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Molecular typing of amyloid fibrils:

Tissue deposition model and clinical heterogeneity of systemic amyloidosis

SUMMARY OF THE THESIS

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INTRODUCTION

Amyloidosis represents a group of diseases characterized by the deposition of amyloid fibrils in tissues, causing dysfunction of affected organs. Amyloid is histologically evidenced by Congo Red staining and is Congo-positive and has green birefringence ("apple green") in polarized light [1]. Amyloid is composed of different proteins that cause numerous forms of amyloidosis. 40 amyloidogenic proteins causing systemic or localized amyloidosis have been identified [2]. The differentiation between forms of amyloidosis is based on typing, which identifies the type of protein in the amyloid composition. The most frequent forms of amyloidosis are light chain (AL) amyloidosis, transthyretin (wild-type and hereditary) amyloidosis, and secondary (AA) amyloidosis [3-4].

Diagnosis of amyloidosis is a laborious process that requires clinical suspicion, identification of amyloid deposits, and amyloid typing. Amyloidosis is a systemic disease that can affect any organ, and the clinical presentation is variable. Patients are often seen by multiple specialists, receive incorrect diagnoses, and have a diagnosis delay of several months or even years. Multiple biopsies are often necessary to establish a definite diagnosis.

The heterogeneity of clinical presentations and the laborious diagnostic process make this pathology underdiagnosed globally. The prognosis of amyloidosis patients is determined by early diagnosis before irreversible organ failures are established by accumulation of amyloid fibrils.

This work focuses on the research of amyloid typing methods, improvement of amyloidosis diagnosis, and analysis of the characteristics of amyloidosis types identified in patients diagnosed over 20 years (2001-2020) at the Hematology and Bone Marrow Transplant Center of Fundeni Clinical Institute.

Given that amyloidosis is a rare and polymorphic disease with diverse clinical presentations, there is no data on incidence, diagnostic methods, or characteristics of amyloidosis patients in Romania. This work aims to describe these characteristics through retrospective analysis of data from a reference center for the diagnosis and treatment of amyloidosis.

I. GENERAL PART

Chapter 1. Amyloid typing

Amyloid typing refers to the identification of the protein involved in the formation of amyloid fibrils (fibrillogenesis) [5]. Even though different proteins are involved in fibrillogenesis, they generate amyloid fibrils that cannot be morphologically differentiated [6]. Thus, Congo red staining can identify amyloid deposits, but cannot differentiate between types of amyloid.

The differentiation between the types of amyloid is done by immunohistochemical, immunofluorescence, immunoelectron microscopy, genetic testing, or proteomic methods [7]. Typing methods can be divided into antibody-based methods (immunohistochemistry, immunofluorescence, and immunoelectron microscopy) and antibody-independent methods (genetic testing and proteomics) [8].

Immunofluorescence and ***immunohistochemistry*** are limited by the availability of antibodies, especially for the rarer forms of amyloidosis. Another problem is the sensitivity and specificity of the antibodies, as amyloid precursors often have genetic mutations and conformational changes, and antibodies against the wild-type protein may be less reactive with the mutant form [7].

Genetic testing is used in cases of hereditary amyloidosis. Mutations associated with amyloidosis have been identified in many proteins (ATTR, AApoAI, AApoAII, ALys, AFib, AGel). Genetic testing is particularly important in ATTR, where it differentiates between two distinct forms of amyloidosis: the familial form (mutant TTR protein) and the non-mutant form (wild-type TTR protein) [7].

Proteomics deals with the entire protein component of an organism or environment (proteome). As a result, the need for special tests for a specific protein is eliminated, avoiding the dependence on specific antibodies for each protein or dependence on the identification of a specific mutation [7].

Chapter 2. Classification of amyloidosis and description of the most common forms of systemic amyloidosis

In the last 27 years, the number of amyloidogenic proteins has increased from 15 to 40 proteins [2,9]. The most frequent ones are light chain amyloidosis, transthyretin amyloidosis (wild-type and hereditary), and secondary amyloidosis [3-4].

2.1 Light chain amyloidosis (AL)

Light chain amyloidosis (AL) is a rare hematologic malignancy with a protein precursor that is the light chains of immunoglobulins, synthesized by clonal plasma cells of the bone marrow [10]. The incidence rate is approximately 1.2 per 100,000 residents per year [11]. It occurs in patients with a median age of 64 years, with slight male predominance (54%) [12]. The diagnosis is often delayed by non-specific clinical presentation, sometimes with a delay of over a year [13].

The diagnosis of AL requires the fulfillment of 4 criteria established by the IMWG: (1) the presence of an amyloid-related clinical syndrome; (2) histopathological confirmation of the presence of amyloid deposits by Congo red staining; (3) evidence that the amyloid deposits are made of light chains of immunoglobulins, and (4) evidence of plasma cell proliferation [14].

The amyloid-related clinical syndrome is variable depending on the affected organ. Amyloid can deposit in virtually any tissue, but there is an increased affinity for certain organs (heart – 70-80%, kidney – 50-70%, nervous system – 20-25%, liver – 15-20% and digestive system – 5-15%) [10].

