The myeloproliferative neoplasms (MPN) Childhood Registry and Biobank

Document type:	**Registry Protocol**
Version Number:	**1.2**
Release date:	**19.11.2022**
Coordinating Investigator:	**Dr. med. Axel Karow**
	Department of Pediatrics and Adolescent Medicine
	Friedrich-Alexander-Universität Erlangen-Nürnberg
	Loschgestrasse 15
	91054 Erlangen, Germany

1 Synopsis

Title of the Registry	MPN Childhood Registry
Coordinating Investigator	Dr. med. Axel Karow, Department of Pediatrics and Adolescent Medicine Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)
Deputy Coordinating Investigator	Prof. Dr. med. Markus Metzler, Department of Pediatrics and Adolescent Medicine Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)
Scientific Committee	Prof. Dr. med. Holger Cario, Department of Pediatrics and Adolescent Medicine, University Medical Center Ulm PD Dr. med. Miriam Erlacher, Department of Pediatrics and Adolescent Medicine Division of Pediatric Hematology and Oncology University of Freiburg
Reference Genetics and Cytogenetics	Dr. rer. nat. Yvonne Behrens; Department of Human Genetics Hannover Medical School
Reference Histology	Prof Dr. med. Maike Büttner-Herold, Institute of Pathology Friedrich-Alexander-University Erlangen-Nürnberg (FAU)
Sponsor	University Hospital, Universitätsklinikum Erlangen
Registry Design	National multicenter observational registry
Registry Timetable	Registry start: December 01, 2022 Establishing the prerequisites for a cross-centre registry in the first year Registration of eligible patients in the second and third year Thereafter in principle without any definite limitation
State of the Art	The classical myeloproliferative neoplasms (MPN) polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) as well as primary hyereosinophilic syndrome (pHES) are clonal hematopoietic stem-cell disorders usually diagnosed in adulthood. In children and adolescents, classical MPN and pHES are significantly less frequent and exhibit distinct biological and clinical features. So far, only few cohorts of pediatric patients with these MPN have been investigated and clinical and genetic features, treatment options, and outcomes in young patients are insufficiently described. Consequently, there is only limited data available for the standardization of diagnostic and therapeutic approaches and largely, adult guidelines are applied in childhood MPN. However, these are not tailored to the specific needs of pediatric patients.

Primary Registry Objectives	• To determine the incidence and epidemiology of classical MPN, PV, ET and PMF as well as pHES in children and adolescents through systematic inclusion of these patients in a national population-based registry • To systematically characterize clinical, hematologic, genetic and cytogenetic features of children and adolescents with PV, ET, PMF and pHES • To identify pediatric patients with MPN and high risk of complications and disease progression and transformation into acute myeloid leukemia (AML) • To improve the therapy for children and adolescents with MPN by discussing treatment options with the treating centers and tailor therapy to the specific and individual needs of pediatric patients
Secondary Registry Objectives	• To prepare future international collaborative prospective studies focusing on the optimization and standardization of childhood MPN-diagnosis and treatment • To develop a biobank system of pediatric patients with MPN
Inclusion Criteria	○ Newly diagnosed PV, ET, PMF or pHES ○ Age < 18 years (up to 17 years and 365 days) at the day of diagnosis ○ Patient treated in a participating center ○ Written informed consent to registry participation
Exclusion Criteria	○ Hereditary or secondary polycythemia, thrombocytosis, myelofibrosis or HES with underlying reasons other than PV, ET, PMF or pHES
Participating Centers	All centers caring for pediatric patients with MPN
Methodology	All patients undergo a standardized routine diagnostic approach including a clinical, hematologic, morphologic, histologic, genetic and cytogenetic assessment at diagnosis, then annually or at suspected disease progression. Data is provided by the participating centers and entered in the electronic CRF (case report forms) by the coordinating study center (CSC) using the REDCap® software.
Statistics	The statistical evaluation is descriptive and exploratory. A detailed procedure is developed before and during the evaluation.

2 List of Abbreviations

AEC	*Absolute Eosinophil Count*
AML	*Acute Myeloid Leukemia*
ANC	*Absolute Neutrophil Count*
BM	*Bone Marrow*
BSA	*Body Surface Area*
BW	*Body weight*
CBC	*Complete Blood Count*
CGH	*Comparative Genomic Hybridization*
CI	*Cumulative Incidence*
CNS	*Central Nervous System*
CRF	*Case Report Form*
CSC	*Coordinating Study Center*
CT	*Computerized Tomography*
CTC	*Common Toxicity Criteria*
DMC	*Data Monitoring Committee*
EU	*European Union*
EFS	*Event Free Survival*
EORTC	*European Organization for Research and Treatment of Cancer*
ET	*Essential Thrombocythemia*
FAB	*French American British Group*
GCP	*Good Clinical Practice*
GGT	*Gamma Glutamic Transferase*
GLP	*Good Laboratory Practice*
GMP	*Good Manufacturing Practice*
HSCT	*Hematopoietic Stem Cell Transplantation*
HE	*Hypereosinophilia*
HES	*Hypereosinophilic Syndrome*
IEC	*Independent Ethics Committee*
ICH	*International Conference on Harmonization*
IPSS	*International Prognostic Scoring System*
LDH	*Lactate Dehydrogenase*
LFT	*Liver Function Test*
MCV	*Mean Corpuscular Volume*
MDS	*Myelodysplastic Syndrome*
MFD	*Matched Family Donor*
MMFD	*Mismatched Family Donor*
MNC	*Mononuclear Cells*
MPN	*Myeloproliferative Neoplasms*
MRI	*Magnetic resonance imaging*
NGS	*Next/New Generation Sequencing*
NIH	*National Institute of Health (US)*
n.s.	*Non-significant*
PB	*Peripheral Blood*
PBSCT	*Peripheral Blood Stem Cell Transplantation*
PI	*Principal Investigator*
PMF	*Primary Myelofibrosis*
PV	*Polycythemia Vera*
QOL	*Quality of Life*
RBC	*Red Blood Cell Count*
SAE	*Serious Adverse Event*
SAP	*Statistical Analysis Plan*
SDV	*Source Data Verification*
SGOT	*Serum Glutamic Oxaloacetic Transaminase*
SGPT	*Serum Glutamic Pyruvic Transaminase*
TRM	*Transplant Related Mortality*
UD	*UD Unrelated Donor*
WBC	*WBC White Blood Cell Count*
WHO	*World Health Organization*

3 Table of Contents

4 Schedule of activities

Visit Name	Pre-Screening	Initial Diagnostic Procedures	Disease monitoring[9]	Yearly Follow-up Visit[10]
Informed consent	X			
Demography	X	X		X
Inclusion/Exclusion criteria	X			
Disease History	X			
Complications/organ involvement related to the disease[1]	X	X		X
Medical History		X		
Family history		X		
Concomitant Medication /Therapy		X		
Disease-associated symptoms[2]				
Physical Findings[3]		X		X
Body Weight		X		X
Body Height		X		X
Birth Weight		X		X
Head Circumference (cm)		X		
Organomegaly		X	X	X
Hematology[4]	X	X	X	X
Clinical Chemistry[5]		X		X
Bone marrow with differential count[6]	X	X	(X)	(X)
Peripheral blood with differential count[6]	X	X	X	X
Cytogenetic analysis of BM/PB		X	(X)	(X)
Direct and indirect Coombs-test		X		
Molecular analyses from BM/PB		X	(X)	X
HLA-typing		X		
Vitamin and iron status[7]		X		X
Patient alive at this visit				X
Karnofsky Score		X		X
MPN-specific treatment			X	X
Toxicity[8]				X
Concomitant treatment		X		X

[1]Complications/organ involvement related to the disease: i. e. thrombosis or bleeding are required at the initial diagnostic procedures and follow-up visits but are optional at the pre-screening visit.

[2]Disease-associated symptoms: fatigue, headache, fever, active infection

[3]Physical Findings: splenomegaly, hepatomegaly, lymphadenopathy, congenital abnormalities, xanthomas, café au lait spots, cranial nerve palsy, respiratory tract symptoms, signs of neurofibromatosis type I.

[4]Hematology: hemoglobin, hematocrit, MCV, reticulocyte count, platelet count, CBC with differential.

[5]Clinical chemistry: bilirubin, ASAT, ALAT, Gamma-GT, LDH, Uric Acid, Creatinine, Alkaline Phosphatase

[6]Bone marrow and Peripheral blood are summarized in Appendix 2, follow-up bone marrow only in case of changing peripheral blood counts and/or suspected progress

[7]Screening vitamin deficiencies: folic acid, vitamin B12; full iron status including serum iron, serum ferritin, and serum transferrin saturation

[8]Toxicity: anemia, neutropenia, thrombocytopenia, skin rash, nausea, vomiting, diarrhea, edema, muscle cramps, headache, LFT elevation, infection

[9]Response assessment and disease monitoring in classical MPN and pHES can be performed as described in 12.3

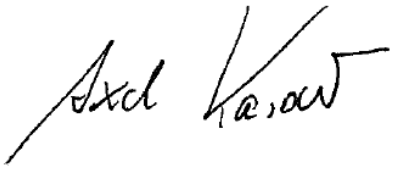
[10]Yearly follow-up will be performed until adulthood.

