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Approach to the Diagnosis and Management of Essential Thrombocytosis in a Resource-Limited Setting

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ABSTRACT

Essential thrombocytosis (ET) is a myeloproliferative neoplasm (together with polycythemia vera, chronic myelogenous leukemia, and primary myelofibrosis) characterized by clonal proliferation of megakaryocytes, usually due to the presence of *JAK2*, *CALR*, or *MPL* mutations. Whereas a bone marrow aspirate and genetic testing for these mutations are necessary for an accurate diagnosis of ET, in resource-limited settings, these are often either inaccessible or limited by prohibitive costs. A nuanced clinical approach is necessary to diagnose and manage ET in these settings. In this study, we present a case of ET diagnosed and managed in a resource-limited rural setting in Kenya with hydroxyurea and aspirin. We highlight the importance of individualized therapeutic targets, the challenges with dose adjustments in the setting of hydroxyurea-induced neutropenia, and how extrapolated data from the use of hydroxyurea elsewhere may help guide such dose adjustments. Finally, we propose a rationalized approach to treating ET in a resource-limited clinical setting.

INTRODUCTION

Essential thrombocytosis (ET), also called essential thrombocythemia or primary thrombocytosis, is one of the myeloproliferative neoplasms (MPN) whose hallmark is the clonal proliferation of various myeloid cells with varying morphology, maturity, and efficiency. The other main MPNs are polycythemia vera (PV), chronic myelogenous leukemia (CML), and primary myelofibrosis (PMF) (Arber *et al.*, 2016). ET is characterized by sustained megakaryocyte proliferation that leads to increased numbers of circulating platelets, often of various sizes (platelet anisocytosis). Figure 1, panel by Schafer *et al.*, shows the appearance of ET on a peripheral blood film and on a bone marrow assay. (Tefferi & Pardanani, 2019). Approximately 85-90% of patients with MPNs will demonstrate mutually exclusive mutations in the *JAK2* (60-65%), *CALR* (20-25%), or *MPL* (5%) genes (Cazzola & Kralovics, 2014; Klampfl *et al.*, 2013; Nielsen *et al.*, 2013). About 10-15% of patients have no such mutations (the so-called 'triple-negative') (Tefferi *et al.*, 2014). ET constitutes approximately a third of all cases of MPNs in developed countries, with a median age at diagnosis of 60 years. Most patients enjoy a normal life expectancy, but survival is decreased in older patients and those with thrombotic complications (Hultcrantz *et al.*, 2015; Roaldsnes *et al.*, 2017; Srour *et al.*, 2016; Tefferi & Barbui, 2017). The main features of ET include a persistently elevated platelet count of >450,000/μL on a complete blood count, a clinical course characterized by thrombotic and/or hemorrhagic events with a possible splenomegaly, and marked thrombocytosis and hyperplasia of megakaryocytes on peripheral blood smear

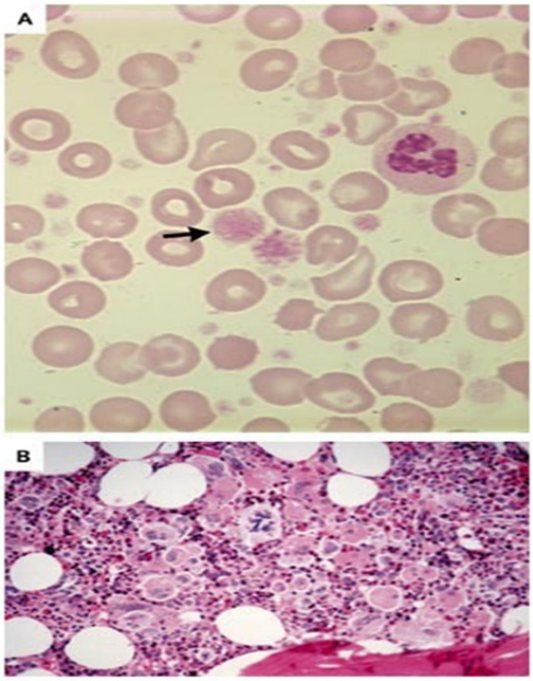

Figure 1: Histologic Features of Essential Thrombocythemia (Schafer, 2004)
In Panel A, a peripheral-blood smear from a patient with essential thrombocythemia contains an increased number of platelets, including giant platelets (arrow; Wright's stain, ×100). In Panel B, a specimen of bone marrow from a patient with essential thrombocythemia contains an increased number of megakaryocytes (hematoxylin and eosin, ×100). (Courtesy of Dr. Scott Murphy, American Red Cross Blood Services, Penn–Jersey Region, Philadelphia.)

