

**"CAROL DAVILA", BUCHAREST DOCTORAL SCHOOL
MEDICINE**

**CORRELATIONS BETWEEN MORPHOLOGICAL,
IMMUNOPHENOTYPICAL, CYTOGENETIC AND MOLECULAR
ASPECTS IN SYSTEMIC MAST CYTOCYTOSIS
SUMMARY OF THE DOCTORAL THESIS**

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List of abbreviations and symbols

CTMC (connective tissue mast cells)

PCR

KIT receptor for stem cell factor (SCF) and MYD88 (adaptor of Toll-like receptors),

MMC (mucosal mast cells)

RAG (recombination genes of immunoglobulin segments)

MCT (tryptase-rich mast cells)

MCTC (tryptase-chymase-rich mast cells)

INTRODUCTION

Systemic mastocytosis is a rare hematological disease, marked by abnormal infiltration of mast cells in various organs, with an extremely heterogeneous clinical and biological manifestation(1) . The difficulties of diagnosis and treatment, together with recent advances in molecular biology, justify the need for in-depth research. This work aims to contribute to the understanding of the pathogenesis of the disease and to facilitate the development of diagnostic methods and personalized therapeutic strategies, by correlating morphological, immunophenotypic, molecular and clinical data.

While systemic mastocytosis is intensively researched internationally in specialized centers, in Romania no studies have been published so far that provide epidemiological data or specific clinico-biological correlations. The present study, conducted in a center of reference for mastocytosis, aims to fill this gap through a retrospective analysis of a group of patients diagnosed with this condition.

The objectives include morphological characterization of mast cells, identification of molecular mutations (with a focus on KIT D816V) and their correlation with clinical manifestations and administered treatments. The methodology uses modern techniques such as specific histology, flow cytometry and PCR, and the data will be statistically analyzed to identify relevant relationships.

The study has an interdisciplinary approach, integrating expertise from hematology, immunology, molecular biology and bioinformatics. Although the small sample size is a limitation, the preliminary results suggest important links between the genetic profile and the clinical phenotype, providing a solid basis for future expansion of the research. Further directions aim at identifying new biomarkers and evaluating targeted therapies, depending on the molecular characteristics of each patient.

GENERAL PART

1. Mast cell – general presentation in human biology

Mast cells are cells that play a role in the immune system in human biology, acting as “guard” cells that respond to multiple external and internal danger signals (2). These cells are found mainly in the skin, mucous membranes and around the vascular endothelium, being capable of affecting almost any organ once activated.

Mastocytosis is a rare hematopoietic pathology characterized by clonal expansion of mast cells and their abnormal activation, which leads to accumulation in various tissues and to the exaggerated release of biological mediators(1). In recent years, awareness of this condition has increased due to the efforts of medical societies and patient organizations, as well as the increasingly frequent practice of serum tryptase analysis in allergology. Thus, the differential diagnosis of diseases with allergic, dermatological, gastrointestinal, or hematological manifestations increasingly includes mastocytosis. In addition, the promotion of new therapeutic options—especially tyrosine kinase inhibitors—mandates physicians to correctly classify patients into one of seven distinct clinical categories, each with its own therapeutic indications and prognosis.

Recent phylogenetic studies emphasize that mast cells are among the first immune cells to emerge in evolution(3). The first metachromatic cells with features suggestive of mast cells were described more than 500 years ago in urochordates, a subphylum of marine chordates, called “test cells” for their role in innate immunity and tissue repair (4–6). In fish—including primitive jawless species—cells with a histochemical and biochemical profile similar to mast cells have been detected, expressing the stem cell factor (SCF) receptor KIT and MYD88 (adaptor of Toll-like receptors), conferring the ability to recognize microorganisms and parasites (7,8). With the transition to vertebrates and the emergence of the RAG (recombinant immunoglobulin segment genes) network, mast cells acquired adaptive immune functions (3).

Mast cells are derived from hematopoietic progenitors transmitted mainly by the bone marrow. Unlike other myeloid cells, they enter the circulation as progenitor cells, expressing FcεRI, KIT and CD34 at early stages. In the presence of SCF, they rapidly differentiate into mature mast cells. In the circulation, the passage is rapid, and tissue infiltration occurs constitutively and is accelerated by inflammatory processes. In tissues, the progenitors differentiate into two main subtypes: CTMC (connective tissue mast cells) – predominant in the skin and connective tissue. Their granules contain heparin, visible

metachromatically with toluidine blue; MMC (mucosal mast cells) – located in the mucosa of the respiratory tract and gastrointestinal tract. Their granules contain chondroitin E sulfate, stainable yellow/green with safranin. In humans, mast cells are classified based on the type of proteases in their granules into: MCT (tryptase-rich mast cells) and MCTC (tryptase- and chymase-rich mast cells), corresponding in many respects to CTMC and MMC in rodents. This differentiation reflects functional specialization: MRGX2 receptors are expressed in CTMC, but not in MMC(9).

