Protocol HRYD-033

Diagnostic project to determine the prevalence of mastocytosis in patients with cytopenia or leucocytosis

Protocol

Version 1.1, 27-December-2018

Sponsor

Martin-Luther-University Halle-Wittenberg, represented by the Rector, who is represented by the Dean of the Medical Faculty

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Protocol Committee

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Statistical consultation

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Project management / data management

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Financing

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1 Background and Rationale

Systemic mastocytosis (SM) is a rare myeloproliferative neoplasm characterized by infiltration of clonally derived mast cells in different tissues, including bone marrow, skin, the gastrointestinal tract, the liver, and spleen [1]. The clinical presentation of mastocytosis is heterogeneous, ranging from mild to life-threatening mediator-related symptoms. Over the past few years, substantial advances have been made in understanding the pathogenesis, evolution, and complexity of mast cell neoplasms. Regardless of the type of SM, the bone marrow is involved in virtually all patients [2]. In patients with indolent SM (ISM) skin involvement is usually found, compared with less frequently detection of skin manifestation in aggressive variants of the disease [3]. Particularly the more aggressive variant with extra cutaneous symptoms is generally seen in adult patients [4].

SM diagnostic criteria were defined by the WHO in 2001 and have been confirmed in the WHO updates of 2008 and 2016. The major SM criterion is the multifocal clustering of mast cells (at least 15 mast cells/cluster) in 1 or more visceral organs (usually documented in the BM). Minor SM criteria include an abnormal morphology of mast cells (immature forms, spindling forms, decentralized oval nuclei or bi- or poly-lobed nuclei, hypogranulated cytoplasm), expression of CD2 and/or CD25 in mast cells, an activating *KIT*-mutation at codon 816 (most frequently *KIT* D816V), and an elevated serum tryptase concentration of >20 ng/ml. The present of the major and at least 1 minor SM criterion or 3 minor SM criteria are needed for the diagnosis of SM [5].

According to the WHO classification 2016 SM is divided into different subtypes (indolent systemic mastocytosis (ISM), smoldering SM (SSM), SM with an associated hematologic (non-MC lineage) neoplasm (SM-AHN), aggressive SM (ASM), mast cell leukemia (MCL), and mast cell sarcoma (MCS) [5].

The signs and symptoms of SM vary based on which organs are affected [6]. The clinical spectrum includes signs of:

- Anemia, thrombocytopenia, leukopenia or leukocytosis
- gastrointestinal symptoms (e.g. abdominal pain, diarrhea, nausea, vomiting)
- itching, hives, and/or flushing of the skin
- Anaphylactoid reactions
- hepatomegaly, splenomegaly, and lymphadenopathy

In many patients with cytopenias and/or leucocytosis the diagnosis of systemic mastocytosis is missed or overseen for a long time as it may coexist with other hematological diseases [SM with associated hematological neoplasia cell lineage disease (SM-AHN)] on one side and/or the lack of awareness of the disease in the medical community. Often atypical presentation can delay the establishment of the diagnosis substantially.

Epidemiological data are missing to define the precise incidence, point prevalence, or cumulative prevalence of mastocytosis in the general population. The prevalence of an overt mastocytosis has been estimated to be about 10 cases per 100,000 inhabitants in the USA [6]. An analysis of the 18 SEER registries presents an age adjusted calculated incidence rate of 0,4 per one million person-years for the diagnosis of systemic mastocytosis, which is much lower than compared with the age-adjusted incidence rates for polycythaemia vera with 10.9 per one million person-years [7].

Detection of mutations in patients with myeloid neoplasms has shown great potential for diagnostic and prognostic purposes. Next-generation sequencing (NGS) is currently implemented for the routine diagnostic profiling. Pan-myeloid targeted NGS fits elegantly in the routine diagnostic approach of myeloid neoplasms allowing for an improved diagnosis, subclassification, and prognosis.