5 Responsibilities

Coordinating Investigator

Dr. med. Axel Karow
Department of Pediatrics and Adolescent Medicine
Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)
Loschgestrasse 15
91054 Erlangen
Germany

Signature



Dr. med. Axel Karow

Deputy Coordinating Investigator

Prof. Dr. med. Markus Metzler
Department of Pediatrics and Adolescent Medicine
Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)
Loschgestrasse 15
91054 Erlangen
Germany

Scientific Committee

Prof. Dr. med. Holger Cario
Department of Pediatrics and Adolescent Medicine
University Medical Center Ulm
Eythstraße 24
89075 Ulm
Germany

PD Dr. med. Miriam Erlacher
Department of Pediatrics and Adolescent Medicine
Division of Pediatric Hematology and Oncology
University of Freiburg
Mathildenstr 1
79106 Freiburg
Germany

Reference Genetics and Reference Cytogenetics:

Dr. rer. nat. Yvonne Behrens
Department of Human Genetics
Hannover Medical School
Carl-Neuberg Strasse 1
30625 Hannover
Germany

Reference Histology

Prof. Dr. med. Maike Büttner-Herold
Institute of Pathology
Friedrich-Alexander-University Erlangen-Nürnberg (FAU)
Krankenhausstr. 8-10
91054 Erlangen
Germany

6 State of the art

6.1 Classical MPN

6.1.1 Background

The classical myeloproliferative neoplasms (MPN) comprise the three *BCR::ABL1*-negative disease entities polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Serious complications associated with MPNs are thromboembolic events and disease progression of and to myelofibrosis and acute myeloid leukemia (AML) (1).
Like other myeloid diseases, e. g. myelodysplastic syndromes (MDS) and chronic myeloid leukemia (CML), MPNs are usually diagnosed in adulthood and the median age at diagnosis of the two more prevalent conditions, PV and ET, is over 60 years. In children and adolescents, however, MPNs are very rare and knowledge concerning the biological and clinical characteristics at diagnosis and in the further course of these diseases including the incidence of vascular and long-term complications remains limited. As a consequence, there is no standardization of diagnostic and therapeutic approaches in pediatric patients with MPN and in most instances, guidelines from adult patients are applied in children and adolescents. These include that the diagnosis of an MPN is based on the identification of a driver mutation and the assessment of a bone marrow trephine. Treatment should be adapted according to a stratification into low risk or high risk according to the patient`s age and history of thrombosis or hemorrhage with the aim to reduce thrombotic complications (2-4). The significance of different therapeutic avenues including antiplatelet and/or cytoreductive treatment in the population of pediatric patients with MPN is largely unknown and information about the potential long-term sequelae of treatment is sparse.
Currently, there is no population-based registry on a national or international level for pediatric patients < 18 years with MPN. The main aims of the MPN Childhood Registry are to validate the epidemiology of MPN in childhood and adolescence, to improve and standardize the diagnostic approach by a centralized morphologic, histologic, cytogenetic and molecular review and to evaluate the different therapeutic modalities and tailor these to the specific needs of pediatric patients.

6.1.2 Epidemiology

A significant variability of data concerning in the incidence of MPN between countries was reported. The global incidence of MPN in children and adolescents has recently been estimated at around 0.82 per 100,000 per year (range 0.1 to 2.25), which is about 100 times lower than in adults (5-7). Thus, pediatric individuals represent only a small proportion of patients with MPN.
However, the more frequent use of routine blood counts in recent years has been associated with an increase in the diagnosis of MPNs in children and adolescents and therefore, the true incidence of these diseases might be underestimated.
The global incidences of PV, ET, and PMF were found to be variable around 0.18, 0.6, and 0.53 per 100,000 per year, respectively. Current studies showed a median age at diagnosis of 12 years for pediatric patients with PV and 9.3 years for pediatric patients with ET. The percentage of female cases in these analyses was 45% in PV and 57.6% in ET (6, 7).

6.1.3 Clinical presentation

There is a high variability of symptoms associated with MPN in children and adolescents. Recent analyses have shown that nearly half of the pediatric patients with MPN were asymptomatic at the time of diagnosis. Headaches, abdominal or bone pain were the most commonly reported symptoms. A small proportion of patients appears to be diagnosed following a thrombotic or hemorrhagic event. The frequency of these complications is lower in pediatric cohorts than in adults. The reason for the original consultation was unclear or unknown in most cases in these analyses.
The most frequent abnormal clinical finding is splenomegaly described in more than half of the cases with ET and a smaller subgroup of individuals with PV. There was no correlation of splenomegaly and abdominal symptoms or thrombosis (6-8).

6.1.4 Hematological characteristics

In a recent cumulative retrospective analysis, Ianotto et al. have assessed full blood counts of pediatric cases with MPN at diagnosis. For PV patients, the median leukocyte count was 13.2 G/L, the median

hemoglobin 180 g/L (maximum level, 189 g/L), the maximum hematocrit was 72.5%, and the platelet count was 799 G/L. For ET patients, the median leukocyte count was 10.6 G/L, the median hemoglobin 131 g/L, and the median platelet count 1192 G/L (maximum 4500 G/L). Notably, in this and other retrospective analyses, the differentiation from hereditary and secondary erythrocytosis and thrombocytosis remained unclear and in a substantial number of cases, the authors rather generally stated that patients fulfilled the diagnostic criteria according to the current World Health Organization classification. However, bone marrow results were described for only about half of all cases comprising mostly short descriptions and general conclusions (6).

6.1.5 Molecular characteristics

As for other myeloid diseases like MDS (9), obvious differences concerning the frequency and the spectrum of genetic driver and non-driver variants between pediatric and adult patients with MPN have been described.
For PV, the percentage of pediatric cases positive for the classical Janus kinase 2 (JAK2) V617F driver mutation was between 37% and 24% and therefore significantly lower than in adult cases, whereas the rate of *JAK2* exon 12 mutations appeared comparable. Accordingly, the percentage of pediatric individuals who did not harbor one of these two driver mutations was higher (10-12). In a larger analysis of pediatric ET cohorts including all driver mutations, the proportions of positivity were also lower than in adult cohorts and found to be 31% for *JAK2*V617F, 10% for *CALR*, and 2% for MPL. Consequently, a higher proportion of 57% of these pediatric cases were triple-negative for driver mutations (13-19).
In next-generation sequencing analyses, a significant proportion of 35% of patients did not carry any non-diver mutation (Figure 1) (13, 15). High-risk mutations associated with an inferior prognosis in primary myelofibrosis in adult patients were uncommon and the clinical significance of non-driver mutations could not be assessed in these studies.
As already addressed, the exclusion of hereditary and secondary cases was critically discussed in these reports. The differences in the mutational landscape found in these analyses of pediatric patients with MPN compared to adult patients requires a prospective evaluation.
Systematic analyses concerning cytogenetic aberrations in pediatric MPN have not been performed so far.

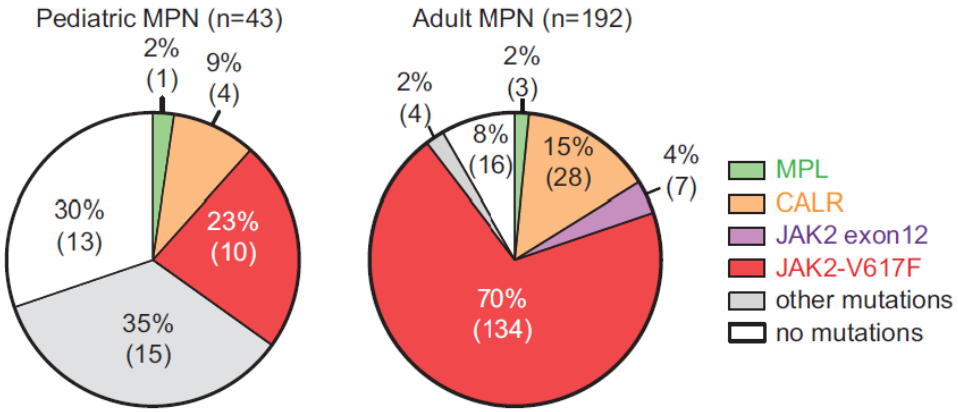


Figure 1: Mutation profiling in pediatric vs. adult patients with MPN according to Karow et al. Leukemia 2015 (15)

6.1.6 Implications of diagnostic criteria in pediatric MPN

The diagnostic criteria for PV, ET, and PMF are shown in table 1 and 2, respectively. However, it needs to be emphasized that the World Health Organization (WHO) diagnostic criteria for MPNs are tailored to adults. The PV criteria, for example, do not consider pediatric age-adjusted normal ranges for hemoglobin (Hb) or hematocrit (Hct) (12). Moreover, as described earlier, a significant proportion of pediatric patients do not exhibit a driver mutation as a major diagnostic criterion for MPNs. Therefore, from the diagnostic point of view, it appears even more important that a bone marrow aspiration as well as a bone marrow biopsy assessing morphology, cellularity and reticulin fibrosis are routinely performed in all children and adolescents with suspected MPN.

Polycythemia vera (PV)	**Essential thrombocythemia (ET)**
Major criteria	
1	
Hemoglobin > 16.5 g/dL (men); Hemoglobin > 16.0 g/dL (women); Hematocrit > 49% (men); Hematocrit > 48% (women) or increased red cell mass (RCM) *	Platelet count ≥ 450 × 10^9/L *
2	
BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)	BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3	
Presence of *JAK2V617F* or *JAK2* exon 12 mutation #	Not meeting WHO criteria for *BCR::ABL1* + CML, PV, PMF, MDS, or other myeloid neoplasms
4	
	Presence of *JAK2*, *CALR* or *MPL* mutation #
Minor criteria	
1	
Subnormal serum erythropoietin level	Presence of a clonal marker (e.g., abnormal karyotype) or absence of evidence for reactive thrombocytosis

Table 1: 2016 World Health Organization diagnostic criteria for polycythemia vera and essential thrombocythemia adapted from Barbui T et al. Blood Cancer J 2015, Arber et al. Blood 2016, and Arber et al. Blood 2022 (3, 20, 21)
*Values above the upper normal pediatric age range; #Possible lack of mutation in children and adolescents

Primary myelofibrosis (PMF)	
Prefibrotic/early PMF	**Overt PMF**
Major criteria	
1	
Megakaryocytic proliferation and atypia, without reticulin fibrosis > grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often decreased erythropoiesis	Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis (grade 2 or 3)
2	
Not meeting WHO criteria for *BCR::ABL1* + CML, PV, ET, MDS or other myeloid neoplasm	Not meeting WHO criteria for *BCR::ABL1* + CML, PV, ET, MDS or other myeloid neoplasm
3	
Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker or absence of minor reactive BM reticulin fibrosis #	Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence, the presence of another clonal marker or absence of evidence for reactive BM fibrosis #
Minor criteria	
1	
Presence of one or more of the following, confirmed in two consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 × 10^9/L • Palpable splenomegaly • LDH level above the upper limit of the institutional reference range	Presence of one or more of the following, confirmed in two consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 × 10^9/L • Palpable splenomegaly • LDH level above the upper limit of the institutional reference range • Leukoerythroblastosis

Table 2: 2016 World Health Organization diagnostic criteria for primary myelofibrosis adapted from Barbui T et al. Blood Cancer J. 2015, Arber et al. Blood 2016, and Arber et al. Blood 2022 (3, 20, 21)
#Possible lack of mutation in children and adolescents

Given the differences described in the disease biology, alternative criteria for PV and ET in children have been proposed, with PV criteria including Hb or RBC count above the 97.5th percentile for age, and ET criteria granting the absence of reactive causes of thrombocytosis equal weight as the identification of a known driver mutation (22).