Mature human mast cells express a complex repertoire of receptors that give them the ability to respond to variable stimuli. These include:

FcεRI (high-affinity receptor for IgE) – essential for allergic reactions, evolved as a defense mechanism against parasites and venoms (10).

Fcγ receptors (for IgG) – include FcγRI, FcγRIIb and occasionally FcγRIII under certain cytokine conditions (11) FcγRI, with high affinity, appears on mast cell processes only in the presence of IFN-γ or in psoriatic lesions, activating mast cells under specific conditions. FcγRIIb, through the ITIM motif, inhibits FcεRI-dependent degranulation when associated with it.

KIT (CD117) – a tyrosine kinase receptor of SCF, crucial for hematopoiesis and mast cell survival. In mastocytosis, approximately 85–90% of adults have the KIT D816V mutation, which leads to constitutive activation of the receptor and uncontrolled proliferation (20,21). Downstream signaling includes JAK/STAT, PLCγ, MAPK/ERK, and PI3K/AKT axes, supporting cell survival, proliferation, and migration (12,13).

MRGX2 (G-coupled receptors) – involved in anaphylactic drug reactions and neuronal inflammation, activating mast cells independently of FcεRI (14–17). They are stimulated by antimicrobial peptides, insect venoms, and neuropeptides such as substance P (27–30). MRGX2 inhibitors may represent future therapeutic targets in mastocytosis.

CD63 – plays the role of a hydrostatic activation marker, as it is rapidly overexpressed on the surface of mast cells after allergen exposure (18,19). CD63 is overexpressed on mast cells in the bone marrow of patients with mastocytosis.

CD203c (E-NPP3) – membrane ectonucleotidase (E-NPP) activity, associated with malignancy and tumor activation(20,21). It is upregulated upon IgE receptor engagement and overexpressed on neoplastic mast cells (22).

CD30 – a member of the TNFR/NGFR family, with dual signaling that can induce either activation or apoptosis. Its expression is normally restricted to activated T and B lymphocytes, but in systemic mastocytosis (indolent and aggressive) CD30 is aberrantly overexpressed on neoplastic mast cells (23,24).

CD25 (IL-2R α chain) – components of the high-affinity receptor for IL-2 (heterotrimer with β and γ). Mast cells in systemic mastocytosis express dysregulated CD25, which is a marker of neoplastic mast cells (25). The pathological significance of the aberrant expression remains unclear.

In myeloproliferative mast cell diseases, the clinical phenotype is determined by both the mass effect of cellular accumulation and the variable release of mediators, generating a polymorphic symptomatic picture. Certain mediators represent therapeutic targets and diagnostic/monitoring markers:

Histamine - is the main biogenic amine released by human mast cells after IgE receptor activation. It can be measured in biological fluids. Elevated serum histamine or its urinary metabolites may indicate systemic mastocytosis and/or mast cell activation (26), but is not considered a diagnostic criterion.

Heparin – glycosaminoglycan produced by mast cells with anticoagulant effect, present in cutaneous mastocytosis; in advanced forms of systemic mastocytosis, excessive release can lead to hemorrhagic complications, although most coagulation parameters remain normal (27,28).

Proteases – stored in mast cell granules and represent a significant proportion of the total protein content of mast cells (29). Once released, they degrade tissue proteins, influencing inflammation, angiogenesis, bone homeostasis, and allergic responses. They can also degrade venoms (bee, scorpion, reptile), reducing toxicity (8).

Serum tryptase – its serum level is a minor diagnostic criterion in systemic mastocytosis (value >20 ng/mL). There are two main isoforms, β (proteolytically active as a homotetramer) and α (less active) (30). An increase in serum level of $\geq 20\%$ from baseline + 2 ng/mL within 4 hours after an allergic reaction indicates mast cell activation (31,32)

Chymase – serine protease involved in the generation of angiotensin II and endothelin 1, participating in inflammation, blood pressure regulation and atherosclerotic plaque instability; degrades lipoproteins, promotes the formation of foamy macrophages and contributes to tissue fibrosis (33,34).

Carboxypeptidase A3 (CPA3) – identified in human mast cells and associated with allergic airway diseases (35).

Prostaglandin D2 (PGD2) and cysteinyl leukotrienes (CysLT: LTC4, LTD4, LTE4) – lipid mediators rapidly synthesized after mast cell activation (36). PGD2, produced from arachidonic acid by COX-1/COX-2, induces vasodilation and bronchoconstriction, and is increased in biological fluids after allergic exposure(37) . CysLTs, produced by mast cells and other inflammatory cells, induce smooth muscle contraction at concentrations much lower than histamine and cause urticaria and erythema. Urinary LTE4 is increased in mastocytosis and correlates with urinary N-methyl-histamine and serum tryptase levels (38,39).