Cardiac involvement requires the fulfillment of an echocardiographic criterion (interventricular septum >12 mm without another cause of hypertrophy) or a biological criterion (NT-proBNP>332 ng/L, in the absence of renal failure or atrial fibrillation) [15-16]. Approximately 30-50% of newly diagnosed AL amyloidosis patients have cardiac involvement [17]. The progress of cardiac imaging has led to a better characterization of cardiac involvement, using global longitudinal strain (GLS) with the typical "apical sparing" appearance on echocardiography and late gadolinium uptake on cardiac MRI [18]. Cardiac biomarkers (NT-proBNP and troponin), increased in AL with cardiac involvement, have been included in cardiac staging systems, with a prognostic role [19-20].

Renal involvement is defined by proteinuria/24h > 0.5 g, with the predominant albuminuria [15]. Single renal involvement is associated with a better prognosis, and the goal is to avoid end-stage renal failure with the need for dialysis [21].

Peripheral nervous system involvement is defined by the identification of symmetrical sensorimotor axonal polyneuropathy in the lower limbs [15] and involves predominantly small, unmyelinated nerve fibers, resulting in dysesthesia, paresthesia, and progressive loss of sensitivity.

Autonomic involvement (vegetative nervous system) is defined by autonomic dysfunction, from asymptomatic orthostatic hypotension to severe hypotension, intestinal and urinary bladder dysfunction. However, orthostatic hypotension (systolic BP $\leq$ 90 mmHg) is not solely caused by autonomic dysfunction, and it is also seen in patients with low cardiac output or hypoalbuminemia, who have a decrease in plasma volume [15]. The main differential diagnosis for peripheral and autonomic involvement is complicated diabetes [22].

Liver involvement is defined by hepatomegaly (>15cm), in the absence of heart failure or an increase in alkaline phosphatase level above 1.5 x the upper normal value [15]. The association of hyperbilirubinemia is a poor prognostic factor, with rapid progression to liver failure [23].

Gastrointestinal involvement is defined by the detection of amyloid deposits in the digestive tract in patients with gastrointestinal clinical manifestations [15]. The main manifestations include dysphagia, weight loss, nausea, vomiting, abdominal pain, symptoms related to malabsorption (diarrhea, steatorrhea, anorexia), bleeding (hematemesis, hematochezia, melena, to massive bleeding that can be fatal) [24-27].

Soft tissue involvement is defined by a series of presentations of amyloid infiltration, including macroglossia, skin involvement, carpal tunnel syndrome, myopathy, claudication, arthropathy and adenopathy [15].

Respiratory involvement is defined by the presence of respiratory symptoms or interstitial imaging pattern with direct evidence of amyloid deposits in the lung [15]. The diagnosis of systemic amyloidosis with lung involvement is rare, but it is reported that interstitial amyloid is frequently present, albeit asymptomatic [28].

2.2. Familial Transthyretin Amyloidosis (ATTRh)

Familial Transthyretin Amyloidosis (ATTRh) is a rare, autosomal dominant hereditary disease caused by point mutations in the transthyretin gene. It affects 5,000-10,000 individuals globally, with a higher frequency in endemic areas such as Portugal, Sweden, and Japan [29]. Over 130 TTR mutations have been identified, each determining a specific phenotype of the disease [30]. The ATTRh phenotype can be classified into neurologic (e.g. Val30Met), cardiac (e.g. Val122Ile) or mixed [31]. The most common mutation is Val30Met, found predominantly in endemic areas [32]. In Romania, the most common mutation identified is Glu54Gln, presenting a mixed phenotype. The first case of ATTRh Glu54Gln was described in 2012 [33].

2.3. Wild-Type Transthyretin Amyloidosis (ATTRwt)

Wild-Type Transthyretin Amyloidosis (ATTRwt) is caused by the deposition of amyloid fibrils made up of wild-type, non-mutant transthyretin. It affects older individuals (mean age 78.6 years) and has a male predominance [34]. The prevalence of this disease has significantly increased in recent years, with an estimated 1.1% of heart failure patients having ATTRwt [35].

2.4. Familial Lysozyme Amyloidosis (ALys)

Familial Lysozyme Amyloidosis (ALys) is a rare, autosomal dominant disease caused by point mutations in the lysozyme gene [36]. The prevalence of the disease is unknown, with variable onset age and phenotype, even in the case of the same mutation or family [37]. Survival is prolonged compared to other types of amyloidosis (median 17.9 years), even without etiological treatment [38-39]. The first case in Romania was described in 2006 [40] and a distinct mutation (Asp67Gly) was identified.

2.5. Secondary Amyloidosis (AA)

Secondary Amyloidosis (AA) is a rare complication of chronic inflammatory diseases, with amyloid fibrils composed of serum protein A (SAP), an acute phase protein synthesized by the liver [41]. Numerous pathologies associated with persistent inflammation are described [42]. The prevalence of AA has decreased in recent years due to the availability of effective antibiotics and anti-inflammatory treatments [43].

II. ORIGINAL PART

Chapter 3. Analysis of Amyloid Typing

Amyloid typing is essential for identifying the type of protein involved, classifying amyloidosis, prognosis, and therapeutic approach.