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and bone marrow assays, respectively (Tefferi & Barbui, 2020). Asymptomatic patients with ET (about 50% of all cases) may be found when persistent thrombocytosis is noted on routine complete blood counts for other reasons. Disease-related symptoms of ET are largely vasomotor due to disturbances in microvascular circulations and include headaches, dizziness, fainting, blurring or transient loss of vision (e.g., amaurosis fugax, ocular migraines), non-specific fatigue, atypical chest pains, myalgias, acral paresthesia, erythromelalgias, and livedo reticularis, etc. (Wolanskyj *et al.*, 2005). Patients may also present with complications related to thrombosis (due to both quantitative and qualitative plate dysfunctions) in various organs, e.g., strokes, acute coronary syndromes, superficial thrombophlebitis, deep vein thrombosis, pulmonary embolism, hepatic or portal vein thrombosis, digital ischemia, thrombosis-related first-trimester pregnancy losses, etc. (Tefferi & Barbui, 2017). Hemorrhagic manifestations tend to occur in cases of extreme thrombocytosis (platelet counts >1000,000/μL) as well as following treatment with high doses of non-steroidal anti-inflammatory drugs (Bellucci *et al.*, 1986; Landolfi *et al.*, 2006). Treatment of ET is based on individualized stratification of the risks and/or occurrence of thrombosis or bleeding (Barbui *et al.*, 2015) and generally includes observation and cytoreductive therapy with medications such as low-dose aspirin and hydroxyurea (first-line therapy) (Rocca *et al.*, 2020; Tefferi & Barbui, 2020). Interferon alfa, anagrelide, ruxolitinib, and apheresis may be used as second-line agents (Birgegård, 2016; Tefferi & Barbui, 2020). Older agents like busulphan and phosphorus-32 (^{32}P) may also be used as second-line agents but have been associated with increased risks of acute myelogenous leukemia (Finazzi *et al.*, 2005; Tefferi & Barbui, 2020).

CASE SUMMARY

History and Physical Examination

A 60-year-old married father of three adults, a tailor by profession from Pipeline Estate, Nakuru County, Kenya, with no preceding significant medical history, first presented to us in February 2022, with concerns of pain and darkish discoloration of the small left toe. This was associated with a ≥6-month' history of bilateral lower limb numbness, paresthesia, and burning pain of both hands and feet in a stocking-and-gloving distribution. He'd also experienced recurrent global headaches, blurring of vision, occasional dizziness, myalgias, general fatigue, reduced effort tolerance, and mild bipedal edema. However, he had no history to suggest heart failure, bleeding diatheses, stroke-like symptoms, or deep venous thrombosis. He had no known family history of any hematological or cardiovascular diseases. The rest of the systemic inquiry was unremarkable.

On examination, he was in fair general health. His lower limb examination revealed multiple macular-to-patchy non-tender, non-blanching discrete skin lesions on both legs and thighs, mild bilateral pitting ankle edema, with the left small toe appearing remarkably darkened but non-tender, and with an associated septic wound on its tip. The neurovascular exam of both legs as well as the rest of the musculoskeletal exam were normal. He had no features of chronic liver disease but had a dull percussion note over Traube's space, indicating splenomegaly. A full neurological exam only showed mild impairment in the peripheries of light touch and vibration, while examination of the other systems was unremarkable.