Cytokines and chemokines – mast cells release TNF, TGF- β , IFN- α , VEGF-A–D, IL-6, IL-11, IL-13, IL-16, IL-18, GM-CSF, NGF, PDGF, as well as chemokines such as IL-8 (CXCL-8), MCP-1, MIP-1 α/β , RANTES, and eotaxin. These molecules regulate local and systemic inflammation, recruiting inflammatory cells, and remodeling tissues.

In conclusion, mast cells play a central role in the physiology of inflammation and allergy, and their proliferative pathology—systemic mastocytosis—reflects not only cellular accumulation, but also the disruption of the mediator balance. In the following chapters of the thesis, the histological, cytometric and molecular methods applied in the study of patients with systemic mastocytosis, the statistical correlations between the studied parameters and clinical outcomes, as well as the bioinformatic interpretation of the data will be detailed. The main limitation of the study is represented by the relatively small sample size, requiring validation of the conclusions in larger cohorts.

1. Systemic mastocytosis - definition and classification

Mastocytosis is a rare, heterogeneous hematological disease characterized by the clonal and atypical accumulation of mast cells in one or more organs (40). It is classified into cutaneous (MC) and systemic (MS) forms, according to WHO guidelines (41). MC is common in children, with a good prognosis, while systemic, chronic and incurable forms predominate in adults (42). Depending on the clinical and biological manifestations, MS is classified into several subtypes: pure medullary mastocytosis (BMM), indolent (ISM), smoldering (SSM), aggressive (ASM), mast cell leukemia (MCL) and the form associated with another hematological neoplasia (SM-AHN) (43). The first three have a favorable or intermediate prognosis, while the latter are considered advanced forms, requiring cytoreductive therapeutic approaches or even allogeneic transplantation.

The diagnosis of MS is based on strict criteria defined by the WHO, which include dense mast cell infiltrates, atypical morphology, the presence of KIT mutation (especially D816V), aberrant expression of CD25 and/or CD2, and elevated serum tryptase levels. The determination of the disease subtype is made according to the presence of B and C signs as follows: B signs (e.g. osteoporosis, hepatomegaly, splenomegaly without dysfunction) and C signs (anemia, liver failure, pathological fractures, malabsorption), these are essential in staging the disease and in guiding the therapeutic decision (43).

Clinically, ISM is dominated by symptoms induced by the release of mast cell mediators—flushing, pruritus, tachycardia, hypotension—often manageable with symptomatic treatment (72–74). In contrast, ASM and SM-AHN are associated with organ dysfunction, require cytoreductive therapies, and are often refractory to conventional treatment (76).

KIT mutations are central to the pathogenesis of mastocytosis. In adults, the KIT D816V mutation is present in >80% of patients with advanced forms and in ~100% of those with ISM. This mutation constitutively activates the KIT receptor and is detectable also in non-mast cell hematopoietic cells (basophils, eosinophils, CD34+), suggesting an early pathogenic event in stem cells. The allelic burden of the KIT D816V mutation correlates with disease severity and is an important marker for monitoring therapy and progression (44).

In addition to KIT mutations, advanced forms of MS are often characterized by additional mutations in non-KIT genes: TET2, ASXL1, SRSF2, RUNX1, CBL, RAS, which contribute to a more aggressive phenotype and a poor prognosis (45).

The clinical subtypes of MS reflect this genetic and biological diversity. In ISM, the KIT D816V mutation is frequently confined to mast cells, and the prognosis is favorable (79,90). In SSM, the mast cell burden is higher and hematopoietic involvement is more extensive (109,110). ASM is characterized by severe organ dysfunction and an increased incidence of additional mutations (111–117). AHN-MS combines MS with a hematologic neoplasia, often myeloid, with a complex molecular and therapeutic profile (113,118). MCL is the most aggressive variant, with poor survival, characterized by extensive mast cell infiltration and the frequent (but not universal) presence of the KIT D816V mutation (113,121). Subvariants of MCL (such as the chronic form, cMCL) are under characterization and pose diagnostic and therapeutic challenges (119–123). Mast cell sarcoma (MCS), although extremely rare, involves mast cells with major morphological atypia and variable genetic profiles (125–126).

In conclusion, systemic mastocytosis is a complex condition, in which diagnosis and treatment are based on the correlation of clinical, histopathological, immunophenotypic and molecular data. The KIT D816V mutation plays a central, but not exclusive, role in the pathogenesis of the disease. Identification of additional mutations and assessment of the allelic load have major prognostic importance. Accurate classification of MS subtypes and appropriate staging allow for a personalized therapeutic approach, especially in advanced forms. In the future, sensitive molecular tests and therapies targeted to the genetic profile could redefine the management of this rare but potentially severely progressive disease.