The objective of this study was to identify the methods of amyloid typing and the implementation of these methods in routine practice. Patients diagnosed with systemic amyloidosis over a 20-year period (2001-2020) at the Hematology Clinic of Fundeni Clinical Institute were included in the study.

251 patients with amyloidosis were identified, of which 190 patients had AL, 44 patients had ATTRh, 8 patients had AA, 5 patients had ALys, and 4 patients had ATTRwt (Fig. 1.)

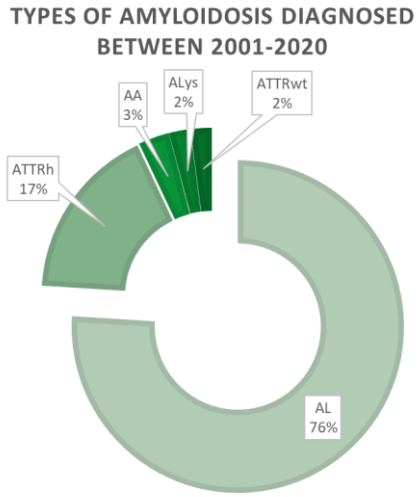


Fig.1. Distribution of patients with amyloidosis included in the study, according to type of amyloidosis (AL, ATTRh, AA, ALys and ATTRwt)

In order to identify amyloid deposits, 89.6% of patients underwent biopsies. Based on the location, the biopsies were divided into minimally invasive biopsies (abdominal fat and salivary gland biopsy), organ biopsies and osteomedullary biopsy. Minimally invasive

biopsies were the most common, representing 50% of all biopsies performed. Among organ biopsies, renal biopsy was the most frequent (55.3% of organ biopsies).

The Congo Red stain was used to highlight amyloid deposits. The highest positivity rate of Congo Red stain was recorded in renal biopsy (94%), followed by abdominal fat biopsy (88.2%) and salivary gland biopsy (80%).

Amyloid typing was performed in 56.6% of patients diagnosed with amyloidosis. The typing methods used were immunofluorescence (46%), DNA sequencing (31%), immunohistochemistry (11%), PCR-RFLP for TTR Glu54Gln (10%), and protein sequencing/mass spectrometry (2%) (Fig.2.).

The typing results showed the prevalence of the lambda light chain (60 cases) and the TTR Glu54Gln mutation (36 patients).

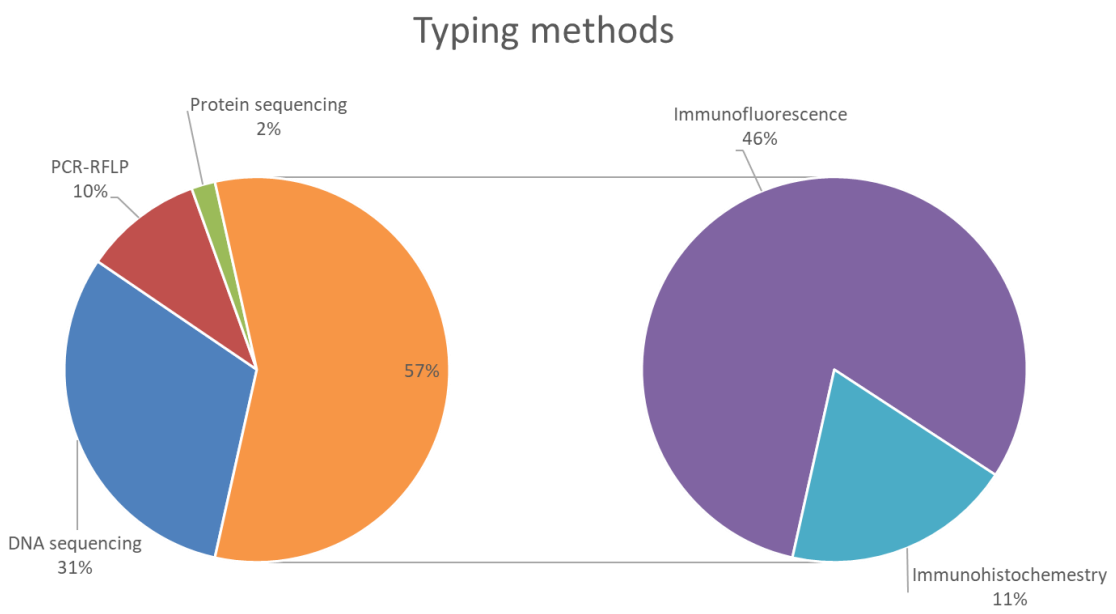


Fig.2. Distribution of patients with amyloidosis based on the method of typing used in the study

Chapter 4. Analysis of differences between amyloidosis based on the diagnostic interval

The incidence of amyloidosis has increased in recent years due to a better knowledge of this pathology and improved diagnostic methods [3].

The present study aims (1) to identify the incidence of amyloidosis cases according to the diagnostic interval, (2) to identify age differences based on the diagnostic period and (3) to establish differences in onset-diagnosis duration based on the diagnostic period.

For comparing purposes, the 20 years were divided into ***4 intervals***: 2001-2005, 2006-2010, 2011-2015 and 2016-2020.