The advent of sequencing techniques has allowed new insights into the molecular basis of myeloid neoplasms. Similar to most sporadic human malignancies, mastocytosis is a complex, dynamic disease, characterized by multiple somatically acquired driver mutations, coexisting competing clones, and disease evolution over time. Recent studies in large, population-based cohorts have identified recurrent mutations in epigenetic regulators (*DNMT3A*, *ASXL1*, *TET2*), and less frequently in splicing factor genes (*SF3B1*, *SRSF2*), to be associated with clonal hematopoietic expansion in elderly seemingly healthy subjects [8]. The term "clonal hematopoiesis of indeterminate potential has been proposed to describe this phenomenon which seems associated with increased risks of hematologic neoplasms. Preliminary data indicate that the rate of progression of clonal hematopoiesis of indeterminate potential to hematologic disease may be similar to the rate of progression of other premalignant states, such as monoclonal gammopathy of undetermined significance to multiple myeloma.

2 Aim of the project

The primary goal of this project is to assess the prevalence of mastocytosis in a prospective cohort of consecutive patients who are referred to the department of Hematology/Oncology, Halle (Saale) with a suspected diagnosis of hematologic malignancy because of cytopenia or leucocytosis. Patients with known or suspected systemic mastocytosis will be excluded.

3 Project design

This project is a routine diagnostic single-center assessment of prevalence of systemic mastocytosis in patients with an unclear leucocytosis and/or cytopenia.

The following data of these patients will be documented pseudonymously in the database after return of the signed and dated informed consent form (ICF).

- Routine diagnostic work-up
- Single-centre analysis (department of Hematology/Oncology, Halle (Saale))
- Numbers of patients n=100
- Project duration: maximum of 2 years

4 Primary endpoint

- Prevalence of systemic mastocytosis in patients with cytopenia and/or leucocytosis

5 Secondary endpoints

- Subtype of SM according to WHO-classification
- Mutational profiling

6 Selection of Project Population

6.1 Inclusion criteria

- Age ≥ 18 years
- Signed informed consent
- Male and female patients are eligible
- Patients referred to the University Hospital Halle
- Leukocytosis and/or cytopenia in one or more cell lineages that are not related to an active lymphoid disease

6.2 Exclusion criteria

- limited legal capacity of the patient
- Age < 18 years
- Active known lymphatic disease at time of informed consent
- Known or suspected systemic mastocytosis

7 Parameters to be collected

The following standard parameters of patients with cytopenia or leukocytosis will be documented at time of diagnostics:

- Patient characteristics (age, gender, ethnicity)
- Medical history, history of allergic reactions, drug history, family history
- Clinical examination (cutaneous manifestation of SM, spleen size, liver size, lymphadenopathy)
- Lab evaluation of routine blood samples including:
    - blood picture and differential blood count, reticulocytes, fragmentocytes
    - liver and renal function (ALAT, ASAT, alkaline phosphatase, GGT, total-bilirubin, conjugated and unconjugated bilirubin)
    - serum tryptase level
    - LDH
    - ferritin, vitamin B12 and folic acid, erythropoietin level
    - serum protein, albumin, protein-electrophoresis
    - coombs test (direct, indirect)
    - antinuclear antibodies
    - tests to exclude the antiphospholipid syndrome
    - viral examination to exclude HIV and viral Hepatitis
    - blood evaluation for paroxysmal nocturnal hemoglobinuria (PNH)
- Blood sample for PCR for BCR-ABL to exclude CML (only in Patients with leukocytosis and left shift in the differential)
- Routine bone marrow examination (in patients in whom the above mentioned laboratory parameters did not yield a diagnosis) including aspirate and biopsy for
    - cytology
    - immunophenotyping
    - cytogenetic analysis

- molecular genetics for myeloid diseases by next generation sequencing (NGS)
- Histological examination of bone marrow biopsy

8 Data collection

All data will be captured in the blinded project specific database by giving every patient a project specific identification number. The data will be transferred in a pseudonymous form from the hospital information and documentation system to the database.

The collection and processing of personal data from patients enrolled in this project will be limited to those data that are necessary to fulfill the objectives of the project. An ICF has been designed to provide patients with information about the type and amount of data collected. The patient has/the patient's legal representative(s) have the right to request through the investigator access to his or her/their wards personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, considering the nature of the request, the conditions of the study, and applicable laws and regulations.