6.1.7 Complications

Overall, complications in MPN, e. g. thrombotic or hemorrhagic events, disease progression and transformation and other malignancies or therapy-associated sequelae appear to be significantly less common in children and adolescents with MPN compared to adult individuals (23, 24). However, these observations should be interpreted with caution because of the limited median follow-up in the respective analyses.
The incidence of thrombosis at diagnosis in children and adolescents was 14.7% and 4% in patients with PV and ET, respectively, and decreased in cases with PV thereafter. A clear predominance of venous vs. arterial events (84.2%) has been described and the majority of events occurred in the splanchnic veins (75%) in particular as Budd-Chiari syndrome (62.5% of venous events) (6). The probability of recurrent thrombotic events in pediatric MPN remains unclear based on the available data. Hemorrhagic events appeared to be very rare in children and adolescents with MPN (1% before and 4.8% after the diagnosis in ET patients and 4% before and after diagnosis in PV patients) and were not associated with the use of antithrombotic drugs (6).
Disease progression and transformation into secondary myelofibrosis and/or acute leukemia as the most serious complications in adult patients seem to occur rarely in pediatric MPN. Evolution into myelofibrosis was reported in only 2% of cases and transformation into acute myeloid leukemia was not described (6).
One previous study has found that a small proportion of pediatric cases with MPN might occur after previous treatment for acute leukemia or lymphoma (10). Data on the association of MPN and solid cancers is sparse and no systematic information on potential implications of previous chemotherapy or

cytoreductive drugs on the occurrence of MPN or cancer is available. Similarly, there are no systematic analyses of the association of MPN with non-malignant disease entities or complications of pregnancy.

6.1.8 Treatment

In most analyses on pediatric MPN, it remained unclear, whether treatment decisions and initiation were made by pediatric or adult hematologists (6). However, the general impression is, that due to the rarity of MPN in childhood and adolescents and the associated lack of age-appropriate diagnostic and therapeutic guidelines, adult hematologists, who are much more familiar with these diseases, are regularly consulted by the pediatrician.
Antithrombotic drugs such as aspirin, vitamin K antagonists and low molecular weight heparin seem to be employed in more than half of the cases with PV and ET and irrespective of the lack of high-risk features, most patients received cytoreductive treatment (6).
In larger analyses, pediatric patients with PV were treated with phlebotomy (45.2%) or received hydroxycarbamide (25.8%) whereas interferon appears to be more rarely employed (6). Children and adolescents with ET were regularly treated with the non-chemotherapeutic agents anagrelide (20.9%) and interferon alpha (4.6%). Notably, ruxolitinib, the inhibitor of *JAK1* and *JAK2*, which, since its approval, has become the standard-of-care treatment for adult patients with PMF and PV has so far been prescribed in single pediatric cases only (25).
Historically, a proportion of pediatric patients with MPN were treated with other conventional therapeutic approaches such as melphalan, busulfan, radiotherapy or allogeneic stem cell transplantation.

6.1.9 Outcome

The mortality rate of MPN in childhood and adolescence is low and the reported deaths were associated with vascular events such as Budd-Chiari syndrome (6).
However, it needs to be emphasized that the follow-up period of pediatric patients with MPN until transition to adult hematology is limited creating a reporting bias and the long-term outcome of MPN occurring early in life has not been systematically investigated yet.

6.2 Hypereosinophilic Syndrome

6.2.1 Background and classification

Hypereosinophilia and Hypereosinophilic Syndrome (HE/HES) belong to a heterogeneous group of myeloproliferative disorders characterized by an absolute eosinophil count (AEC) of ≥ 1.5 x G/L with associated end-organ involvement, extensive tissue hypereosinophilia or bone marrow hypereosinophilia of ≥ 20% with peripheral blood eosinophilia (26-30).
Hypereosinophilia and HES are also very rare in children and adolescents and data on the disease entity in these age groups is sparse. The incidence of pediatric HE/HES has been estimated with a rate of 3.5 per 100.000 children and adolescents per year (31). Due to variable clinical presentation, also HES might be underdiagnosed.
Based on the associated mechanism, HE/HES is classified as primary or secondary. For this registry, only children and adolescents with primary myeloid HE/HES (pHES) are eligible. However, primary HE/HES can be diagnosed only in a small minority of cases with HE/HES in adult and pediatric patients alike (8% and 11%, respectively [7]) caused by a malignant clonal myeloid or eosinophilic stem cell neoplasm. Underlying genetic events affect fusion genes or mutations involving tyrosine kinase pathways such as platelet-derived growth factor-alpha (PDGFR-α), platelet-derived growth factor-beta (PDGFR-β), fibroblast growth factor receptor 1 (FGFR1), and JAK2. Further causes of primary HE/HES comprise chronic eosinophilic leukemia, acute myeloid leukemia, systemic mastocytosis, MDS, classical MPNs (PV, ET and PMF) or MDS/MPN overlap disorders such as chronic myelomonocytic leukemia (CMML). The classical lymphocyte variant of primary HE/HES is associated with aberrant clonal T cells. Except for classical MPNs, these latter disease entities are not included in the registry.
Secondary HE/HES caused by defined underlying conditions are far more common than primary HE/HES. These cases are characterized by polyclonal expansion of eosinophils driven by increased production of cytokines, such as interleukin (IL) -3, IL-5 or granulocyte-macrophage colony-stimulating factor (GM-CSF). Secondary HE/HES are induced by a wide variety of conditions including allergies, infections, neoplasms, inflammatory syndromes, immunodeficiencies, drug hypersensitivities, and others.

6.2.2 Diagnosis of Pediatric HES

Hypereosinophilia/hypereosinophilic syndrome in children and adolescents with AEC ≥ 1.5 x G/L on more than 2 occasions more than 4 weeks apart with or without associate clinical symptoms should be further evaluated.
Owed to the potential severity of the disease, a primary hematological malignancy should be ruled out initially. Apart from a detailed history taking and physical examination including evaluation of fever, lymphadenopathy, organomegaly, pallor, and bleeding sings, the primary diagnostic approach includes the identification of potential additional organ involvement. The basic laboratory tests comprise blood count with differential, blood smear, lymphocyte subsets, serum-immunoglobulins with IgE, C-reactive protein, liver and renal function, urinalysis, lactate dehydrogenase, uric acid, serum tryptase, serum vitamin B12, and a basic metabolic panel.
Initial diagnostic imaging should include chest X-ray or chest/abdominal/pelvic CT or MRI.
Performing a bone marrow puncture for morphologic and cytogenetic/genetic analysis and a bone marrow biopsy for histological assessment should also be part of the diagnostic approach if hematological malignancy is suspected. Organ involvement should be addressed for example by targeted tissue biopsy, bronchoscopy including bronchoalveolar lavage, echocardiogram and electrocardiogram.
Further evaluation of secondary HE/HES comprises parasite serology with respect to possible exposure and diagnostics concerning immunodeficiency, autoinflammation and connective tissue disorders.
Pediatric patients with HE/HES may have a normal initial evaluation and should therefore be followed up including peripheral blood counts every two to six months.
Figure 2 represents a flowchart of the diagnostic evaluation in pediatric HE/HES.