Diagnostic Evaluation

During that first encounter, it was noted on a complete blood count that his total platelet count was markedly elevated at 2306 $x10^3/\mu L$ (the normal range is 150-450 $x10^3/\mu L$), while the total leucocyte count and hemoglobin were both normal at 10.4 $x10^3/\mu L$ and 14 g/dl, respectively. He had normal random blood glucose, normal renal and liver panels, a negative HIV rapid test, a negative COVID-19 rapid antigen test, a normal chest x-ray and electrocardiogram, and a normal erythrocyte sedimentation rate of 8 mm/hr. Ultrasonography showed a mild splenomegaly of 14cm and early bilateral leg varicose veins, but no deep venous thrombosis. He was assessed to have features of a possible underlying myeloproliferative disorder, predominantly of the ET phenotype. He got lost to follow-up until nine months later, when he re-presented with a worsening of similar symptoms. His platelet count had further increased to 2733 $x10^3/\mu L$. A peripheral blood film showed extreme thrombocytosis with both giant and small platelets, consistent with primary (essential) thrombocytosis. Due to severe financial and logistical challenges, it was impossible to do a bone marrow aspirate or any genetic tests.

Management and Follow-Up

His clinical syndrome was most consistent with ET, with predominant vasomotor symptoms and associated sensory peripheral neuropathy. He was put on oral hydroxyurea (HU) starting at a dose of 1g daily and aspirin at 75mg daily. For the neuropathy, he was put on pregabalin 75mg daily (and amitriptyline 25mg nocte was later added during follow-up). The septic wound was surgically managed to full healing. While on HU, he developed symptomatic hyperuricemia due to HU-induced platelet degradation (with a clinical syndrome of non-tophaceous acute gouty polyarthritis), which was successfully managed with allopurinol and analgesics. Besides, the dose of HU was adjusted to a maximum tolerable dose of 1.5 g/day, aiming to keep an absolute neutrophil count of ≥1.5 $x10^3/\mu L$. Significantly, whenever his absolute neutrophil count reduced to <1.5 $x10^3/\mu L$, he developed a recurrent triad of oropharyngeal candidiasis, septic grade 2 tonsillitis, and cystitis. This was fully treated with antimicrobials and supportive therapy.

The HU in each case was stopped for a week and subsequently restarted at a much lower dosage, resulting in the resolution of the neutropenia and the recovery of the target absolute neutrophil count. However, the platelets

and the uric acid levels rose precipitously when HU was stopped (or dosage reduced), with a corresponding recurrence of mild-to-moderate vasomotor symptoms and gouty polyarthritis. These have been fully controlled with a corresponding adjustment of the various drug dosages. He is currently on follow-up at the medical clinic with ongoing clinical and laboratory monitoring to target the individualized therapeutic goals described earlier and clinical surveillance for infections, transformation to other MPNs, and any adverse effects of the various drugs. Table 1 below shows a composite summary of his complete blood count, uric acid levels, and relevant interventions.

Table 1: Summary of the Complete Blood Count, Uric Acid Levels, and Interventions

Date	Total Platelets	Total WBC	Hemoglobin	Uric acid	Interventions
	(150-450 x10^3/μL)	(4-11 x10^3/μL) N=neutrophils, L=lymphocytes ANC=absolute neutrophil count	(11-16 g/dl)	(3.4-7mg/dl)	HU=hydroxyurea, ASA=aspirin, ALP=allopurinol, CTX= cotrimoxazole, OD= once daily Abx= antibiotics
22/2/2022	2306	10.4	14.0	-	
15/11/2022	2733	11.4	11.7	2.3	HU 1g +ASA 75mg OD started
22/11/2022	2472	10.9	12.1	-	HU 1g +ASA 75mg OD
2/12/2022	2124	9.7	12.0	13.2	HU 1.5g +ASA 75mg + ALP 100mg OD
29/12/2022	942	7.0	12.4	7.9	HU 1.5g +ASA 75mg + ALP 200mg OD
27/1/2023	918	3.6(N=67%, ANC=2412/μL, L=28%)	12.3	12.9	HU 1.5g +ASA 75mg + ALP 300mg OD
10/3/2023	745	3.1 (N=64%, ANC=1984/μL, L=28%)	13.0	4.6	HU 1.5g +ASA 75mg + ALP 300mg OD
5/5/2023	675	2.2 (N=63%, ANC=1386/μL, L=31%)	12.6	3.0	HU 1g +ASA 75mg + ALP 100mg OD + Abx
3/7/2023	977	2.6 (N=59%, ANC=1534/μL, L=34%)	13.3	4.6	HU 1.5g +ASA 75mg+ ALP 100mg OD
14/8/2023	673	2.8 (N=57%, ANC=1596/μL, L=38%)	13.4	4.0	HU 1.5g +ASA 75mg+ ALP 100mg OD
11/9/2023	535	2.5 (N=59%, ANC=1475/μL, L=30%)	13.0	3.5	HU 0.5g +ASA 75mg+ ALP 100mg OD + Abx
9/10/2023	1445	4.0	13.0	8.6	HU 1g +ASA 75mg + ALP 200mg OD
8/11/2023	899	3.8 (N=63%, ANC=2394/μL, L=31%)	12.7	2.5	HU 1.5g +ASA 75mg + ALP 200mg OD