It was observed that the number of diagnosed patients increased consecutively for each period. Over half (59.4%) of the patients with amyloidosis were diagnosed in the last 5 years (2016-2020) (Fig.3.).

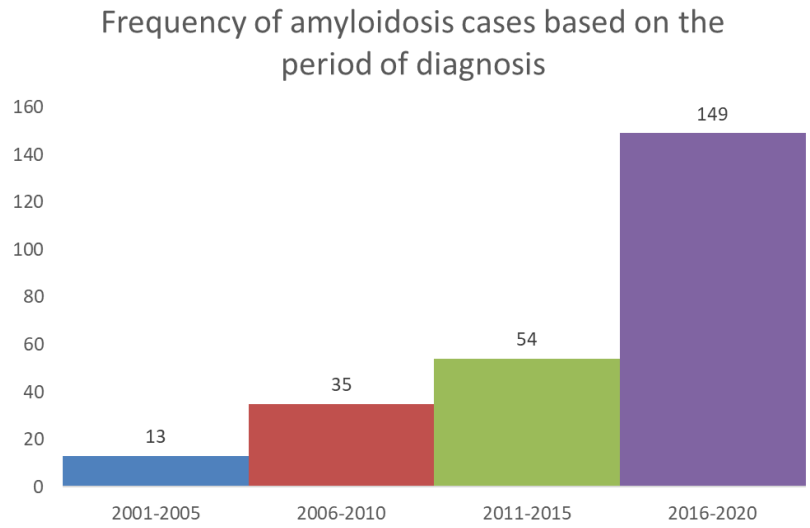
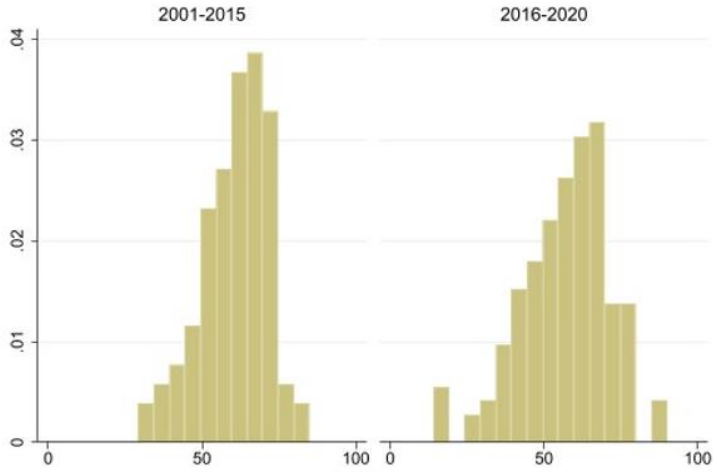


Fig. 3. Repartition of patients with amyloidosis according to the diagnostic period

A statistically significant increase in the number of non-AL amyloidosis was recorded starting in 2016. Among non-AL amyloidosis, ATTRh showed the highest increase

(from 1.2% to 15.7%). Also, a decrease in ***the average age at diagnosis*** of 3.9 years is observed, which is explained by the increase in the incidence of non-AL amyloidosis (Fig. 4.).



	Patients no.	Mean	SD
2001-2015	102	60,7	10,5
2016-2020	143	56,9	14,4
Total	245	58,5	13

P=0.0219 (t-test)

Age difference of 3.9 years between patients diagnosed before and after 2015

Fig. 4. Histograms of patient ages and difference between the average ages of patients diagnosed with amyloidosis in the periods 2001-2015 and 2016-2020

No significant statistical difference was recorded in the ***onset-diagnosis duration*** for patients diagnosed before and after 2016. However, it is noted that the onset-diagnosis duration is 35.9 months longer for non-AL patients compared to AL patients, regardless of the diagnostic period.

Chapter 5. Characterization of Identified Amyloidosis Types

5.1. Light Chain Amyloidosis (AL)

Light Chain Amyloidosis (AL) is the most common form of amyloidosis. It is a systemic disease that can affect any organ and the clinical presentation is polymorphic. The prognosis is the least favorable among systemic amyloidosis. The median survival is 4 months in case of advanced cardiac involvement, but the evolution of specific therapy has improved survival from 13 months to 4.6 years after 2010 [44].

The objectives of the study were (1) to evaluate the general characteristics of AL patients, (2) to evaluate the predominant organ involvement in AL patients, (3) to evaluate the cytogenetic risk factors in AL patients, and (4) to evaluate the survival of AL patients.

Data analysis in the present study was performed using the STATA BE 17 program. The t-student test was used for continuous variables and the χ^2 test was used for discrete variables for statistical significance analysis. The median survival was calculated using the Kaplan-Meier curves, and the log-rank test was used to determine the statistical significance between the survival curves.

The study included 190 AL patients, with median age 62 years and slight male predominance (52,6%). Most of the patients resided in Bucharest, but patients were observed from 34 different counties (Fig.5.).