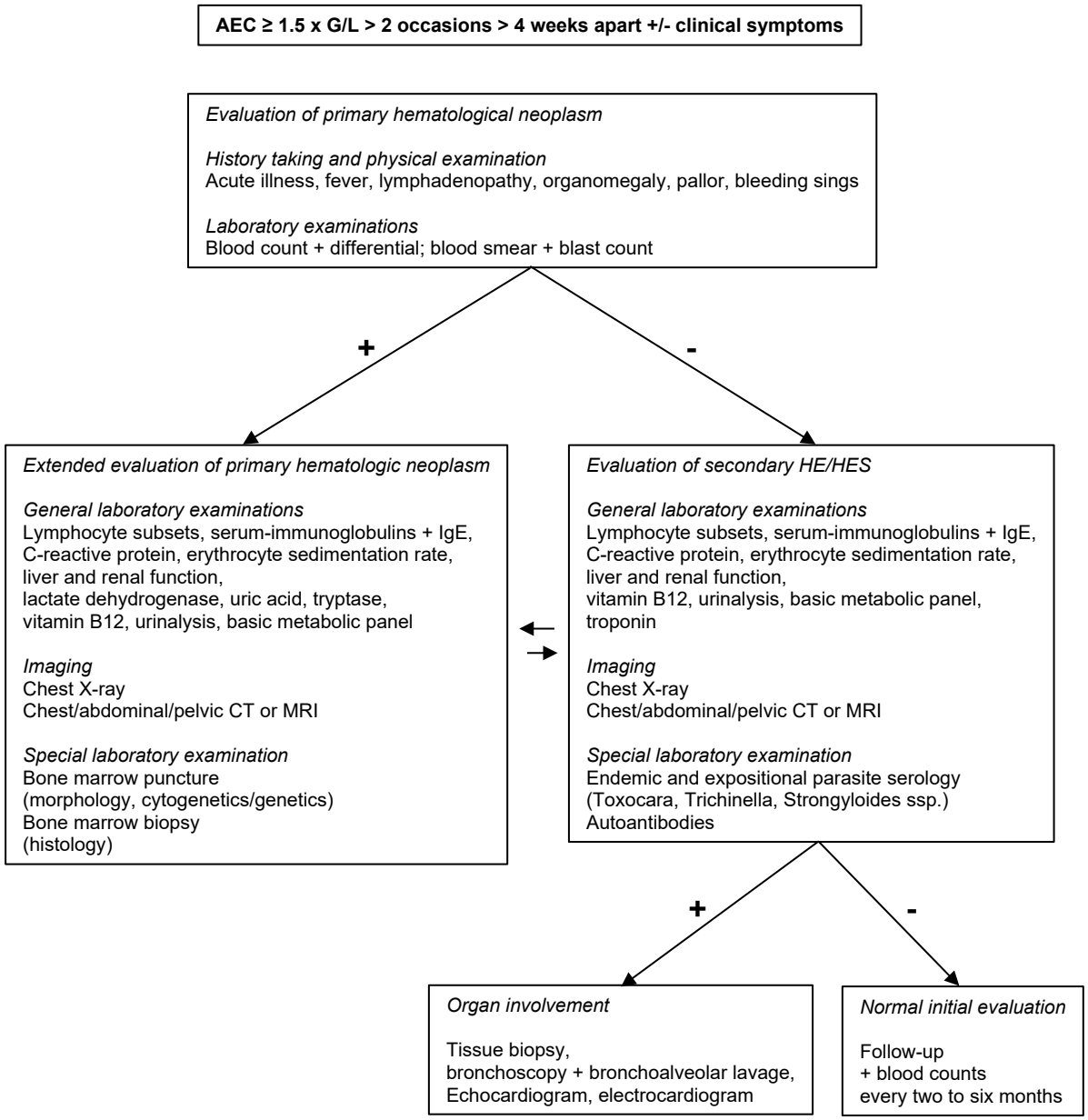


Figure 2: Diagnostic evaluation in pediatric patients presenting with HE/HES

6.2.3 Treatment of Pediatric HES

As for adults, first-line treatment in pediatric HE/HES is systemic high-dose steroid therapy. The usually applied dose is 1-2 mg/ kg body weight per day (29). Response to steroids is variable depending on the underling condition but usually occurs rapidly within a few days. In case response is lacking and the AEC and associated clinical symptoms do not improve, therapy should be extended.
Patients with underlying myeloid HES and FIP1L1- PDGFR-α fusion gene show a high response rate to imatinib (approved for pediatric CML and applied at a dose of 340 mg/m^2). However, in cases without a PDGFR-α alteration, response is rare (32, 33).
To date, mepolizumab, a monoclonal antibody acting through IL-5 antagonism, is the only biologic therapeutic agent approved by the U. S. Food and Drug Administration, which has shown effectiveness in a phase 3 randomized placebo-controlled trial in adolescents ≥ 12 years and adults with HE/HES (34). Mepolizumab is applied subcutaneously every 4 weeks at a dose of 300 mg.

Additional therapies including hydroxyurea, interferon-alpha and benralizumab, a humanized anti-human IL5-Rα antibody, can be used off-label (29). Other non-steroid agents such as Janus kinase inhibitors and dexpramipexole are currently being studied in the context of HE/HES treatment (35, 36). Generally, response to treatment is associated with the HE/HES subtype and the concomitant therapy of underlying conditions in secondary HE/HES. Like steroid therapy, IL-5 antagonism is presumably more efficient in non-myeloid HE/HES.

6.2.4 Complications and prognosis of Pediatric HES

Often, HE/HES becomes chronic and the median duration of the disease is 65.5 months in pediatric patients (37). Resolution of HE/HES-associated clinical symptoms and AEC < 0.5 G/L through HES-directed therapies is achieved in only a minority of cases. Adverse prognostic events include AEC > 100 G/L, congestive heart failure, underlying myeloproliferative neoplasm and resistance to steroids (29). Development of secondary malignancies poses an additional long-term risk in a proportion of patients. In children, the mortality associated with HE/HES is low (37).

7 Objectives

7.1 Rationale of the Registry

The rationale of the registry is to generally adapt and standardize the diagnostic approach for children and adolescents with MPN through a centralized morphologic, histology, genetic and cytogenetic review provided by the infrastructure of this registry.
This will provide us with the opportunity to systematically acquire data on the epidemiology and the clinical, hematological and genetic characteristics of childhood MPN, to identify patients with high risk of disease progression and to tailor the treatment to the distinctiveness of children and adolescents.

7.2 Primary Objectives

The primary objectives of the registry are:

- To determine the incidence and epidemiology of PV, ET, PMF and pHES in children and adolescents through systematic inclusion of these patients in a national population-based registry
- To systematically characterize clinical, hematologic, genetic and cytogenetic features of children and adolescents with PV, ET, PMF and pHES
- To identify pediatric patients with high risk of complications and disease progression and transformation into acute myeloid leukemia (AML)
- To improve the therapy for children and adolescents with MPN by discussing treatment options with the treating centers and tailor therapy to the specific and individual needs of pediatric patients

7.3 Secondary Objectives

The secondary objectives of the registry are:

- To prepare future international collaborative prospective studies focusing on the optimization and standardization of childhood MPN-diagnosis and treatment
- To develop a biobank system of pediatric patients with MPN

8 Allocation of the MPN Registry to other GPOH Registries

The MPN Childhood Registry is a project of a cooperative study group of pediatric hematologists, pathologists and geneticists mandated by the Society for Pediatric Oncology and Hematology (GPOH). Embedded in the GPOH-structure, it complements the diagnostic spectrum of the rare myeloid entities MDS, SAA, and CML and the hematologic disorders sickle cell disease and hemoglobinopathies (Figure 3).

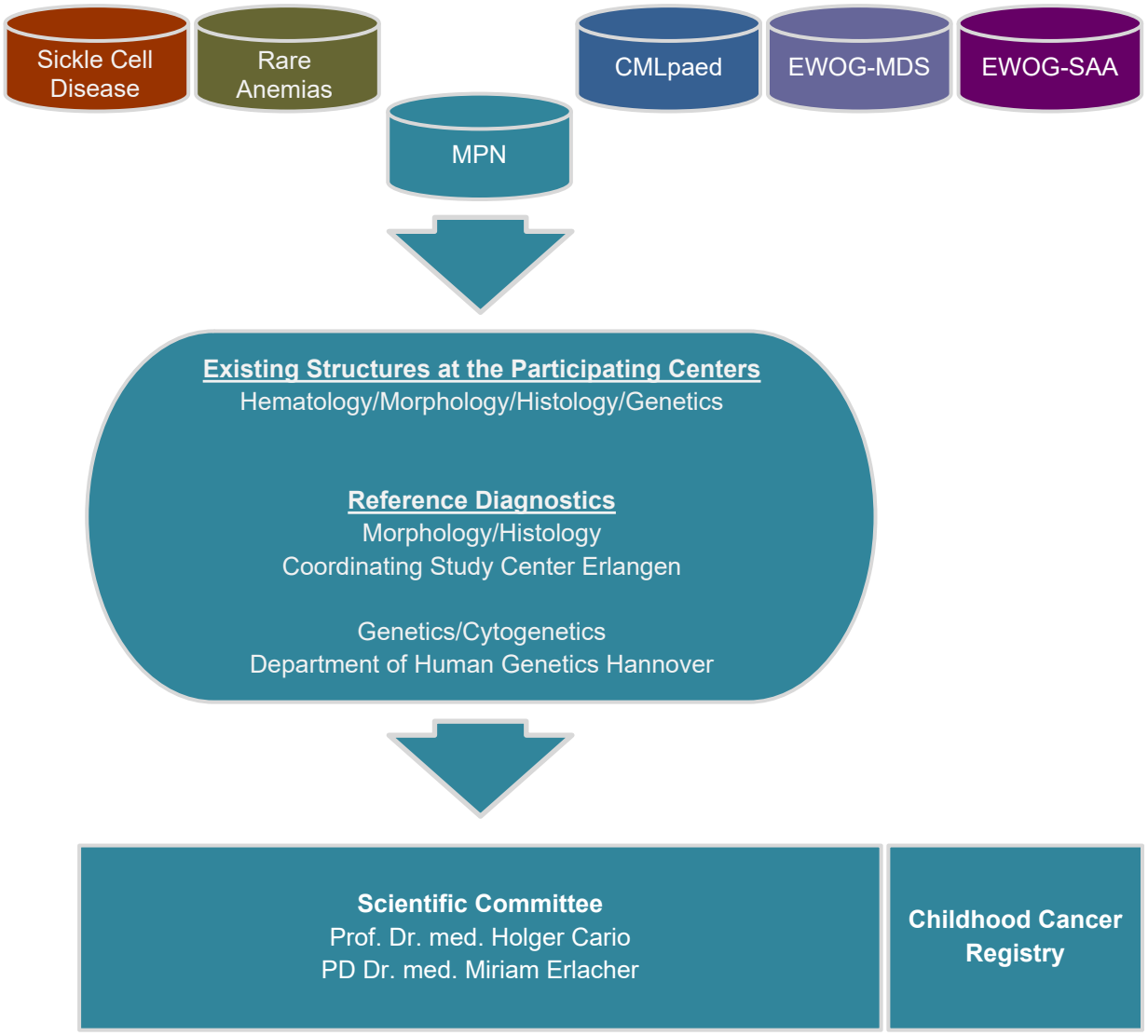


Figure 3: Allocation of the MPN Childhood Registry to other GPOH Registries

9 Investigational Plan

9.1 Registry/Study Design

This is a German national multicenter, retro-, and prospective, non-randomized, non-interventional registry. A registry biobank will be established.
The total duration of the registry is not defined, patients can remain in the registry until adulthood.

9.2 Participating Centers

All centers caring for pediatric patients with MPN.

9.3 Reference Laboratories

The reference clinical and morphological assessments in this registry are carried out by the coordinating study center at the Department of Pediatrics and Adolescent Medicine of the Friedrich-Alexander-Universität Erlangen-Nürnberg.
For histological assessment, bone marrow biopsies are sent to the coordinating study center and forwarded in-house for reference review to the institute of pathology of the Friedrich-Alexander-Universität Erlangen-Nürnberg.
Reference genetics including a somatic variant profiling based on targeted NGS approach and reference cytogenetics are performed at the Institute of Human Genetics at Hanover Medical School.

10 Patient population

10.1 Study Population

In the first year, we estimate 50 patients to be registered ("existing patients" and new diagnoses). Due to a lack of epidemiological data, further recruitment cannot be reliably estimated. However, based on the knowledge derived from previous investigations, we expect to be able to register between 10 and 20 patients per year. The number of registered patients should not be limited. Any center caring for pediatric patients with MPNs is invited to enroll patients.

10.2 Inclusion Criteria

Patients enrolled in this registry are to meet the following Inclusion Criteria:

- Newly diagnosed PV, ET, PMF or pHES according to the WHO classification and diagnostic criteria for myeloproliferative neoplasms (3)
- Age < 18 years
- Patient treated in a participating center
- Written informed consent to registry participation

10.3 Exclusion Criteria

Specific Exclusion Criteria for registration are:

- Hereditary or secondary polycythemia, thrombocytosis, myelofibrosis or HES with underlying reasons other than PV, ET, PMF or pHES.