Key: Note the adjustment of HU dosages whenever the absolute neutrophil count dropped below 1500/μL, and the subsequent precipitous increase in platelet counts and uric acid levels

RESULTS AND DISCUSSION

A diagnostic evaluation of suspected ET includes a medical history focused on the occurrence of thrombotic and hemorrhagic manifestations, with a careful search for concomitant cardiovascular risk factors in the setting of thrombotic complications. The diagnostic criteria of ET by the World Health Organization and the International Consensus Classification (Arber *et al.*, 2022) require either all four of the major criteria or the first three major criteria plus the minor criterion, as shown in Table 2 below.

Table 2: WHO diagnostic criteria for Essential Thrombocythemia (Arber *et al.*, 2022)

Major criteria
1. Platelet count ≥450 × 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyper-lobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR*, or *MPL* mutation
Minor criterion
1. Presence of a clonal marker or absence of evidence for reactive thrombocytosis
Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion.

Key: CML: chronic myeloid leukemia; ET: essential thrombocythemia; PMF: primary myelofibrosis; PV: polycythemia vera; WHO: World Health Organization

In our patient, it was not possible to do a bone marrow aspiration and trephine biopsy (BMAT) or any genetic tests for *JAK2*, *CALR*, or *MPL* mutations due to severe financial challenges. These services are not readily available in the vast majority of our health centers, and their access upon referrals to regional reference laboratory centers is severely hindered by their prohibitive costs.

The main differential diagnosis for ET is reactive thrombocytosis (RT). Platelets are also members of the acute phase reactant superfamily, which will be elevated in many inflammatory and other conditions, e.g., infections, iron deficiency anemia, acute bleeding, trauma, asplenia, etc.) Patients with RT will have evidence of these conditions as well as elevation of other acute phase reactants (e.g., C-reactive protein, erythrocyte sedimentation rate, ferritin, etc.). (Schafer, 2004). Other MPNs must also be excluded prior to initiating treatment for ET. In our patient, a targeted clinical evaluation ruled out alternative diagnoses. He had no features of inflammation or trauma; he had a normal ESR; he maintained a persistently normal leucocyte count (pre-treatment) and normal hemoglobin; and his platelets were too high to be compatible with just a RT. A validated simple laboratory scoring system based on a complete blood count that distinguishes ET from RT is consistent with our assessment of ET in this case (Shen *et al.*, 2021). At any rate, one would expect massive splenomegaly with CML (and severe leukocytosis) and PMF (and severe anemia), while PV would have marked polycythemia.