Fig.5. Map of the distribution of residence of AL patients included in the study

The most frequent (>30%) symptoms of onset were edema, paresthesia, sicca syndrome, weight loss and physical fatigue. Lambda chain was the amyloidogenic protein in 72% of cases, serum protein immunofixation was positive in 77.4% of cases, median dFLC was 153 mg/l, and the median plasma cell infiltration was 10%. The frequencies of organ involvement were: kidney (67.9%), heart (59.5%), PNS (53.2%), ANS (39.5%), soft tissue (39.5%), liver (25.3%), digestive tract (9%), and lung (3.7%).

Considering the diversity of clinical manifestations at diagnosis depending on the involved organs and based on literature data, we defined the dominant organ involvement for the purpose of identifying suggestive clinical characteristics. The definition of ***predominant organ*** followed the most amyloid–impacted organ, the diagnostic criteria being attached in the paper.

The distribution of predominant organs was renal (40%), cardiac (36%), liver (7.9%), nervous system (6.8%), soft tissue (4.7%), digestive tract (2.6%), and pulmonary (1.6%) (Fig.6.).

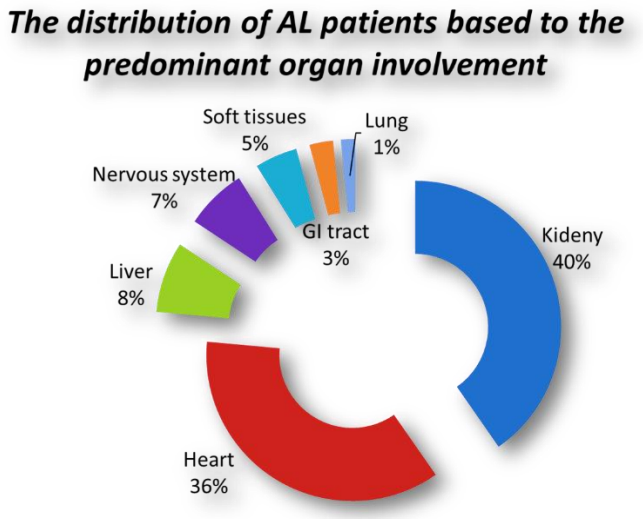


Fig.6. The distribution of AL patients based to the predominant organ involvement

Dominant renal involvement was characterized by statistically significant more frequent onset with edema and less frequently with dyspnea and cough.

Patients with ***dominant heart involvement*** had a higher proportion of light chain lambda (80.9% vs. 67.5%). Statistically significant more frequent onset symptoms recorded in this group were dyspnea, sicca syndrome, syncope, and cough.

Dominant PNS involvement was significantly more frequently associated with onset with paresthesia.

Patients with ***dominant soft tissue involvement*** had a significantly higher degree of plasma cell infiltration (26% vs. 13.4%) and a longer average onset-to-diagnosis duration (25 months vs. 12 months). Cutaneous involvement and macroglossia were the most common forms of soft tissue involvement.

Dominant liver involvement had a statistically significant more frequent onset with abdominal bloating and asthenia and less frequently with edema and sicca syndrome. The median value of hepatic stiffness was significantly higher in patients with hepatic predominance (49.6 KPa vs. 20.6 KPa). Coagulation factor X had a significantly lower average level (50% vs. 73.5%).

PNS, digestive and pulmonary dominant involvements were present in too few patients to perform statistical analysis.

Cytogenetic abnormalities were evaluated in 25 patients and were present in 32% of cases. Del17p, t (14;16), t (4;14) and hyperdiploidy were identified in 7, 3, 2 and 1 case respectively. Patients with del17p had significantly more frequent digestive involvement.

The death rate of AL patients was 65.8%, and ***the median survival*** was 19 months (Fig.7.). Survival was lower in patients with absent monoclonal heavy chain, in patients who presented with asthenia, weight loss, anorexia or coughing, who showed cardiac involvement, autonomic involvement, cardiac and liver dominance. On the other hand, patients with dominant renal involvement had a better survival rate. The presence of amyloid deposits in the bone marrow was also associated with lower survival. Patients who received treatment had a better survival rate, and among the treatments given, exposure to proteasome inhibitors, immunomodulators and autologous bone marrow transplantation were associated with better survival.

