral, and lower limb arteries; any hypercoagulable state in pregnancy; systemic infection; additional malignancy; and major surgery.

4. Treatment should not be harmful to the patient (the principle of *non nocere*). If we consider the possible leukemogenicity of any cytostatic drug, including HU (as discussed), the drug may be administered for prolonged periods of time only in patients whose life expectancy is not substantially longer than the median time of transition to s-AML (~15 years[86]). Arbitrarily, HU may be given as frontline therapy to patients older than 60 years.

On the basis of the above-summarized principles, a treatment algorithm for primary therapy of MPD-T was established (Table 1). The therapeutic goal of thromboreductive therapy with HU, ANG, or IFN should be the normalization of platelet counts (below 400×10^9/L) in high-risk patients with an indication to thromboreducing agents, especially in those with additional thrombophilic risks. In low-risk patients without additional thrombophilic risk factors (whose indication to cytoreductive therapy was based solely on the excessive platelet count), the goal to reach the counts below 600×10^9/L seems satisfactory. Maintenance treatment is always necessary.

COMMENTS AND EXPLANATIONS FOR THE TREATMENT ALGORITHM

1. We have introduced the category MPD-T with progressive thrombocythemia, based on the experience that patients with increases of platelets $>200 \times 10^9$/L in 2 months will always achieve counts for which thromboreducing therapy is indicated. Early introduction of therapy minimizes the time when they are at higher risk of thrombosis, according to the study showing that the thrombotic risk is dependent on the time of exposure to elevated platelets.[48]
2. The recommended dosages are the following: ASA, 50 to 100 mg daily (or 100 mg every other day); ANG, 0.5 to 5.0 mg/d; IFN, 1 to 30 MIU/wk; HU: 0.5 to 2.0 mg/d.
3. In case of an insufficient effect of these doses or the occurrence of major side effects, IFN may be a substitute for ANG and vice versa. In case of an insufficient effect or slight toxicity or side effects, another drug (of the three thromboreductive agents) can be added in combination, allowing the reduction of the dosage of the first-line drug.
4. The choice between ANG and IFN is left to the treating physician, also considering the preferences of the individual patient (e.g., his or her compliance to self-administer IFN). The decision to use IFN or PEG-IFN is also up to the physician. IFN may be more advantageous in early IMF and PV for several reasons: it may prevent or reduce splenomegaly more effectively,[124] and reduction of leukocyte and erythrocyte counts may also be an advantage in IMF or PV. In true ET, ANG may be the drug of choice

in the younger patients. However, this is dealt with only as a matter of speculation, but not as a valid recommendation.

5. At high platelet counts ($>1500\times10^9$/L, and especially at $>2000\times10^9$/L), traversing the risky state of the "double jeopardy"[39] of both the hemorrhagic and thrombotic risks in parallel[9,36,44] (Fig. 1) as soon as possible is mandatory in order to achieve platelet counts below 1000×10^9/L. At this level, the sole risk is thrombosis, which can be further reduced by addition of ASA. For the purpose of quick thromboreduction, we advocate HU even in younger patients (<60 years); in our experience, its dosing is the most predictable of all three drugs (HU, ANG, and IFN) considered. Thus, with HU, less time is spent compared with the need to titrate the effective dosage of ANG or IFN. Once the younger patient achieves platelet count $<1000\times10^9$/L, we switch from HU to either ANG or IFN.
6. If the patient receiving ANG or IFN therapy has no significant side effects and exceeds the age of 60 years, continued administration of the respective medication is allowed.
7. ASA may be optionally discontinued in low-risk patients if thromboreductive maintenance therapy steadily keeps the platelet counts below 400×10^9/L. ASA is not given to patients receiving anticoagulants (warfarin and similar drugs), which is planned to be life-long therapy in patients with a history of venous thromboembolism.
8. Bleeding has to be managed using ethamsylate, plasma derivatives, and nonspecific agents. Antifibrinolytics or activated coagulation factor concentrates should be avoided or used exceptionally and cautiously. This holds true especially in patients with a history of thromboembolism, given that these therapeutic interventions might provoke its recurrence. Naturally, administration of antiaggregants must be stopped.

CONCLUSION

With the appearance of new drugs (ANG and IFN) in the treatment of MPD-T, and lacking robust randomized studies with the older and novel drugs, it was increasingly difficult to manage MPD-T in practice. The need for some guidelines was evident. Therefore, a group of Czech experts discussed the published literature along with their own experience and came to a consensus. Herein, we have reviewed our understanding of the problem and present practice guidelines that were approved by the Czech Hematological Society. We are aware that in the absence of firm scientific evidence, some solutions are based on expert opinion (e.g., the problem of potential leukemogenicity of HU; and the fact that the well-known thrombophilic risks in the general population will be even higher in the setting of MPD-T). We envision that the situation will soon be even more complex, once the tyrosine kinase inhibitors targeting *JAK2* (or perhaps kinases that are not yet known) become available. Nevertheless, in accordance with Marchioli et al,[125] we look forward to the new scientific evidence in the field to emerge that would necessitate the re-evaluation of these guidelines.

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ABBREVIATIONS

ANG	anagrelide
ECP	European clinical and pathological (criteria)
ET	essential thrombocythemia
HU	hydroxyurea
IFN	interferon-α
IMF	idiopathic myelofibrosis
MPD-T	myeloproliferative disorder with thrombocythemia
PV	polycythemia vera

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