The goals of treatment in ET are to alleviate vasomotor symptoms, reduce the complications related to thrombosis and/or hemorrhage, and control splenomegaly (if present). Decisions on therapeutic choices are based on individual risk stratification and the availability and tolerability of the various agents. Low-dose aspirin (40-100 mg orally administered once daily) and HU (at 15 mg/kg/day orally administered at a usual starting dose of 500mg once or twice daily) are first-line agents (Tefferi & Barbui, 2020). Aspirin can reduce vasomotor symptoms and thrombo-hemorrhagic complications in most cases of ET. Higher doses are associated with increased risks of gastritis and gastrointestinal bleeding. HU is effective in reducing platelet counts, controlling splenomegaly, and reducing vasomotor symptoms in ET. The dose of HU is adjusted to a target platelet count of 100,000-450,000/μL while limiting leukocytopenia and anemia by doing weekly-to-monthly complete blood count monitoring (Tefferi & Barbui, 2020). The response rates of HU vary from about 80% complete response to 15% partial response using the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-NRT) and the European LeukemiaNet (ELN) criteria (Hernández-Boluda *et al.*, 2011). Adverse effects of HU include oral ulcers, skin rashes, hyperpigmentation, long-term lung and liver toxicities, etc. HU is contraindicated in pregnancy and lactation. HU-induced neutropenia due to myelosuppression may manifest as recurrent infections presenting with fever, sore throat, urinary tract infections, etc. (Antonioli *et al.*, 2012; Luchtman-Jones *et al.*, 2016). These effects are reversible by withholding HU for 1 to 2 weeks and then re-initiating at a lower dosage (Wang *et al.*, 2011). When our patient got recurrent HU-induced neutropenia-related infections, the HU was withheld for one week in each case and successfully re-started at lower dosages tailored to therapeutic targets.

There are no studies that specifically address the modification of doses of HU for ET in cases of severe HU-induced neutropenia. However, extrapolation of expert consensus recommendations from the use of HU in sickle cell anemia in children may give guidance on the definition of HU-related myelosuppression and toxicities, as well as dose adjustments to the maximum tolerated dosages (MTD) in such cases (McGann *et al.*, 2016; McGann & Ware, 2015). This is demonstrated in Table 3 below.

Based on these recommendations, an absolute neutrophil count of ≥1500/μL was adopted for our patient as the target for defining the maximum tolerated dosage of the HU, with the uppermost dosage of 1.5 g/day. So far, the recommended target platelet count of 100,000-450, 000/μL while on HU treatment has not been achieved due to the concurrence of HU-related neutropenia, as highlighted.

Interestingly, the patients' subjective symptoms have almost completely resolved on HU and aspirin.

Therefore, in resource-limited clinical settings, we propose a practical and rational approach to the diagnosis and treatment of ET, as summarized in the algorithm shown in Table 4 below.

Table 3: Hematological parameters used to define the hydroxyurea maximum tolerated dose (MTD) with mild marrow suppression (McGann *et al.*, 2016; McGann & Ware, 2015)

Parameter	Toxicity criteria	Escalation criteria	Target MTD value
Absolute neutrophil count (ANC, x 10^9/l)	< 1.0	> 3.0	1.5–3.0
Absolute reticulocyte count (ARC, x 10^9/l)	< 80 (unless Hb ≥ 9.0)	> 200	100–200
Platelets (x 10^9/l)	< 80	> 150	> 80
Hemoglobin (Hb gm/dl)	Hb <4.0 or Hb <6.0 unless ARC >100	>6.5	

Table 4: An Algorithm for the Diagnosis and Management of Essential Thrombocytosis in a Resource-Limited Setting

A. Platelet count: 450-1000 x 10^3/µL
1. Rule out RT: inflammatory conditions, trauma, iron deficiency anemia, etc., elevated CRP, ESR, procalcitonin, and other acute phase reactants.
2. If no evidence of RT, go to B.
B. Platelet count: >1000 x 10^3/µL
1. Clinically: vasomotor symptoms, thrombotic and/or hemorrhagic complications.
2. Peripheral blood film: presence of marked/severe/extreme thrombocytosis with platelet anisocytosis.
3. Clinically rule out other MPNs using a complete blood count, targeted clinical examination, and peripheral blood film.
4. Bone marrow aspirate and trephine biopsy, where possible: findings meeting the major criteria for ET diagnosis.
5. Make a presumed or definitive diagnosis of ET and go to C.
C. Diagnosis of ET
1. Start on low-dose aspirin and hydroxyurea.
2. Therapeutic target platelet counts of 150-450 x 10^3/µL, and an absolute neutrophil count of ≥ 1.5 x 10^3/µL.
3. Individualize therapeutic targets.
4. Monthly follow-ups with complete blood counts (and any other relevant tests).
5. Monitor for therapeutic targets, drug adverse effects, and disease transformations (e.g., into leukemias).