2. Treatment of Systemic Mastocytosis

Systemic mastocytosis (SM) is a rare hematological disorder that requires a differentiated therapeutic approach, adapted to the disease subtype and severity of symptoms. Current therapeutic strategies include control of symptoms mediated by mast cell degranulation, reduction of mast cell burden in advanced forms, and targeted therapies targeting specific KIT gene mutations, especially D816V.

Symptomatic therapy is based on H1 and H2 antihistamines (e.g. cetirizine, famotidine), leukotriene antagonists (montelukast), corticosteroids, sodium cromoglycate, and omalizumab, an anti-IgE monoclonal antibody effective in severe allergic episodes. Treatment of bone involvement involves bisphosphonates, denosumab, and peginterferon alfa-2a. Treatment of anaphylactic reactions involves the administration of adrenaline, and sodium cromoglycate is useful in gastrointestinal and skin symptoms (46).

Tyrosine kinase inhibitors (TKIs) are the mainstay of targeted therapy. Midostaurin (100 mg/day), approved in 2017, targets KIT D816V and has demonstrated an overall response rate of 60%, although gastrointestinal adverse effects have necessitated dose adjustments in many cases(47). Avapritinib (200 mg/day), a type 1 selective inhibitor, has shown superior results: an ORR of 75%, complete remission in 36% of cases, and significant reduction in serum tryptase (48). However, cognitive adverse events have been reported, more frequently in patients with thrombocytopenia or MS-AHN.

Newer-generation inhibitors still in the investigational stage, such as bezuclastinib and elenestinib, have improved safety profiles and provide potent inhibition of KIT D816V. Elenestinib has been shown to reduce serum tryptase levels by up to 68% in patients with ISM, and bezuclastinib, in the APEX trial, achieved an ORR of 56% and a >50% reduction in tryptase, VAF, and mast cell infiltration in >90% of patients(49,50).

Other cytoreductive therapies include cladribine, interferon-alpha, and hydroxyurea, used in refractory or advanced forms. Allogeneic hematopoietic stem cell transplantation (ASCT) is considered a curative option for severe cases, especially for SM-AHN, but with limited benefit in MCL and ASM. Avapritinib has demonstrated comparable or superior survival to ASCT in certain subgroups, redefining the indications for transplantation.

Prognosis is assessed using scores such as MAPS, MAPSS, IPSM, GPSM or MARS, which integrate clinical (age, hemoglobin, platelets, alkaline phosphatase) and genetic (ASXL1, RUNX1, SRSF2, DNMT3 mutations) parameters (51).

These scores allow stratification of patients according to risk, guiding therapeutic decisions and estimating median survival. Furthermore, scoring systems are useful in defining the types of therapeutic response: complete remission, major response, partial response, clinical improvement, stable or progressive disease (52).

The treatment of systemic mastocytosis is undergoing a significant transformation with the development of highly selective targeted therapies with favorable safety profiles. Molecular and clinical stratification of patients allows for a personalized therapeutic approach, and new prognostic scores contribute to optimizing disease management and maximizing survival. Ongoing studies will clarify the place of combination therapies and new TKIs in the standard of care for MS.

SPECIAL PART

3. Working hypothesis and general objectives

Systemic mastocytosis is a rare pathology that affects the mast cell through its clonal proliferation. It is a heterogeneous disease, with complex symptoms: allergic, dermatological, endocrinological, gastroenterological and hematological, but can affect any organ.

Given the low diagnosis rate at the national level and the lack of widespread awareness of this condition, patients often do not have access to early diagnosis, or to individualized treatment that can only be administered in a reference center with experience in the management of these cases.

The hypothesis of this study was based on the fact that systemic mastocytosis is a rare disease that is underdiagnosed. *By implementing a national reference center, can the management of these patients be improved?*

Management involves correct diagnosis and reducing diagnostic time, improving access to medication and both specific and supportive treatment, providing the patient with access to a multidisciplinary team (allergist, dermatologist, gastroenterologist, orthopedist, ICU, emergency room, hematologist) to improve quality of life and general access to medical services tailored to the patient's needs.

Starting from this hypothesis, the proposed objectives are:

- - structuring and establishing a retrospective database, with over 160 patients diagnosed between 2006 and 2025.
- - identifying significant correlations between the clinical form of mastocytosis and tryptase levels, marrow infiltrate, organ involvement and therapeutic need, including symptomatic therapy as well as specific therapy.

In Romania, systemic mastocytosis remains largely understudied, with limited statistical data available to guide prognosis and treatment strategies. Addressing this gap requires detailed investigation into the morphological, immunophenotypic, cytogenetic, and molecular characteristics of the disease to establish their direct impact on patient outcomes. Developing a risk score based on diagnostic features could pave the way for personalized therapeutic approaches, tailored to the specific attributes of pathological mast cells found in bone marrow involvement. Such advancements hold the potential to enhance disease prognosis, improve life expectancy, and elevate the overall quality of life for individuals living with systemic mastocytosis.