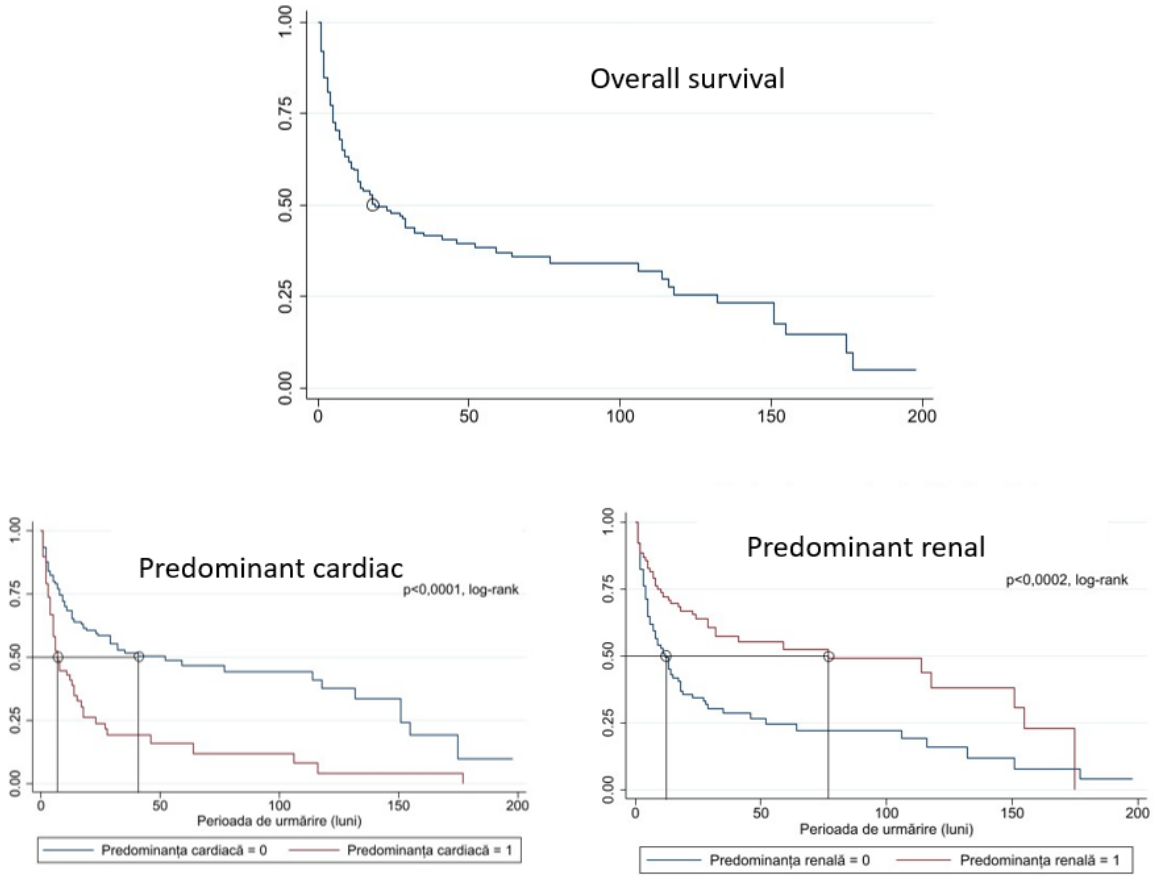


Fig.7. Survival curves based on the presence of cardiac and renal dominant involvement compared to overall survival

5.2. Familial Transthyretin Amyloidosis (ATTRh)

ATTRh is a rare genetic disease with variable phenotype depending on the type of mutation. The goals of this study were to identify the mutations present in Romania and to characterize the phenotype determined by the Glu54Gln mutation.

44 patients diagnosed with ATTRh over 20 years (2001-2020) at the Hematology Clinic of Fundeni Clinical Institute and the Neurology Clinic of the Emergency University Hospital in Bucharest were included in the study.

5 TTR mutations were identified: ***Glu54Gln, Glu89Lys, Glu89Val, Val30Met*** and ***Ile107Val***. Most patients (81.8%) presented the Glu54Gln variant.

A difference in ***patient origin*** was observed based on genotype. Patients with Glu54Gln ATTRh came from Northeastern Romania, from the Suceava, Botosani and Iasi

counties. Patients with Val30Met ATTRh came from Southwestern Romania, from Dolj County, those with Ile107Val from Northeastern Romania from Neamt County, those with Glu89Lys from Northwestern Romania from Maramures County, and those with Glu89Val from Northwest Romania, from Cluj County (Fig.8 and Fig. 9.).

Fig. 8. Geographic distribution of ATTRh patients in Romania based on mutation type

Fig. 9. Geographic distribution of Glu54Gln ATTRh patients in Suceava, Botosani and Iasi counties based on mutation type

The prevalence of ATTRh in 2020 was 1.81 per million inhabitants in Romania, 3.93 per 100,000 inhabitants in Suceava County and 2.04 per thousand inhabitants in Todireşti.

The Glu54Gln ATTRh phenotype is mixed, with early onset (average 43.2 years at onset) and cardiac involvement (100%), PNS (95.2%), ANS (81%), and ocular involvement (23%). The median survival was 60 months.

5.3. Familial Lysozyme Amyloidosis (ALys)

ALys is a rare genetic disease with an unknown prevalence. The purpose of this study to characterize the phenotype and genotype of ALys in Romania.

Five patients diagnosed with ALys Asp67Gly were identified at the Fundeni Hematology Clinic between 2001 and 2020. All patients came from the same family (Fig.10.). The average age at diagnosis was 46.2 years, with an average onset-diagnosis duration of 97.2 months and a M:F ratio of 1.5. Symptoms of onset were abdominal bloating, epistaxis, edema, asthenia, diarrhea, vasculitis, paresthesia, pruritus, and nausea. The affected organs were the liver, spleen, and soft tissues (all patients), digestive tract (4

patients), subdiaphragmatic lymph nodes (3 patients), heart (2 patients), and kidney (2 patients). The peripheral nervous system was involved in one patient, but the diagnosis was clinical – presented peripheral paresthesia.