11 Patient Enrollment and Registration

11.1 Time of Enrollment

It is planned to initiate registration on January 01, 2023.
In principle, registration is without any definite time limitation until adulthood is reached.

11.2 Minimal Requirements for Enrollment (Pre-Screening)

Patients meeting the diagnostic criteria for PV, ET, PMF, and pHES according to the current WHO guidelines modified for age (see also section 6) in the pre-screening process and the inclusion criteria are eligible for the registry.
Before a patient can be enrolled, the following minimal requirements and data sets need to be available:

- Informed consent for the registration in the MPN Childhood Registry signed by the patient and/or the patients` legal representative(s) is available
- Patient identification by name and gender
- Date of birth
- Date of diagnosis
- Hemoglobin level, platelet and white blood cell count
- Bone marrow with differential count

11.3 Mode of Enrollment

As soon as the diagnosis of PV, ET, PMF, or pHES is confirmed, the treating physician obtains the written informed consent of the patient and/or the patient's legal representative(s). The patient will be registered on a patient identification list located at the coordinating study center (CSC) and baseline characteristics including the full name, date of birth, and gender will be recorded. All of the Inclusion and none of the Exclusion Criteria of the study must be fulfilled.

The patient receives a consecutive patient identification number which is given by the CSC and consists of 2 letters reflecting the country code and 4 digits standing for consecutively registered patients.
With the registration, the documenting center confirms that a valid declaration of consent exists and is available.

12 Patient evaluation and data Acquisition

12.1 Study Schedule

In cases with suspected primary MPN, the patient undergoes the diagnostic procedures as outlined below. Once the diagnosis has been confirmed by reference diagnostics, a written informed consent is obtained from the patients and/or the patients` legal representative(s), and the patient is entered in the registry. Defined initial and follow-up data sets will be requested from the participating center by the CSC.
All diagnostic procedures are part of the routine examinations. No additional punctures other than required for routine diagnostic examinations are performed for the registry. For registry purposes 5-10 ml of peripheral blood and 2-3 ml of bone marrow are collected at routine examinations after the diagnosis of MPN has been confirmed and the patient has been included in the registry. Additionally, a buccal swab or 10-15 hair follicles will be collected once after registration.

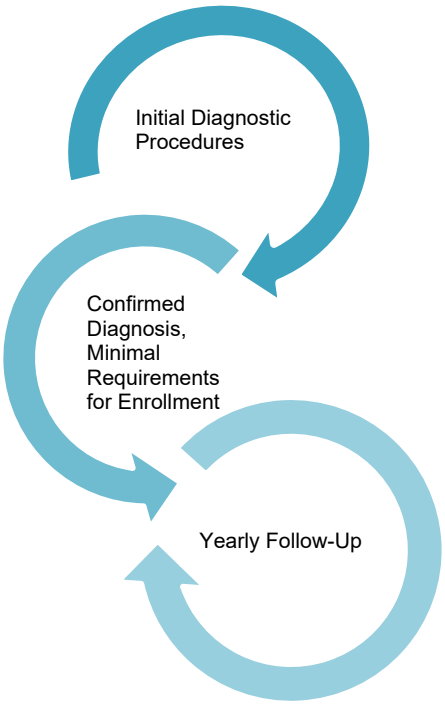


Figure 4: Study Design

12.2 Initial Diagnostic Procedures

All initial diagnostic procedures described here are part of routine diagnostics, which are generally required in case hematological diseases are suspected. They are carried out as part of patient care and are therefore not registry-specific. Patients are included in the registry only after the diagnosis of MPN has been confirmed. In the following, an overview over these common procedures is provided. The Schedule of Activities (section 4) lists the diagnostic procedures, which are performed.

12.2.1 Demographic Data and medical History

Patient demographics: full date of birth, date of diagnosis, age at diagnosis, sex, relevant medical history/current medical conditions (until date of signature of informed consent), disease details will be recorded.

12.2.2 Physical Findings

Physical findings including splenomegaly, hepatomegaly (with the spleen and liver size below costal margin by palpation or absolute organ size by ultrasound), lymphadenopathy, signs of thrombosis or bleeding, congenital abnormalities, xanthomas, café au lait spots, cranial nerve palsy, respiratory tract symptoms. Body weight in kilogram (kg), body height in centimeters (cm), and head circumference (in cm) with percentiles measured at the time of the initial diagnostics.

12.2.3 General Laboratory Examinations

Blood collection is based on local recommendations or guidelines for limitations of acceptable blood volume extraction limits based on participant age and/or weight. All clinical laboratory assessments are provided in the Schedule of Activities (section 4).

12.2.4 Specific Laboratory Examinations

Polycythemia vera and essential thrombocythemia can be suspected in case of persistent and otherwise unexplained elevation of the hemoglobin level, the hematocrit, and the platelet count above the normal values for age.

Underlying cardiovascular, infectious or inflammatory conditions or iron deficiency must be ruled out initially. Familial blood counts should also be evaluated.
If a primary non-familial cause remains likely, blood and bone marrow material including unstained smears and a bone marrow biopsy are sent to the CSC Erlangen for morphologic and pathologic review. In addition, NGS prognostic panel-based somatic variant screening including the genes *ASXL1, CBL, CALR, EZH2, IDH2, HRAS, JAK2, KRAS, MPL, NRAS, SF3B1, SRSF2, TP53*, standard fluorescence R-banding analysis (cytogenetics) and fluorescence in situ hybridization (FISH) are performed at the department of human genetics, Hanover medical school.
If the diagnostic criteria for PV and ET or other myeloid neoplasms such as CML or MDS are not met, but primary myelofibrosis is suspected based on persistent anemia, leukocytosis, palpable splenomegaly or elevated LDH-level, the diagnostic procedures are as described above.

Hypereosinophilia/Hyper eosinophilic syndrome should be further evaluated in children and adolescents with AEC ≥ 1.5 x G/L on more than 2 occasions more than 4 weeks apart with or without associate clinical symptoms according to the flowsheet shown in Figure 2 (6.2.2).
If a primary hematological disorder is suspected, blood and bone marrow material including unstained blood and bone marrow smears and a bone marrow biopsy are sent to the CSC Erlangen for morphologic and histopathologic review.
Molecular genetic detection of fusion genes (RNA panel sequencing) of *JAK2, FGFR1, PDGFRA, PDGFRB and mutation analysis of ASXL1, CKIT, DNMT3A, JAK2, SRSF2, STAT5B, TET2* are performed at the department of human genetics, Hanover medical school.

According to the obtained results, patients will be assigned to one of the following groups:

- Polycythemia vera (PV)
- Essential Thrombocythemia (ET)
- Primary Myelofibrosis (PMF)
- Primary Hypereosinophilic Syndrome (pHES)

In case PV, ET, PMF or pHES are diagnosed based on these diagnostic procedures, screening for inclusion in the registry is initiated.

12.3 Laboratory Tests/Special Examinations during Study Period

Like the initial diagnostic procedures, also the examinations during the study period are routine diagnostic procedures commonly required for the follow-up of confirmed hematologic diseases. They are performed in the context of patient care and thus, they are not registry-specific. The following paragraph provides a general routine scheme for follow-up procedures referred to by the diagnostics during the study period in Table 3.
Response to treatment in PV, ET, and PMF could initially be assessed through weekly, bi-weekly or at least monthly clinical evaluation including determination of peripheral blood counts until the normal values are almost reached. Thereafter and in cases without treatment, clinical evaluation and determination of peripheral blood counts monthly to every 3 months appear sufficient and the control intervals can even be stretched in the further course. In case of changing peripheral blood counts and/or suspected progression, repetition of BM morphology and histology as well as cytogenetic and genetic analysis should be considered.
Treatment response in pHES could primarily be assessed through weekly, bi-weekly or at least monthly clinical evaluation and determination of blood counts until stable hematologic response is achieved. In cases harboring a translocation, additional FISH-analysis can be performed from bone marrow and/or peripheral blood to verify cytogenetic response. Once stable hematologic and cytogenetic response is reached, determination of blood counts could be continued monthly to at least every 3 months under therapy.
Moreover, individual assays can be established to enable assessment of molecular response.

Diagnostics	Initially	During study period[1]	Address
Hematology	X	(X)[1]	
Morphology **→ 10 PB smears** **→ 10 BM smears** unstained **→ 5-10 ml Heparin-PB** **→ 2-3 ml Heparin-BM** at room temperature	X	(X)[1]	Dr. med. A. Karow Studienleitung MPN Universitätsklinikum Erlangen Kinder- und Jugendklinik Klinisches Labor/Zellbiologie Loschgestr. 15 D-91054 Erlangen
Histology **→ 1 BM biopsy** 10% formalin	X	(X)[1]	BM biopsy will be forwarded internally to Prof. Dr. med. M. Büttner-Herold Pathologisches Institut Universitätsklinikum Erlangen
Cytogenetics/FISH/Genetics **→ 5 ml Heparin-BM** **→ 5 ml EDTA-BM** at room temperature	X	(X)[1]	Dr. rer. nat. Yvonne Behrens Medizinische Hochschule Hannover Institut für Humangenetik OE 6300 Carl-Neuberg-Str. 1 D-30625 Hannover

Table 3: Initial special laboratory examinations and disease monitoring
[1]A general routine scheme for diagnostic procedures during the study period in classical MPN and pHES is provided in 12.3

The following data is recorded for patient receiving disease-specific and/or concomitant treatment or no treatment at each at the treating center or at least once per year:

- Treatment/Medication: body weight, length; start/end/ongoing therapy, drug, dosage
- Every bone marrow examination along with complete blood count with differential (including reticulocytes)
- In the absence of a bone marrow examination the first peripheral blood count indicating progress
- In the absence of progress, the last peripheral blood count
- Bone marrow with differential
- Genetics/Cytogenetics: Every analysis (metaphase and FISH)
- NGS panel-based somatic variant screening: Every analysis
- Date of last examination
- Karnofsky score

- Survival: stable disease, complete remission (CR), progress/relapse (date, site, kind of progress/relapse)
- Complications including thrombotic or hemorrhagic events or organ involvement
- Toxicity
- Secondary malignancy (date of diagnosis, diagnosis)
- Death (date, cause)

12.4 Patients Undergoing HSCT

For patients undergoing HSCT, the recorded data largely corresponds to the EBMT-follow-up.
In addition, MPN-specific aspects including pretransplant disease course, treatment, and status of disease, hematological values, bone marrow investigation, and genetic/cytogenetic results before transplantation, conditioning regimen, donor, graft manipulation, engraftment, remission status posttransplant, GvHD, and other complications will be documented.