4. General research methodology

The study was retrospective and observational, conducted at the Mastocytosis Reference Center of the Bucharest University Emergency Hospital, between January 2006 and March 2025. 162 adult patients who met the WHO 2022 criteria for systemic mastocytosis (MS), with complete clinical, biological and molecular documentation, were included. Exclusion criteria included cases with incomplete data, age under 18 years or lack of diagnostic confirmation. Diagnosis was established based on bone marrow biopsy, immunohistochemistry and molecular testing for the KIT D816V mutation. Initial evaluation included serum tryptase levels, advanced imaging and, depending on indication, endoscopy.

Data were extracted from the hospital electronic system and dedicated archives and cross-checked by two investigators. Demographic, clinical, molecular and treatment variables were collected, and survival was followed up until March 31, 2025. Statistical analyses were performed in SPSS v29 and JASP 0.19.3, using Mann-Whitney U, Kruskal-Wallis, χ^2 and Fisher tests, considering p values < 0.05 as significant.

For the immunophenotypic analysis of mast cells, flow cytometry was used, a technique that allows the identification of specific CD markers (e.g. CD25, CD2) in combination with CD117 and the exclusion of CD34+ precursors. Bone marrow samples were collected by aspiration puncture from the iliac crest, processed and stained with toluidine blue for morphological evaluation. Immunophenotyping included a classic and an extended panel of markers, in order to differentiate normal from pathological mast cells. The technical steps included cell lysis, successive centrifugations, labeling with fluorescent monoclonal antibodies and reading with Gallios Flow Cytometer.

An extensive bibliographic analysis was also performed by querying international databases (PubMed, Embase, ClinicalTrials.gov, WHO ICTRP, Cochrane, Web of Science) to identify targeted therapies in MS, including KIT-inhibitors and transplantation. The study complies with the principles of the Declaration of Helsinki and was approved by the SUUB Ethics Committee (protocol code 88221/31.12.2024).

6. Evaluation of the epidemiological, genetic, molecular profile of the patient with systemic mastocytosis in Romania

6.1. Introduction

This study provides a descriptive analysis of the epidemiological and molecular characteristics of patients with MS in Romania, using data from the registries of the Bucharest Mastocytosis Reference Center, Bucharest University Emergency Hospital. MS is a rare hematological disorder, determined by the aberrant accumulation of mast cells in various organs, and understanding its national profile is essential for optimizing diagnosis and treatment. The study explores the demographic distribution, prevalence and associated genetic mutations, with a special focus on the KIT D816V mutation, directly involved in the pathogenesis of the disease.

6.2. Material and method

This retrospective observational cohort study was conducted at the Reference Center for Mast Cell Disorders within the Bucharest University Emergency Hospital, analyzing 162 adult patients diagnosed with systemic mastocytosis (MS) between 2006 and 2025. Patients with suggestive clinical presentation, determined serum tryptase level and confirmed KIT mutation were included. The diagnosis was established by bone marrow biopsy, immunohistochemistry and PCR molecular testing for KIT D816V, and data were collected from electronic and paper archives, verified by two investigators. The evaluation also included imaging and endoscopic investigations. The treatments administered and the evolution of the patients were documented until March 2025. The primary objectives were the distribution of MS subtypes according to WHO and overall survival, and the secondary objectives included organ involvement and therapeutic strategies. Statistical analyses were performed with SPSS and JASP, using appropriate methods for continuous and categorical variables, with a significance threshold of $p < 0,05$.

6.3. Results

The geographical distribution of systemic mastocytosis (MS) cases in Romania shows significant variations between counties, with a high concentration in university centers and areas with access to specialized diagnosis. Bucharest records the highest number of patients (28.8%), followed by Ilfov, Braşov and Constanţa. In contrast, many counties reported only one case, suggesting either a low incidence or underdiagnosis. These data highlight the need for a strategy to expand access to diagnosis in underrepresented regions.

Demographically, females predominate (approximately double that of males), and the median age at diagnosis is similar between sexes. Interestingly, the actual age was significantly higher in women. Mean serum tryptase values exceed the diagnostic threshold, with a general trend of higher levels in men, especially in the extreme ranges (>300 ng/mL).

Digestive disorders were slightly more common in women, especially gastritis, while skin disorders (macules, papules, plaques, vesicles) were reported more often in men, with a clear male predominance in the case of vesicles. Cytoreductive treatment was administered differently by gender: cladribine, imatinib and interferon were administered exclusively to men, while midostaurin and Thromboreductin were used more frequently in women.

Regarding symptomatic treatment, a significantly more frequent use was noted in women, especially of Montelukast, cetirizine and other antihistamines. This may reflect either differences in symptomatology or a different therapeutic approach depending on the sex. In the analysis of biological values, the mean levels of LDH, alkaline phosphatase and bone marrow infiltration were similar between the sexes, without statistically significant differences. The mean dose of sodium cromoglycate was the same in men and women (800 mg/day), with no consensus on the effective dose.