Key: ET=essential thrombocytosis, RT=reactive thrombocytosis, CRP=C-reactive protein, ESR=erythrocyte sedimentation rate, and MPNs=myeloproliferative neoplasms.

CONCLUSION

In resource-limited settings, a diagnosis of ET must still be made through a high index of suspicion and a nuanced approach that incorporates compatible clinical features, persistent thrombocytosis (especially with platelet counts >1000 x 10^3/µL), and a simple peripheral blood smear that shows extreme thrombocytosis with platelet anisocytosis. This is followed by a meticulous, targeted evaluation to rule out reactive thrombocytosis and other MPNs. A BMAT biopsy and genetic testing for *JAK2*, *CALR*, or *MPL* mutations are ideally required for proving a diagnosis of ET, but in resource-limited settings, obtaining these tests is usually hopelessly vetoed by their inaccessibility and prohibitive costs. HU and aspirin are widely available in most clinical settings and should judiciously be used to treat ET with careful monitoring based on individualized treatment targets.

RECOMMENDATIONS

HU-induced neutropenia is a dose-limiting adverse effect of HU therapy that may complicate ET treatment and hinder the attainment of target platelet levels. Unfortunately, no robust data currently exists in the literature that would give guidance on dose adjustments in such cases. More clinical trials and expert consensus recommendations are necessary to bridge this gap, especially with a focus on resource-limited clinical contexts.

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REFERENCES

Antonioli, E., Guglielmelli, P., Pieri, L., Finazzi, M., Rumi, E., Martinelli, V., Vianelli, N., Luigia Randi, M., Bertozzi, I., De Stefano, V., Za, T., Rossi, E., Ruggeri, M., Elli, E., Cacciola, R., Cacciola, E., Pogliani, E., Rodeghiero, F., Baccarani, M., . . . Vannucchi, A. M.

(2012). Hydroxyurea-related toxicity in 3,411 patients with Ph'-negative MPN. *Am J Hematol, 87*(5), 552-554. https://doi.org/10.1002/ajh.23160

Arber, D. A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M. J., Le Beau, M. M., Bloomfield, C. D., Cazzola, M., & Vardiman, J. W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood, 127*(20), 2391-2405. https://doi.org/10.1182/blood-2016-03-643544

Arber, D. A., Orazi, A., Hasserjian, R. P., Borowitz, M. J., Calvo, K. R., Kvasnicka, H. M., Wang, S. A., Bagg, A., Barbui, T., Branford, S., Bueso-Ramos, C. E., Cortes, J. E., Dal Cin, P., DiNardo, C. D., Dombret, H., Duncavage, E. J., Ebert, B. L., Estey, E. H., Facchetti, F., . . . Tefferi, A. (2022). International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood, 140*(11), 1200-1228. https://doi.org/10.1182/blood.2022015850

Barbui, T., Vannucchi, A. M., Buxhofer-Ausch, V., De Stefano, V., Betti, S., Rambaldi, A., Rumi, E., Ruggeri, M., Rodeghiero, F., Randi, M. L., Bertozzi, I., Gisslinger, H., Finazzi, G., Carobbio, A., Thiele, J., Passamonti, F., Falcone, C., & Tefferi, A. (2015). Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia. *Blood Cancer J, 5*(11), e369. https://doi.org/10.1038/bcj.2015.94

Bellucci, S., Janvier, M., Tobelem, G., Flandrin, G., Charpak, Y., Berger, R., & Boiron, M. (1986). Essential thrombocythemias. Clinical evolutionary and biological data. *Cancer, 58*(11), 2440-2447.