12.5 Duration of Study Participation

There is no maximum time of follow-up nor are there any defined dropout criteria for registered patients. For patients lost to follow-up the last known set of data should be retrieved and recorded.

13 Management of Patient Data and Patient Material

13.1 Recording of Data

The following data sets should be recorded in the provided CRFs according to the GCP-guidelines:

1. Basic data: One-off registration and collection of unchangeable basic data.
2. Annual survey: The clinical, laboratory, and treatment data since the last survey are documented here.
3. Completion of the documentation: Here it is documented when a patient withdraws the consent to participate in the registry, has moved abroad, or has died.

The annual data collection is not time-limited in order to be able to make statements about long-term trends. However, data collection and storage will be terminated when all registry-specific issues have been finally clarified or can no longer be clarified.
Potential transfer of data from and to registries the patient is participating in requires that the patient has consented to the participation of all registries/studies involved.

13.2 Database

The baseline and annual follow-up data are entered into the paper-based CRFs by the participating centers and sent to the CSC.
The data will then be entered and stored in the Research Electronic Data Capture (REDCap®). The personally identifying data (IDAT) are stored separately from the medical data (MDAT) in accordance with §40 BDSG. REDCap® is provided by the Center of Medical Information and Communication Technology (MIK), University Hospital Erlangen and guarantees that the data is stored, secured and validated in accordance with the law. Technical details of the database used can be found in the REDCap® process description.

13.3 Use of Patients` Material

Peripheral blood, bone marrow aspirate, and bone marrow biopsies are retrieved for routine reference diagnostics to confirm the diagnosis of MPN and to examine the course of the disease.
If the patient/ the patient`s legal representative gave informed consent for the use of material for non-commercial research purposes regarding the disease, material from peripheral blood and bone marrow remaining after completion of routine diagnostics is stored. In this case, a buccal swab or hair follicles is/are additionally collected from the patient once after registration and also stored. The remaining material from peripheral blood and bone marrow and the material from a buccal swab or hair follicles are used for scientific studies on genetic and functional alterations in childhood MPN. The coordinator's research group has the right to conduct the research.
No patient is undergoing an additional invasive procedure in order to gain material for research only.
Every patient/ legal representative may state if she/ he wants to be informed about the research results.
Future studies using the material of the biobank will be advised separately by an ethics committee, since the technical possibilities and data protection conditions can change unpredictably.

13.4 Asservation of Patients` Material and Biobanking

At diagnosis, at regular routine control intervals, at disease progress, prior to possible HSCT and at relapse, material from peripheral blood and bone marrow will be retrieved.

The following material will be used primarily for routine diagnostic purposes:

- 6 smears from PB
- 6 smears from BM
- 2 biopsies from BM

In addition, the following material will be collected as backup for routine diagnostics and/or for research purposes:

- Minimum of 5 ml of heparinized/ EDTA-PB
- Minimum of 5 ml of heparinized/ EDTA-BM
- Buccal swab or 10-15 hair follicles (once after initial diagnosis)

The samples are sent from the treating center to the CSC, where they will be processed and stored as part of the certified Central Biobank Erlangen (CeBE) linked to the registry-specific pseudonym.

The material will be stored as follows:

- Smears from PB and BM will be frozen at -80 °C.
- Cells in PB and BM will be separated by a Ficoll gradient. Mononuclear cells (MNC) will be frozen according to standard procedures.
- DNA from buccal swab or hair follicles and DNA and RNA from MNC will be extracted and stored according to standard procedures. DNA from granulocytes in the Ficoll pellet will be extracted after red cell lysis and stored according to standard procedures.

To obtain germline DNA, hair follicles or a buccal swab should be retrieved from all patients after diagnosis. Cytogenetic material is stored at the reference laboratory.

Upon evaluated application to the CSC, material can be made available for research purposes. Until then, only the study group and, if necessary, for processing and storage, the biobank staff will have access to the data and biomaterials. The aim of the biobank is to store the samples for the investigation of the pathophysiology of MPN in childhood and adolescence. Findings from these investigations should be used as soon as they are relevant for the treatment of individual patients and made available to the

attending physician. If the patient/legal representatives withdraw from participation and/or refuse further storage, the samples will be destroyed.

The biobanking procedures of this protocol will be embedded in the certified Central Biobank Erlangen (CeBE), a voluntary non-profit organizational association of quality-assured biobanks located in Erlangen. These institutions share a common organization, common procedures and platform structures concerning IT, data, quality and stakeholder management under the patronage of the medical faculty of the Friedrich-Alexander Universität Erlangen-Nürnberg (FAU) and the University Hospital Erlangen (UKER). The primary objective of the CeBE is to collect, characterize, register, archive and process human biosamples at high quality for research purposes and to support and promote scientific projects of the medical faculty of the University of Erlangen-Nürnberg, the University Hospital of Erlangen and all other member institutions of the CeBE in accordance with the Declaration of Taipei. The Department of Pediatrics and Adolescent Medicine of the Friedrich-Alexander-Universität Erlangen-Nürnberg is an integral part and thus a contractual user of the CeBE.
Biosamples and data are submitted to the CeBE upon written request of the user via the sub-biobanks of the CeBE according to the valid data and bio sample usage regulations, unless otherwise regulated. The prerequisites for this are a defined scientific project, a relevant, valid ethics vote and the written consent of the patients and/or their legal representatives or subjects. The data and biosamples Use & Access Committee (UAC) of the Erlangen University Hospital decides on the assignment under consideration of the respective consents and all applicable data protection regulations. Contributing clinical institutions have unrestricted access to samples and data they have brought in and must always be consulted by other working groups and consent before a project using these samples and/or data can be approved. The sale or the transfer of collected biosamples or their derivatives for commercial use without appropriate specific approval from the board of directors and the contributing clinical facility is excluded.

14 Quality Assurance

Quality assurance within the MPN Childhood Registry will be conveyed through the Advisory Board and an authorized supervision as described below.

14.1 Scientific Committee

The Scientific Committee including a senior advisor meets at least once every two years and discusses study issues, interim data, and analyses in order to guarantee the scientific value of the study.

14.2 Authorized Supervision

The members of the CSC including the principal investigator will have the duty of being authorized supervisors.
The authorized supervisors will stay in regular contact with the participating centers mostly by e-mail and telephone to gather information about the compliance with the study protocol requirements, completeness and plausibility of the data in the CRF and conformity with the original data, the updated patient identification lists, and the archiving system. Thus, the progress of the registry will be controlled and problems will be realized and addressed early.
The authorized supervisors sign that they will handle all data that are under professional secrecy or show the patient's identity confidentially and will use the data only for the purpose the patient gave informed consent for. No data disclosing the identity of patients will leave the CSC as a result of the monitoring procedure.

14.3 Data monitoring

Monitoring of the data with verification of the source data is not planned. However, entered data is subjected to a plausibility and completeness check by the CSC. Ambiguities and discrepancies are clarified with the documenting center. Audit trails regulate document access and entry of data in REDCap® on a personal basis.

15 Statistics

Statistical evaluations of the patient data sets registered to date are envisaged one year after the start of recruitment and then at annual intervals. The demographic data, incidence, disease subgroups, treatment and course of the disease will be addressed. Scientific findings are published in relevant medical journals.
Due to the aims of the registry, the statistical methods will be largely descriptive and exploratory and carried out according to standard methods.

16 Conditions for Protocol Amendments

16.1 Changes in the Protocol

Any modifications of this protocol require a written protocol amendment and the joint and signed agreement of the coordinating investigator, the deputy coordinating investigator and the members of the advisory board. After approval, an amendment becomes effective as an integral part of the protocol and all participating centers will be informed of the amendment by the CSC.

17 Ethical and Legal Considerations

The registry will be conducted in accordance with the Declaration of Helsinki in the currently valid version. Before the initiation of the registry, the registry protocol, the patient information and the proposed informed consents are submitted to the ethics committee of the Medical Faculty Erlangen. In addition, the participating centers submit the documents to their responsible ethics committees for consultation.
Modifications to the registry protocol changing its purpose or the handling of patient data or material will be reported to the responsible ethics committee. Changes that only affect the input interface for data in REDCap® do not require a new vote by the ethics committee.
By written consent to this protocol, the investigator declares to ensure compliance and to grant full access to all documentation by authorized individuals.

17.1 Patient Information and Informed Consent

The signing of the informed consent by the patient and/or the patient's parents/legal guardians is a prerequisite for registry participation. The registry does not pose any risks for the participants, since no registry-specific interventions are foreseen.
Prior to inclusion in the MPN Childhood Registry, each patient and/or the patient's parents/legal guardians will be carefully informed by the treating physician on the nature, objectives, expected benefits, potential risks and duration of the registry. The patient and/or parents/legal representatives must be given sufficient time and opportunity to decide whether to participate and to clarify any open questions before consenting. The declaration of consent is personally dated and signed by the patient and/or both parents/legal representatives and the attending physician. Patient participation in the study is voluntary. In the case of underage patients, the parents/legal representatives will be informed and they will document their consent by signing the informed consent form. The presumed will of the patient must be considered. If the patient is able to recognize the nature, meaning and scope of the consent and to express her/his will accordingly, she/he will also be informed in an age-appropriate manner and asked to give consent to participate in the registry. For this purpose, an information leaflet tailored to age groups in child-friendly and age-appropriate wording is provided.
Patients who reach the age of 18 while participating in the registry can continue to participate in the registry if they (again) consent to participate.
The patient and/or the patient's parents/legal guardians will receive a copy of the written informed consent once signed, and the original version of the informed consent has to be kept in the investigator`s file.