Bone involvement was more frequently reported in women, but men had a higher proportion of osteopenia. DEXA monitoring is limited in Romania due to poor access to this investigation.

Bivariate analyses revealed significant correlations between mastocytosis subtypes and clinical or biological parameters. Cutaneous mastocytosis (CM) correlated positively with serum tryptase and marrow infiltration, but negatively with symptomatic treatment and antihistamines. ISM had an inverse correlation with tryptase and marrow infiltration, but positively with symptomatic treatments and bone involvement. SSM correlated inversely with tryptase and marrow infiltration, but positively with organomegaly (hepato- and splenomegaly) and bone involvement. ASM showed positive correlations with the use of cladribine and imatinib, but negatively with tryptase, marrow infiltration and alkaline phosphatase. SM-AHN correlated negatively with skin involvement.

Tryptase correlated positively with bone marrow infiltration and alkaline phosphatase, but inversely with hepato- and splenomegaly. Bone marrow infiltration

correlated negatively with symptomatic treatment and antihistamines, but positively with alkaline phosphatase. Symptomatic treatment correlated very strongly with antihistamine use ($r = 0,841$).

Kaplan-Meier analysis revealed a negative impact on survival among patients who received cytotoxic therapy, with a median survival of 204 days compared to an estimated median survival of 456 days in those without such treatment. The difference was statistically significant ($p = 0,005$), supporting the hypothesis of a more reserved prognosis for patients with advanced forms requiring cytoreductive therapy.

6.4. Discussions

The results of the bivariate analysis in the studied cohort of patients with systemic mastocytosis revealed multiple relevant correlations between the subtypes of the disease and clinical and biological parameters. Cutaneous mastocytosis was paradoxically associated with increased levels of tryptase and marrow infiltration, suggesting a possible systemic activity. In contrast, indolent systemic mastocytosis showed an inverse correlation with tryptase and the KIT D816V mutation, but an association with bone involvement and the use of symptomatic treatment, highlighting a significant symptomatic profile, although with a favorable prognosis. The smoldering subtype presented contradictory correlations: low levels of tryptase, but significant visceral involvement, which confirms its intermediate status. Aggressive systemic mastocytosis was associated with cytotoxic treatments and with liver and spleen involvement, but also with decreased levels of tryptase, possibly through mechanisms of cellular exhaustion. In SM-AHN, the lack of skin involvement was noted. Kaplan-Meier analysis showed significantly reduced survival in patients treated with cytotoxic agents, suggesting a selection of these patients in severe forms. These findings emphasize the importance of an integrated evaluation, tailored to the disease subtype, and the need for more effective and better tolerated therapies.

7. Current treatment in current practice in Aggressive Systemic Mastocytosis in Romania

7.1. Introduction

In Romania, aggressive systemic mastocytosis is a rarely diagnosed and underreported condition, mainly due to the high degree of clinical heterogeneity, lack of awareness and limited access to molecular diagnostic methods. In addition, modern approved treatments for advanced systemic mastocytosis are available in a limited number of centers, and the inclusion of patients in international clinical trials is limited. These aspects contribute to significant variations in medical practice and may negatively influence the prognosis of patients.

This study aims to describe how this condition is diagnosed and treated in current practice in Romania, through a retrospective analysis of a cohort of patients evaluated in a national reference center. The main objective is to highlight the particularities of treatment, the barriers to access to modern therapies and the results obtained with available therapies. Through this analysis, we aim to identify concrete directions for improving the standard of care, as well as the integration of therapeutic strategies aligned with international recommendations.

7.2. Materials and method

The diagnosis of systemic mastocytosis (SM) was established according to the World Health Organization (WHO) guidelines valid at the time of diagnosis and subsequently confirmed according to the updated WHO guidelines and the International Consensus Classification (ICC) recommendations. Response criteria were assessed using the International Working Group–Myeloproliferative Neoplasms Research and Treatment and European Competence Network on Mastocytosis (IWG-MRT-ECNM) response criteria for advanced systemic mastocytosis.

All patients were referred to our multidisciplinary outpatient clinic for evaluation of aggressive mastocytosis, and patients with suspected MS underwent a complete diagnostic evaluation, which included bone marrow aspirate and bone marrow biopsy, as well as KIT D816V mutation testing.

Information on clinical symptoms was obtained from electronic medical records and medical interviews with patients. Prognostic score was assessed using the International

Prognostic Scoring System for Advanced Systemic Mastocytosis, based on data available at the time of diagnosis, recorded in the electronic medical record.

7.3. Results

This clinical study presents four representative cases of advanced systemic mastocytosis or at high risk of progression, each illustrating the diagnostic and therapeutic challenges encountered in practice.