Birgegård, G. (2016). The Use of Anagrelide in Myeloproliferative Neoplasms, with Focus on Essential Thrombocythemia. *Curr Hematol Malig Rep, 11*(5), 348-355. https://doi.org/10.1007/s11899-016-0335-0

Cazzola, M., & Kralovics, R. (2014). From Janus kinase 2 to calreticulin: the clinically relevant genomic landscape of myeloproliferative neoplasms. *Blood, 123*(24), 3714-3719. https://doi.org/10.1182/blood-2014-03-530865

Finazzi, G., Caruso, V., Marchioli, R., Capnist, G., Chisesi, T., Finelli, C., Gugliotta, L., Landolfi, R., Kutti, J., Gisslinger, H., Marilus, R., Patrono, C., Pogliani, E. M., Randi, M. L., Villegas, A., Tognoni, G., & Barbui, T. (2005). Acute leukemia in polycythemia vera: an analysis of 1638 patients enrolled in a prospective observational study. *Blood, 105*(7), 2664-2670. https://doi.org/10.1182/blood-2004-09-3426

Hernández-Boluda, J. C., Alvarez-Larrán, A., Gómez, M., Angona, A., Amat, P., Bellosillo, B., Martínez-Avilés, L., Navarro, B., Teruel, A., Martínez-Ruiz, F., & Besses, C. (2011). Clinical evaluation of the European LeukaemiaNet criteria for clinicohaematological response and resistance/intolerance to hydroxycarbamide in essential thrombocythaemia. *Br J Haematol, 152*(1), 81-88. https://doi.org/10.1111/j.1365-2141.2010.08430.x

Hultcrantz, M., Wilkes, S. R., Kristinsson, S. Y., Andersson, T. M., Derolf Å, R., Eloranta, S., Samuelsson, J., Landgren, O., Dickman, P. W., Lambert, P. C., & Björkholm, M. (2015). Risk and Cause of Death in Patients Diagnosed With Myeloproliferative Neoplasms in Sweden Between 1973 and 2005: A Population-Based Study. *J Clin Oncol, 33*(20), 2288-2295. https://doi.org/10.1200/jco.2014.57.6652

Klampfl, T., Gisslinger, H., Harutyunyan, A. S., Nivarthi, H., Rumi, E., Milosevic, J. D., Them, N. C., Berg, T., Gisslinger, B., Pietra, D., Chen, D., Vladimer, G. I., Bagienski, K., Milanesi, C., Casetti, I. C., Sant'Antonio, E., Ferretti, V., Elena, C., Schischlik, F., . . . Kralovics, R. (2013). Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med, 369*(25), 2379-2390. https://doi.org/10.1056/NEJMoa1311347

Landolfi, R., Cipriani, M. C., & Novarese, L. (2006). Thrombosis and bleeding in polycythemia vera and essential thrombocythemia: pathogenetic mechanisms and prevention. *Best Pract Res Clin Haematol, 19*(3), 617-633. https://doi.org/10.1016/j.beha.2005.07.011

Luchtman-Jones, L., Pressel, S., Hilliard, L., Brown, R. C., Smith, M. G., Thompson, A. A., Lee, M. T., Rothman, J., Rogers, Z. R., Owen, W., Imran, H., Thornburg, C., Kwiatkowski, J. L., Aygun, B., Nelson, S., Roberts, C., Gauger, C., Piccone, C., Kalfa, T., . . . Ware, R. E. (2016). Effects of hydroxyurea treatment for patients with hemoglobin SC disease. *Am J Hematol, 91*(2), 238-242. https://doi.org/10.1002/ajh.24255

McGann, P. T., Tshilolo, L., Santos, B., Tomlinson, G. A., Stuber, S., Latham, T., Aygun, B., Obaro, S. K., Olupot-Olupot, P., Williams, T. N., Odame, I., & Ware, R. E. (2016). Hydroxyurea Therapy for Children With Sickle Cell Anemia in Sub-Saharan Africa: Rationale and Design of the REACH Trial. *Pediatr Blood Cancer, 63*(1), 98-104. https://doi.org/10.1002/pbc.25705