8.1 Data protection

A Privacy Impact Assessment evaluating the current risks and protective measures taken has been performed. Investigators are aware of the severe impact unauthorized access or publication of sensitive medical data would have on the victim's sense of privacy and psyche. Data stored in the hospital information and documentation system has a direct impact on treatment and diagnostic decisions. Loss or falsification of this data would have serious consequences for the patient's health. Appropriate measures are in place to protect confidential data. Since source documents are stored in the hospital information and documentation system, they can only be accessed through password-protected user profiles, which are assigned by the Central Service 1 of the University Hospital Halle based on competencies, tasks and the completion of a training course. The information and documentation system also provides an audit trail, noting changes to the source documents. Additionally, both the project database and source documents can only be accessed from devices with password-protected user profiles within the clinic network of the University Hospital Halle. Backup files of data stored on such devices are generated weekly, monthly and every two hours and the whole system is protected by current anti-virus software. The members of the study team are accustomed to working with sensitive data and ensuring its confidentiality.

9 Statistics

Statistical analysis will be done by Dr. med. Eva Kantelhardt, Institute for Medical Epidemiology, Biometrics and Informatics, Martin-Luther-University of Halle-Wittenberg, Magdeburger St. 8, 06112 Halle (Saale), Germany.

9.1 Justification of Trial Design

Trial design was based on the following:

a) In many patients with cytopenias and/or leucocytosis the diagnosis of systemic mastocytosis is missed or overseen for a long time as it may coexist with other hematological diseases.
b) Often atypical presentations substantially delay the establishment of the diagnosis.
c) The lack of awareness of the disease in the medical community is one main reason for a delayed or missed diagnosis.

Thus epidemiological data are missing to define the precise incidence, point prevalence, or cumulative prevalence of mastocytosis as only diagnosed cases could logically be counted [6,7].

Our personal experience at the University Hospital Halle (Saale) shows that with an appropriate awareness for the probability of systemic mastocytosis as a differential diagnostic possibility, around 10% of patients with cytopenias and/or leucocytosis are actually suffering from systemic mastocytosis!

9.2 Justification of Sample Size

Consequently, no formal sample size calculation using power analysis can be performed. Based on our experience we estimate a prevalence of 5-10% of patients with Mastocytosis among the mentioned patient group. An estimated sample size of approximately n=111 with 10% drop-out thus n=100 patients in the population under observation will allow the study to determine an expected percentage of 7 % of patients diagnosed with systemic mastocytosis with a two-sided 95% confidence interval (CI) of about and 5% margin of error. For an exploratory study, these margins of error are considered sufficiently precise and acceptable.

9.3 Statistical Methods

A complete statistical analysis of all parameters will be performed at the completion of the project (a maximum of two years after the start of the project. The project will be terminated earlier if 100 patients were included prior to the end of the two years).

All statistical analyses in this project will be exploratory and descriptive only. Data will be appropriately summarized and analysed using tabulation and graphs with respect to demographic/baseline characteristics and diagnostic results. Standard descriptive summary statistics (including number of patients [n], mean, standard deviation, median, minimum, maximum, and quartiles) will be calculated for continuous variables. Categorical data will be presented in frequency tables using counts and percentages.

10 Milestones

10/2018	Final version of the protocol
12/2018	Vote from the Ethics Committee of the Medical Faculty of the Martin-Luther-University of Halle-Wittenberg. Comments from the Ethics Committee to be answered.
01/2019	Second version of the protocol submitted to Ethics Committee
02/2019	Final vote from the Ethics Committee
03/2019 - 03/2021	Inclusion of patients
03/2021 - 09/2021	Finalization of the database, statistical analysis

11 Patients' consent and vote from the ethics committee

- All patients have to give a written informed consent to this project after being informed in detail about the project by an investigator in a timely manner.
- The present project will be performed in accordance to all applicable laws and regulations and abiding to ICH GCP principles concerning conduct, evaluation and documentation of clinical investigations according to the declaration of Helsinki (2013).
- Investigators confirm adherence to all applicable legal conditions including the data protection law currently effective at the time of protocol signature, regulations and regulatory requirements by signing the project protocol.
- A vote from the Ethics Committee of the University of Halle-Wittenberg will be obtained for the specific analyses as part of this descriptive project.

12 References

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13 Appendices

13.1 Protocol Signature Page

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PD Dr. med. Haifa Kathrin Al-Ali Coordinating Investigator	Signature	14/Jan/2019 Date
Dr. med. Nadja Jäkel Coordinating Investigator	Signature	14 JAN 2019 Date
Dr. med. Eva Kantelhardt (with apl. Prof. Dr. Wienke) Statistical consultation	Signature	14.1.19 Date