The first case describes a 57-year-old patient with aggressive systemic mastocytosis (ASM), initially treated with cladribine, followed by rapid disease progression. Due to the lack of access to targeted therapies, she received imatinib without clinical benefit. Subsequently, she was the first patient in Romania treated with midostaurin, achieving a partial remission according to IWG-MRT-ECNM criteria, with a reduction in marrow infiltrate and ascites, and an improvement in quality of life.

The second case presents a 44-year-old patient diagnosed with MSA after two years of bone pain, with biopsy of an osteolytic vertebral lesion confirming mast cell infiltration. She was treated with midostaurin, bisphosphonates, and anti-RANKL, achieving reduction of pain and marrow infiltrate. Although she experienced severe nausea, the side effect was controlled with granisetron, and the patient maintained the response to treatment for over 3 years.

The third case involves a 75-year-old man with multiple lymphadenopathy, hepatosplenomegaly, and ascites. The diagnosis of MSA was confirmed by lymph node and bone marrow biopsy. Under treatment with midostaurin (at a reduced dose due to comorbidities), the patient showed regression of the lymphadenopathy and a partial remission, maintaining the same response at the May 2025 follow-up.

The fourth case concerns a 53-year-old female patient, initially diagnosed with systemic smoldering mastocytosis (SSM) after an allergological evaluation that revealed elevated serum tryptase. She was initially treated with cladribine, with a favorable response. After 5 years, the disease progressed to ASM, requiring treatment with midostaurin. Digestive adverse effects (nausea and diarrhea) were effectively managed, allowing maintenance of the full dose and obtaining a partial clinical response.

All cases illustrate the favorable impact of midostaurin in progressive MSA and SSM, contributing to disease stabilization and improvement of systemic symptoms, even in the presence of comorbidities or adverse reactions.

7.4. Discussions

Midostaurin is an effective multikinase inhibitor against the KIT D816V mutation, becoming the standard treatment in advanced systemic mastocytosis (MSA). Unlike traditional symptomatic therapies (antihistamines, corticosteroids, cladribine), midostaurin offers clinically proven benefits – reduction of mast cell infiltrate, improvement of organ function and prolongation of survival. The presented clinical cases confirm its efficacy in various forms of MSA, including in the presence of ascites, bone involvement or adenopathies, even in elderly patients or with comorbidities, with increased tolerability in adjusted dose. At the molecular level, midostaurin inhibits KIT and essential signaling pathways (PI3K/AKT, MAPK, STAT5), inducing apoptosis of malignant mast cells and cell cycle arrest. It also significantly reduces the release of inflammatory and vasoactive mediators (histamine, leukotrienes), with a favorable effect on symptoms and tissue remodeling. The drug has a good safety profile, without global immunosuppression, and remains the mainstay therapy in MSA, pending emerging therapies such as avapritinib.

8. Immunophenotypic profile of mast cells in patients with advanced mastocytosis in the Romanian population

8.1. Introduction

Multiparametric flow cytometry immunophenotyping is an essential method for the diagnosis and monitoring of systemic mastocytosis, due to its ability to rapidly identify neoplastic mast cells by detecting aberrant expression of the markers CD2 and CD25. Under normal conditions, these molecules are not expressed on mast cells, and their presence is specific to clonal mast cells. Compared to other methods, flow cytometry offers higher sensitivity. This technique complements the WHO criteria for the diagnosis of MS and can even replace biopsy in some cases, especially when the latter is inconclusive. Recent studies have shown that almost all patients with MS have aberrant expression of CD2 and CD25. Although it has some technical limitations, immunophenotyping remains a valuable tool for both early diagnosis and prognostic stratification, being able to significantly increase access to diagnosis in centers with limited resources.

8.2. Materials and method

In an initial group of patients diagnosed with systemic mastocytosis (indolent or advanced), eight patients were selected for mast cell immunophenotyping by flow cytometry. This method allows the analysis of the expression of specific markers of malignant mast cells, such as CD25 and CD2, through a gating process based on the intense expression of CD117 and the exclusion of myeloid precursors CD34+. Flow cytometry is an advanced technique that simultaneously analyzes multiple cellular characteristics (size, granulation, antigens) while the cells circulate through a laser beam. Immunophenotyping, commonly applied in hematology, uses fluorescently labeled monoclonal antibodies that bind to molecules on the cell surface (CD). These interactions generate optically detected signals, which are interpreted digitally. The procedure involves combining labeled antibodies with blood samples stored at controlled temperatures, thus allowing for the precise identification of clonal mast cells, contributing to the diagnosis and monitoring of systemic mastocytosis.

8.3. Results

The eight cases analyzed by flow immunophenotyping revealed a high variability in the percentage of clonal mast cells and aberrant expression of CD25 and CD2 markers, reflecting the heterogeneity of systemic mastocytosis (MS). Case 1 illustrates the progression from a smoldering to an aggressive form of MS, with moderate expression of CD25 (21%) and CD2 (33%) markers on identified mast cells. Case 2, with the highest mast

cell burden, showed intense expression of CD25 (90%) and a severe course, with death within 3 years, despite treatment with cladribine. In contrast, cases 3 and 5, diagnosed with indolent forms, showed a reduced marrow infiltrate and variable expression of markers, but a better survival.