McGann, P. T., & Ware, R. E. (2015). Hydroxyurea therapy for sickle cell anemia. *Expert Opin Drug Saf, 14*(11), 1749-1758. https://doi.org/10.1517/14740338.2015.1088827

Nielsen, C., Birgens, H. S., Nordestgaard, B. G., & Bojesen, S. E. (2013). Diagnostic value of *JAK2* V617F somatic mutation for myeloproliferative cancer in 49 488 individuals from the general population. *Br J Haematol, 160*(1), 70-79. https://doi.org/10.1111/bjh.12099

Roaldsnes, C., Holst, R., Frederiksen, H., & Ghanima, W. (2017). Myeloproliferative neoplasms: trends in incidence, prevalence and survival in Norway. *Eur J Haematol, 98*(1), 85-93. https://doi.org/10.1111/ejh.12788

Rocca, B., Tosetto, A., Betti, S., Soldati, D., Petrucci, G., Rossi, E., Timillero, A., Cavalca, V., Porro, B., Iurlo, A., Cattaneo, D., Bucelli, C., Dragani, A., Di Ianni, M., Ranalli, P., Palandri, F., Vianelli, N., Beggiato, E., Lanzarone, G., . . . De Stefano, V. (2020). A randomized

double-blind trial of 3 aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia. *Blood, 136*(2), 171-182. https://doi.org/10.1182/blood.2019004596

Schafer, A. I. (2004). Thrombocytosis. *N Engl J Med, 350*(12), 1211-1219. https://doi.org/10.1056/NEJMra035363

Shen, C. L., Hsieh, T. C., Wang, T. F., Huang, W. H., Chu, S. C., & Wu, Y. F. (2021). Designing a Scoring System for Differential Diagnosis From Reactive Thrombocytosis and Essential Thrombocytosis. *Front Med (Lausanne), 8*, 736150. https://doi.org/10.3389/fmed.2021.736150

Srour, S. A., Devesa, S. S., Morton, L. M., Check, D. P., Curtis, R. E., Linet, M. S., & Dores, G. M. (2016). Incidence and patient survival of myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms in the United States, 2001-12. *Br J Haematol, 174*(3), 382-396. https://doi.org/10.1111/bjh.14061

Tefferi, A., & Barbui, T. (2017). Polycythemia vera and essential thrombocythemia: 2017 update on diagnosis, risk-stratification, and management. *Am J Hematol, 92*(1), 94-108. https://doi.org/10.1002/ajh.24607

Tefferi, A., & Barbui, T. (2020). Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *Am J Hematol, 95*(12), 1599-1613. https://doi.org/10.1002/ajh.26008

Tefferi, A., Lasho, T. L., Finke, C. M., Knudson, R. A., Ketterling, R., Hanson, C. H., Maffioli, M., Caramazza, D., Passamonti, F., & Pardanani, A. (2014). *CALR* vs *JAK2* vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia, 28*(7), 1472-1477. https://doi.org/10.1038/leu.2014.3

Tefferi, A., & Pardanani, A. (2019). Essential Thrombocythemia. *N Engl J Med, 381*(22), 2135-2144. https://doi.org/10.1056/NEJMcp1816082

Wang, W. C., Ware, R. E., Miller, S. T., Iyer, R. V., Casella, J. F., Minniti, C. P., Rana, S., Thornburg, C. D., Rogers, Z. R., Kalpatthi, R. V., Barredo, J. C., Brown, R. C., Sarnaik, S. A., Howard, T. H., Wynn, L. W., Kutlar, A., Armstrong, F. D., Files, B. A., Goldsmith, J. C., . . . Thompson, B. W. (2011). Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). *Lancet, 377*(9778), 1663-1672. https://doi.org/10.1016/s0140-6736(11)60355-3

Wolanskyj, A. P., Lasho, T. L., Schwager, S. M., McClure, R. F., Wadleigh, M., Lee, S. J., Gilliland, D. G., & Tefferi, A. (2005). *JAK2* mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. *Br J Haematol, 131*(2), 208-213. https://doi.org/10.1111/j.1365-2141.2005.05764.x