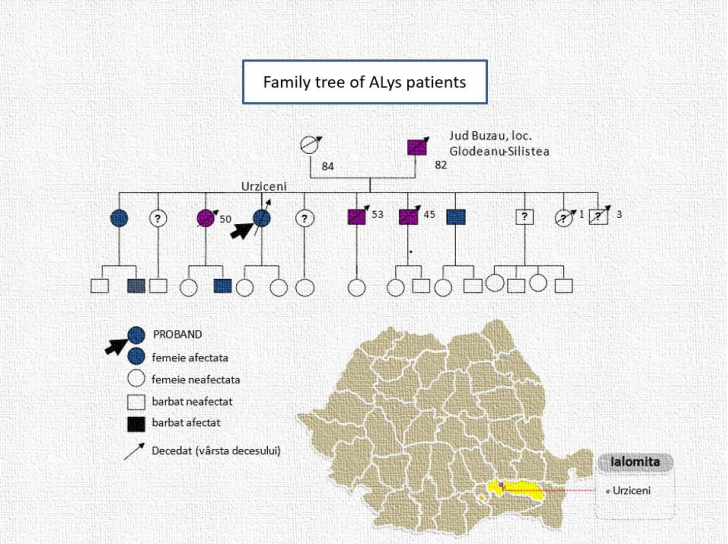


Fig.10. Family tree of the ALys patients diagnosed in Romania

5.4. Other types of amyloidosis

5.4.1 Secondary Amyloidosis (AA)

AA amyloidosis is a complication of chronic inflammatory diseases [41]. The objective of this study was to evaluate the characteristics of patients diagnosed with AA over 20 years (2001-2020) at the Hematology Clinic of Fundeni Clinical Institute.

Eight patients with AA were identified, with an average age at diagnosis of 47.4 years, an average onset-diagnosis duration of 85.3 years and a M:F ratio of 0.3 years. The most frequent onset symptoms were edema, meteorism, diarrhea, arthralgia, and paresthesia.

The underlying inflammatory pathology was composed of familial febrile syndromes (Mediterranean fever and Muckle-Wells syndrome), familial polyarthritis, systemic lupus erythematosus, ankylosing spondylitis, and common variable immunodeficiency. The most frequent organs involved were kidneys, digestive tract, soft tissues, and liver.

The median survival of AA patients was 46 months (Fig.11.).

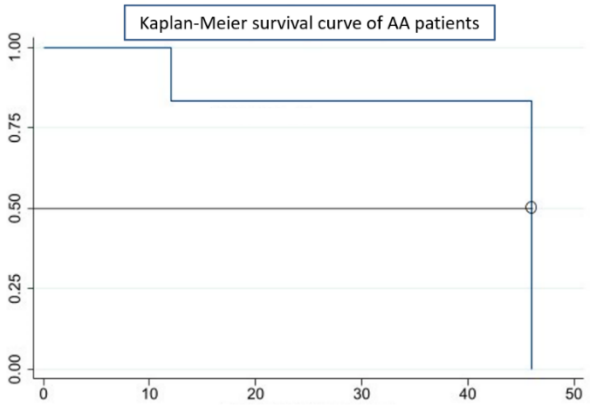


Fig.11. Kaplan-Meier survival curve of patients with AA amyloidosis. Median survival of 46 months

5.4.2. Wild-type Transthyretin Amyloidosis (ATTRwt)

ATTRwt is an age-related pathology, previously referred to as senile amyloidosis. The aim of this study was to characterize patients with ATTRwt diagnosed over a 20-year period (2001-2020) at the Hematology Center of Fundeni Clinical Institute.

Four patients with ATTRwt were identified, with a mean age at diagnosis of 80.5 years, a mean duration from onset to diagnosis of 21.3 months, and a M:F ratio of 3. The main onset manifestations were edema and dyspnea. Cardiac involvement was present in all patients, followed by soft tissue involvement (2 patients), PNS involvement (1 patient), liver involvement (1 patient) and splenic involvement (1 patient) (Fig.12.).

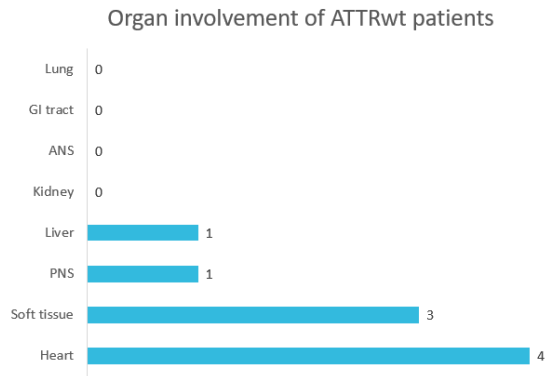


Fig.12. Organ involvement in ATTRwt patients

Chapter 6. Conclusions

Over 20 years, 251 patients with amyloidosis were diagnosed at the Hematology Center of Fundeni Clinical Institute. Five types of systemic amyloidosis were identified: AL, ATTRh, ATTRwt, ALys, and AA.

To identify amyloid deposits, 89.6% of patients underwent biopsies, which were divided into minimally invasive biopsies (abdominal fat and salivary glands biopsy), organ biopsies, and osteomedullary biopsy. Minimally invasive biopsies were the most common, representing 50% of all biopsies performed. Among organ biopsies, renal biopsy was the most frequent (55.3% of organ biopsies).