Case 4 is notable for its association with aggressive T-cell lymphoma, despite a low mast cell load (0.04%), but with intense expression of CD25 (71%) and CD2 (58%) markers; the patient had a rapidly fatal evolution. Case 6 emphasizes the importance of immunophenotyping in the diagnosis of forms with normal tryptase and marked allergic symptomatology, in which CD25/CD2 expression was essential for establishing the diagnosis. Case 7, the first treated with midostaurin in Romania, presented 1.6% mast cells with almost universal expression of CD25 (98%), reflecting an aggressive form with a positive response. Case 8, with a massive mast cell load on biopsy and aspirate (up to 70%), also presented intense expression of CD25 (98%) and CD2 (37%), confirming leukemic transformation. In conclusion, flow immunophenotyping has proven essential both in establishing the diagnosis and in assessing disease severity and evolutionary potential, providing valuable data for therapeutic stratification.

8.4. Discussions

The results obtained confirm the observations in the literature regarding the more frequent and intense expression of the CD25 marker compared to CD2 on clonal mast cells, underlining its importance as a clonality marker. Case 6 highlights the value of immunophenotyping even in indolent forms of systemic mastocytosis, where other diagnostic criteria are insufficient. Although immunophenotyping identifies mast cells with a malignant phenotype, their percentage of total marrow cells does not accurately reflect the medullary mast cell infiltrate observed histologically and cannot replace the information provided by bone marrow biopsy.

However, immunophenotyping offers the advantage of a multiparametric analysis and can assess the degree of expression of the diagnostic markers CD25 and CD2, which may suggest a correlation between their expression and disease aggressiveness. Due to the small number of cases, it was not possible to statistically demonstrate this correlation, but the development of a multicenter study is proposed. In the future, the assessment of the therapeutic response to midostaurin could include immunophenotyping for the detection of minimal residual disease, an innovative approach in mastocytosis, inspired by other malignant hematopathies such as acute leukemia or multiple myeloma.

Conclusions and personal contributions

Within the doctoral thesis "Clinical-biological definition of the patient profile with systemic mastocytosis in Romania - correlations with the therapeutic approach", three complementary studies were conducted that provide an integrated picture of this rare condition.

The first study retrospectively analyzed data from over 160 patients diagnosed between 2006 and 2025 at the only center of expertise in the country. It assessed the geographical distribution, epidemiological, clinical and therapeutic data, as well as the use of symptomatic and cytoreductive treatments. Bivariate analysis revealed patterns useful for risk stratification and treatment adaptation. A frequent delay in diagnosis and a high variability in treatments were found, highlighting the need for individualization of therapy in specialized centers.

The second study evaluated the therapeutic experience in aggressive systemic mastocytosis, identifying obstacles to access to innovative therapies. Although cytotoxic treatments were initially used, subsequent access to targeted therapies such as midostaurin demonstrated significant benefits, including for patients with low performance status. The third study explored the utility of immunophenotyping of mast cells in the bone marrow, demonstrating the sensitivity of the technique in detecting aberrant expression of CD25 and CD2 – essential markers in the diagnosis of OMS. This method has proven valuable even in indolent forms and is emerging as a promising option in monitoring therapeutic response, although it requires validation in larger studies.

Personal contributions include database development, correlation of biological markers with clinical subtypes, application of international prognostic scores on the Romanian cohort, analysis of treatments used and highlighting the regional distribution of cases. The paper provides a complex perspective on systemic mastocytosis in Romania, integrating clinical, biological and therapeutic data, and proposes concrete directions for improving the management of these patients. The need for health policies that facilitate access to early diagnosis, modern treatments and the development of networks of centers of expertise is emphasized.

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List of papers published by the doctoral student, with links to the respective publications .

Delia Soare , Dan Soare , Camelia Dobrea , Eugen Radu, Horia Bumbea - Clinical and Biological Characteristics of Four Patients with Aggressive Systemic Mastocytosis Treated with Midostaurin , Biomedicines 2025, - în curs de publicare

Delia Soare, Poliana Leru, Horia Bumbea - Innovative Therapeutic Approaches in Systemic Mastocytosis, MAEDICA – a Journal of Clinical Medicine 2025; - <https://doi.org/10.26574/maedica.2025.20.2.246>

Delia Soare , Dan Soare , Brîndușa Petruțescu, Oana Firescu, Poliana Leru, Corina Silvia Pop, Lucian Negreanu, Vlad Voiculescu, Horia Bumbea - Real-World Characteristics of Systemic Mastocytosis in Romania: Insights from a Reference-Center–Based Descriptive Study , JOURNAL of MEDICINE and LIFE, 2025 - în curs de publicare