17.2 Data protection

Since May 25th, 2018, data protection has been regulated by law on the European level through the General Data Protection Regulation (EU-GDPR). Patient data is collected, processed and stored in the registry for research purposes. All persons who have access to the stored data and confidential information are also subject to professional secrecy, the Federal Data Protection Act (FDPA) and the State Data Protection Act (SDPA).
Confidentiality is maintained throughout the registration period and beyond. Medical data is only accessible to experts in the context of scientific work. The registry data is collected from the treating center. For this purpose, paper CRFs are completed with patient plain data by the treating center and sent to the CSC, where they are entered in the REDCap® database and stored double pseudonymized with restricted access. Double pseudonymization will be ensured by double encryption with a different code in each case. The decryption lists will be kept in the CSC. Pseudonymized registry data can be passed on to scientists outside of the study cooperation for the above-mentioned research purposes upon written request. Personally identifiable data will not leave the coordinating study center. Third parties do not have access to original documents. The collection and storage of data is terminated when the registry-specific issues have been finally clarified or can no longer be clarified, or the affected patient/legal guardian(s) withdraw the consent for the registry and do not agree to further storage and processing.
Depending on patient characteristics, for example when a patient undergoes an allogeneic stem cell transplant, the treating physician or the registry management can ask the patient to participate in other registries, such as the PRST (= Pediatric Registry for stem cell transplantation)/ EBMT (= European Group for Blood and Marrow Transplantation).
The transfer of data between different registries requires that the patient has consented to the participation of all registries/studies involved.

Any data transferred to third parties for comparative analyses in this registry will be completely anonymized. Therefore, the patients/legal representatives do not have to consent to the transfer of this data separately.

Consent to sample storage in a biobank and sample transfer for research purposes
The storage of samples collected in the registry takes place in the certified Central Biobank Erlangen (CeBE). Consent to store and process patient samples is included in the consent to participate in the MPN Childhood Registry.
The registry management is responsible for administration and storage of samples. The samples are only made available for research purposes upon specific request. The samples and the donor data are stored double pseudonymized and the identifying "keys" remain within the CSC. The data are subject to medical confidentiality and the provisions of the Federal Data Protection Act (FDPA) and the General Data Protection Regulation (EU-GDPR). Storage and processing terminate when the registry-specific issues have been finally clarified or can no longer be clarified, or the affected patients/legal guardians withdraw from the registry and do not agree to further storage and processing.
If genetic tests are to be carried out, a separate consent must be obtained from the patient/legal guardian in accordance with the Genetic Diagnostics Act.

17.3 Patient withdrawal

The consent of the legal guardian or the patient themselves can be withdrawn at any time without giving reason and without any disadvantages for further medical care. Data and/or material that has already been obtained will then be destroyed if the patients/guardians do not agree to the evaluation and further storage. Date of withdrawal, all recorded results at this time, and, if known, the reasons for discontinuance are to be documented in the CRF. If possible, a final examination has to be done.

17.4 Termination criteria for the overall study

The registry will be terminated if events occur or information becomes known making it unjustified to continue the registry. The registry will be terminated if no financial means are available for continuation. If the patient has consented, data and biomaterials can be pseudonymized for research purposes and stored beyond the duration of the study.

17.5 Insurance

Because due to the registry, no novel or controlled therapeutic measures or interventions take place, patient insurance is not foreseen.

18 Study Documents and Archiving of Records

18.1 Investigator`s File

The investigator's file contains all relevant documents including regulatory and study documents, correspondence with ethics committee and general information. The investigator's file has to be accessible for audits and authorized inspections and will be kept within the CSC according to the ICH-GCP-guidelines and legal regulations for a minimum of 15 years.

18.2 Documentation of Patient Data

Case Report Form (CRF):
The registry data will be documented continuously, accurately, plausibly and as completely as possible by the investigator or the representatives in the CRF.

Documentation of data in the patient`s file:
The participation of the registry, the frequency of the visits, disease-associated data, examinations, diagnostic evaluations and concomitant treatment will be documented in the patient`s file by the investigator or the representatives.

Patient Identification List:
The investigator has to keep a patient identification list according to the ICH-GCP-guidelines allowing precise correlation of the patient`s identity to her/his inclusion in the registry.
The patient identification list will contain the following information:

- Full patient name
- Date of birth
- Gender
- Fulfillment of the inclusion and exclusion criteria of the registry

18.3 Archiving of Records

According to the German law and the ICH-GCP-guidelines, the complete investigational records will be stored safely in the Master File at the CSC for a minimum of 15 years.
These will contain originals of complete documentation and copies of outgoing correspondence.

19 Administrative Considerations

19.1 Financing

The registry will be supported by public grants. There is no support from the industry.

19.2 Data Evaluation and Reporting

The data is evaluated internally on a yearly basis. The planning, implementation and evaluation are oriented to the guidelines and recommendations for ensuring Good Epidemiological Practice (GEP). Evaluations of data from the registry database can be requested from the registry management. The transfer will be contractually regulated and limited to anonymous, aggregated or evaluated data.

19.3 Publication of Results

On a national level, the publication rules of the GPOH apply (GPOH study rules, 9th version, version 05/2010).
For international collaborations on childhood MPN, any formal presentation or publication of data collected as a direct or indirect result of this registry will be considered as a joint publication by the collaborators. It requires the agreement of the Principal Investigator and all Collaborators. Authorship will be determined by mutual agreement.

20 References

1. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2017 update on diagnosis, risk-stratification, and management. Am J Hematol. 2017 Jan;92(1):94-108. Epub 2016/12/20. doi:10.1002/ajh.24607. Cited in: Pubmed; PMID 27991718.

2. Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, Harrison C, Hasselbalch HC, Hehlmann R, Hoffman R, Kiladjian JJ, Kroger N, Mesa R, McMullin MF, Pardanani A, Passamonti F, Vannucchi AM, Reiter A, Silver RT, Verstovsek S, Tefferi A, European L. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. J Clin Oncol. 2011 Feb 20;29(6):761-70. Epub 2011/01/06. doi:10.1200/JCO.2010.31.8436. Cited in: Pubmed; PMID 21205761.

3. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016 May 19;127(20):2391-405. Epub 2016/04/14. doi:10.1182/blood-2016-03-643544. Cited in: Pubmed; PMID 27069254.

4. Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, Gangat N, Fjerza R, Belachew AA, Lasho TL, Ketterling RP, Hanson CA, Rambaldi A, Finazzi G, Thiele J, Barbui T, Pardanani A, Vannucchi AM. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood. 2014 Oct 16;124(16):2507-13; quiz 2615. Epub 2014/07/20. doi:10.1182/blood-2014-05-579136. Cited in: Pubmed; PMID 25037629.

5. Barbui T. How to manage children and young adults with myeloproliferative neoplasms. Leukemia. 2012 Jul;26(7):1452-7. Epub 2012/01/19. doi:10.1038/leu.2012.12. Cited in: Pubmed; PMID 22252311.

6. Ianotto JC, Curto-Garcia N, Lauermanova M, Radia D, Kiladjian JJ, Harrison CN. Characteristics and outcomes of patients with essential thrombocythemia or polycythemia vera diagnosed before 20 years of age: a systematic review. Haematologica. 2019 Aug;104(8):1580-1588. Epub 2019/01/27. doi:10.3324/haematol.2018.200832. Cited in: Pubmed; PMID 30679326.

7. Kucine N. Myeloproliferative Neoplasms in Children, Adolescents, and Young Adults. Curr Hematol Malig Rep. 2020 Apr;15(2):141-148. Epub 2020/03/17. doi:10.1007/s11899-020-00571-8. Cited in: Pubmed; PMID 32172359.

8. Martin K. Risk Factors for and Management of MPN-Associated Bleeding and Thrombosis. Curr Hematol Malig Rep. 2017 Oct;12(5):389-396. Epub 2017/09/28. doi:10.1007/s11899-017-0400-3. Cited in: Pubmed; PMID 28948496.

9. Pastor V, Hirabayashi S, Karow A, Wehrle J, Kozyra EJ, Nienhold R, Ruzaike G, Lebrecht D, Yoshimi A, Niewisch M, Ripperger T, Gohring G, Baumann I, Schwarz S, Strahm B, Flotho C, Skoda RC, Niemeyer CM, Wlodarski MW. Mutational landscape in children with myelodysplastic syndromes is distinct from adults: specific somatic drivers and novel germline variants. Leukemia. 2017 Mar;31(3):759-762. Epub 2016/11/24. doi:10.1038/leu.2016.342. Cited in: Pubmed; PMID 27876779.

10. Cario H, McMullin MF, Pahl HL. Clinical and hematological presentation of children and adolescents with polycythemia vera. Ann Hematol. 2009 Aug;88(8):713-9. Epub 2009/05/27. doi:10.1007/s00277-009-0758-y. Cited in: Pubmed; PMID 19468728.

11. Giona F, Teofili L, Moleti ML, Martini M, Palumbo G, Amendola A, Mazzucconi MG, Testi AM, Pignoloni P, Orlando SM, Capodimonti S, Nanni M, Leone G, Larocca LM, Foa R. Thrombocythemia and polycythemia in patients younger than 20 years at diagnosis: clinical and biologic features, treatment, and long-term outcome. Blood. 2012 Mar 8;119(10):2219-27. Epub 2012/01/21. doi:10.1182/blood-2011-08-371328. Cited in: Pubmed; PMID 22262773.

12. Teofili L, Giona F, Martini M, Cenci T, Guidi F, Torti L, Palumbo G, Amendola A, Leone G, Foa R, Larocca LM. The revised WHO diagnostic criteria for Ph-negative myeloproliferative diseases are not appropriate for the diagnostic screening of childhood polycythemia vera and essential thrombocythemia. Blood. 2007 Nov 1;110(9):3384-6. Epub 2007/07/24. doi:10.1182/blood-2007-06-094276. Cited in: Pubmed; PMID 17644735.

13. Fu R, Liu D, Cao Z, Zhu S, Li H, Su H, Zhang L, Xue F, Liu X, Zhang X, Cheng T, Yang R, Zhang L. Distinct molecular abnormalities underlie unique clinical features of essential thrombocythemia in children. Leukemia. 2016 Mar;30(3):746-9. Epub 2015/06/30. doi:10.1038/leu.2015.167. Cited in: Pubmed; PMID 26118316.

14. Giona F, Teofili L, Capodimonti S, Laurino M, Martini M, Marzella D, Palumbo G, Diverio D, Foa R, Larocca LM. CALR mutations in patients with essential thrombocythemia diagnosed in childhood and adolescence. Blood. 2014 Jun 5;123(23):3677-9. Epub 2014/06/07. doi:10.1182/blood-2014-04-572040. Cited in: Pubmed; PMID 24904106.

15. Karow A, Nienhold R, Lundberg P, Peroni E, Putti MC, Randi ML, Skoda RC. Mutational profile of childhood myeloproliferative neoplasms. Leukemia. 2015 Dec;29(12):2407-9. Epub 2015/08/01. doi:10.1038/leu.2015.205. Cited in: Pubmed; PMID 26223499.

16. Langabeer SE, Haslam K, McMahon C. CALR mutations are rare in childhood essential thrombocythemia. Pediatr Blood Cancer. 2014 Aug;61(8):1523. Epub 2014/02/14. doi:10.1002/pbc.24984. Cited in: Pubmed; PMID 24523226.

17. Putti MC, Pizzi M, Bertozzi I, Sabattini E, Micalizzi C, Farruggia P, Ramenghi U, Cesaro S, Russo G, Peroni E, Rugge M, Fabris F, Randi ML. Bone marrow histology for the diagnosis of essential thrombocythemia in children: a multicenter Italian study. Blood. 2017 Jun 1;129(22):3040-3042. Epub 2017/04/15. doi:10.1182/blood-2017-01-761767. Cited in: Pubmed; PMID 28408463.

18. Randi ML, Geranio G, Bertozzi I, Micalizzi C, Ramenghi U, Tucci F, Notarangelo LD, Ladogana S, Menna G, Giordano P, Consarino C, Farruggia P, Zanazzo GA, Fiori GM, Burnelli R, Russo G, Jankovich M, Peroni E, Duner E, Basso G, Fabris F, Putti MC. Are all cases of paediatric essential thrombocythaemia really myeloproliferative neoplasms? Analysis of a large cohort. Br J Haematol. 2015 May;169(4):584-9. Epub 2015/02/27. doi:10.1111/bjh.13329. Cited in: Pubmed; PMID 25716342.

19. Sekiya Y, Okuno Y, Muramatsu H, Ismael O, Kawashima N, Narita A, Wang X, Xu Y, Hama A, Fujisaki H, Imamura T, Hasegawa D, Kosaka Y, Sunami S, Ohtsuka Y, Ohga S, Takahashi Y, Kojima S, Shimada A. JAK2, MPL, and CALR mutations in children with essential thrombocythemia. Int J Hematol. 2016 Aug;104(2):266-7. Epub 2016/05/23. doi:10.1007/s12185-016-2022-2. Cited in: Pubmed; PMID 27209416.

20. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Rationale for revision and proposed changes of the WHO diagnostic criteria for polycythemia vera, essential thrombocythemia and primary myelofibrosis. Blood Cancer J. 2015 Aug 14;5:e337. Epub 2016/02/03. doi:10.1038/bcj.2015.64. Cited in: Pubmed; PMID 26832847.

21. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, Wang SA, Bagg A, Barbui T, Branford S, Bueso-Ramos CE, Cortes J, Dal Cin P, DiNardo CD, Dombret H, Duncavage EJ, Ebert BL, Estey E, Facchetti F, Foucar K, Gangat N, Gianelli U, Godley LA, Goekbuget N, Gotlib JR, Hellstrom-Lindberg E, Hobbs G, Hoffman R, Jabbour EJ, Kiladjian JJ, Larson RA, Le Beau MM, Loh ML, Lowenberg B, Macintyre EA, Malcovati L, Mullighan CG, Niemeyer CM, Odenike O, Ogawa S, Orfao A, Papaemmanuil E, Passamonti F, Porkka K, Pui CH, Radich JP, Reiter A, Rozman M, Rudelius M, Savona MR, Schiffer C, Schmitt-Graeff A, Shimamura A, Sierra J, Stock W, Stone RM, Tallman MS, Thiele J, Tien HF, Tzankov A, Vannucchi AM, Vyas P, Wei AH, Weinberg OK, Wierzbowska A, Cazzola M, Dohner H, Tefferi A. International Consensus Classification of Myeloid Neoplasms and Acute Leukemia: Integrating Morphological, Clinical, and Genomic Data. Blood. 2022 Jun 29. Epub 2022/06/30. doi:10.1182/blood.2022015850. Cited in: Pubmed; PMID 35767897.

22. Kucine N, Al-Kawaaz M, Hajje D, Bussel J, Orazi A. Difficulty distinguishing essential thrombocythaemia from polycythaemia vera in children with JAK2 V617F-positive myeloproliferative neoplasms. Br J Haematol. 2019 Apr;185(1):136-139. Epub 2018/05/17. doi:10.1111/bjh.15386. Cited in: Pubmed; PMID 29767848.

23. Tefferi A, Vannucchi AM, Barbui T. Polycythemia vera treatment algorithm 2018. Blood Cancer J. 2018 Jan 10;8(1):3. Epub 2018/01/13. doi:10.1038/s41408-017-0042-7. Cited in: Pubmed; PMID 29321547.

24. Tefferi A, Vannucchi AM, Barbui T. Essential thrombocythemia treatment algorithm 2018. Blood Cancer J. 2018 Jan 10;8(1):2. Epub 2018/01/13. doi:10.1038/s41408-017-0041-8. Cited in: Pubmed; PMID 29321520.

25. Coskun ME, Height S, Dhawan A, Hadzic N. Ruxolitinib treatment in an infant with JAK2+ polycythaemia vera-associated Budd-Chiari syndrome. BMJ Case Rep. 2017 Jul 14;2017. Epub 2017/07/16. doi:10.1136/bcr-2017-220377. Cited in: Pubmed; PMID 28710306.

26. Rosenberg CE, Fulkerson PC, Williams KW. Diagnosis and Management of Pediatric Hypereosinophilic Syndrome. J Allergy Clin Immunol Pract. 2022 May;10(5):1131-1138. Epub 2022/02/20. doi:10.1016/j.jaip.2022.02.007. Cited in: Pubmed; PMID 35181546.

27. Schwartz JT, Fulkerson PC. An Approach to the Evaluation of Persistent Hypereosinophilia in Pediatric Patients. Front Immunol. 2018;9:1944. Epub 2018/09/21. doi:10.3389/fimmu.2018.01944. Cited in: Pubmed; PMID 30233571.

28. Butt NM, Lambert J, Ali S, Beer PA, Cross NC, Duncombe A, Ewing J, Harrison CN, Knapper S, McLornan D, Mead AJ, Radia D, Bain BJ, British Committee for Standards in H. Guideline for the investigation and management of eosinophilia. Br J Haematol. 2017 Feb;176(4):553-572. Epub 2017/01/24. doi:10.1111/bjh.14488. Cited in: Pubmed; PMID 28112388.

29. Klion AD. How I treat hypereosinophilic syndromes. Blood. 2015 Aug 27;126(9):1069-77. Epub 2015/05/13. doi:10.1182/blood-2014-11-551614. Cited in: Pubmed; PMID 25964669.

30. Ogbogu PU, Klion AD. Hypereosinophilic disorders. J Allergy Clin Immunol Pract. 2015 Mar-Apr;3(2):304-5; quiz 306. Epub 2015/03/11. doi:10.1016/j.jaip.2014.09.024. Cited in: Pubmed; PMID 25754721.

31. Burris D, Rosenberg CE, Schwartz JT, Zhang Y, Eby MD, Abonia JP, Fulkerson PC. Pediatric Hypereosinophilia: Characteristics, Clinical Manifestations, and Diagnoses. J Allergy Clin Immunol Pract. 2019 Nov - Dec;7(8):2750-2758 e2. Epub 2019/05/28. doi:10.1016/j.jaip.2019.05.011. Cited in: Pubmed; PMID 31128377.

32. Ogbogu PU, Bochner BS, Butterfield JH, Gleich GJ, Huss-Marp J, Kahn JE, Leiferman KM, Nutman TB, Pfab F, Ring J, Rothenberg ME, Roufosse F, Sajous MH, Sheikh J, Simon D, Simon HU, Stein ML, Wardlaw A, Weller PF, Klion AD. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. J Allergy Clin Immunol. 2009 Dec;124(6):1319-25 e3. Epub 2009/11/17. doi:10.1016/j.jaci.2009.09.022. Cited in: Pubmed; PMID 19910029.

33. Khoury P, Desmond R, Pabon A, Holland-Thomas N, Ware JM, Arthur DC, Kurlander R, Fay MP, Maric I, Klion AD. Clinical features predict responsiveness to imatinib in platelet-derived growth factor receptor-alpha-negative hypereosinophilic syndrome. Allergy. 2016 Jun;71(6):803-10. Epub 2016/01/23. doi:10.1111/all.12843. Cited in: Pubmed; PMID 26797802.

34. Roufosse F, Kahn JE, Rothenberg ME, Wardlaw AJ, Klion AD, Kirby SY, Gilson MJ, Bentley JH, Bradford ES, Yancey SW, Steinfeld J, Gleich GJ, group HESMs. Efficacy and safety of mepolizumab in hypereosinophilic syndrome: A phase III, randomized, placebo-controlled trial. J Allergy Clin Immunol. 2020 Dec;146(6):1397-1405. Epub 2020/09/22. doi:10.1016/j.jaci.2020.08.037. Cited in: Pubmed; PMID 32956756.

35. King B, Lee AI, Choi J. Treatment of Hypereosinophilic Syndrome with Cutaneous Involvement with the JAK Inhibitors Tofacitinib and Ruxolitinib. J Invest Dermatol. 2017 Apr;137(4):951-954. Epub 2016/11/27. doi:10.1016/j.jid.2016.10.044. Cited in: Pubmed; PMID 27887955.

36. Panch SR, Bozik ME, Brown T, Makiya M, Prussin C, Archibald DG, Hebrank GT, Sullivan M, Sun X, Wetzler L, Ware J, Fay MP, Dunbar CE, Dworetzky SI, Khoury P, Maric I, Klion AD. Dexpramipexole as an oral steroid-sparing agent in hypereosinophilic syndromes. Blood. 2018 Aug 2;132(5):501-509. Epub 2018/05/10. doi:10.1182/blood-2018-02-835330. Cited in: Pubmed; PMID 29739754.

37. Williams KW, Ware J, Abiodun A, Holland-Thomas NC, Khoury P, Klion AD. Hypereosinophilia in Children and Adults: A Retrospective Comparison. J Allergy Clin Immunol Pract. 2016 Sep-Oct;4(5):941-947 e1. Epub 2016/05/01. doi:10.1016/j.jaip.2016.03.020. Cited in: Pubmed; PMID 27130711.

Appendix 1: Patient Information/Informed Consent

Appendix 2: Invoice and Report Forms

Appendix 4: Approval by Ethics Committee