Congo Red stain was used to highlight amyloid deposits. The highest rate of Congo Red positivity was recorded in renal biopsy (94%), followed by abdominal fat biopsy (88.2%) and salivary gland biopsy (80%).

Amyloid typing is an important step in amyloidosis diagnosis but is a laborious method that requires specialized laboratory and personnel. The most commonly used typing methods were immunofluorescence (46%) and DNA sequencing (31%).

The increase in the incidence of amyloidosis was also captured in our 20-year study. Over half (59.4%) of amyloidosis patients were diagnosed in the last 5 years (2016-2020). Similar to literature data, the increase of the incidence of amyloidosis is due to non-AL amyloidosis, especially familial transthyretin amyloidosis.

As amyloidosis is a rare disease, the first step in establishing a diagnosis is to consider it as a possible differential diagnosis in patients with suggestive clinical syndromes. Although this pathology has become more widely known in recent years, patients continue to be suspected (sometimes even diagnosed and treated) of numerous other illnesses before the correct diagnosis of amyloidosis is established. A useful measure of this phenomenon is the time from onset to diagnosis, which has not shown a significant statistical change before and after 2015. In addition, non-AL amyloidosis, which are rarer, less well-known, and have a slower evolution, have a much longer mean onset-to-diagnosis duration than AL amyloidosis (48.6 months vs. 12.6 months).

Patients with AL amyloidosis have the highest frequency in the study cohort, representing 76% of diagnosed amyloidosis cases. Demographic data corresponds to literature data, with slight male predominance and the median age of 62 years.

To evaluate the underdiagnosis of AL amyloidosis, the incidence in Romania was estimated at 336.6 cases per year (based on literature data). Given the increase cases over the last 5 years, the average incidence of AL cases in the study for the period 2015-2020 was calculated to be 19 patients per year.

The most common symptoms at onset in AL patients were edema, paresthesia, sicca syndrome, weight loss and asthenia. The most affected organs were the kidney (67.9%), heart (59.5%), PNS (53.2%), ANS (39.5%) and soft tissues (25.3%).

Considering the systemic nature of AL amyloidosis, we defined and analyzed patients based on the predominant organ. The most frequent predominant organs were kidney (40%), heart (36%), liver (8%) and nervous system (7%). We monitored the specific characteristics of each organ predominance in order to identify red flags.

The presence of monoclonality in the kappa chain may be indicative of the absence of cardiac dominance. Onset with asthenia and abdominal distension in a patient with AL may suggest liver dominance. The association of a monoclonal heavy chain in the AL patient may be a sign of PNS dominance. Soft tissue dominance is associated with a higher plasma cell infiltration and a longer onset-to-diagnosis duration. These results require confirmation in prospective studies.

In terms of cytogenetic risk, the patients studied most frequently presented 17p deletion (87.5% of identified abnormalities). The main limitation of this study is the absence of testing for the t (11;14) translocation, which is most common in AL patients.

The median survival of AL patients was 19 months. In particular, the study patients with AL had a lower survival rate in the absence of a heavy monoclonal chain and in the presence of amyloid deposits on the bone marrow biopsy. Also, patients who presented with asthenia, weight loss, loss of appetite or coughing had lower median survival.

Familial transthyretin amyloidosis was the second most common form of amyloidosis among the studied patients. The incidence of ATTRh patients in Romania was 1.81/million inhabitants, placing the country among non-endemic areas. Also, similar to non-

endemic areas, the most prevalent mutations are non-Val30Met mutations. In Romania, the most frequent one is Glu54Gln, a Romanian-specific mutation with mixed phenotype and early onset. The median survival of ATTRh Glu54Gln patients was 60 months.

Familial lysozyme amyloidosis is a very rare hereditary disease, with only 10 globally described amyloidogenic mutations. Identification of the Asp67Gly mutation, found in Romania, was made in 2005 by the Fundeni Group, and the cases included in our study are the only ones diagnosed worldwide. There was only one death recorded, with a median follow-up period of 13.5 years.

AA amyloidosis is a complication of chronic inflammatory diseases, patients are often followed by the rheumatologist before the onset of amyloidosis. The low frequency of AA cases in our study is likely due to underdiagnosis. The median survival of the patients in our study was 46 months.

Similar to AA patients, patients diagnosed with ATTRwt had a very low frequency. The probable cause is the predominantly cardiac presentation, in the elderly patient, investigated by cardiologists and treated as heart failure of another cause.

Chapter 7. Personal contributions

This represents the largest study of amyloidosis patients in Romania, evaluating patients diagnosed with systemic amyloidosis over a 20-year period (2001-2020). We analyzed typing methods and results, evaluated the incidence of systemic amyloidosis cases based on the diagnostic period, and analyzed patient characteristics based on the type of amyloidosis.

We showed that amyloidosis is underdiagnosed in Romania by highlighting the reduced incidence of cases in a national expertise center. As an expression of the role of the Hematology Clinic Fundeni as an amyloidosis expertise center, the distribution of AL amyloidosis patients' residence involves 34 different counties, but the majority (32,1%) still come from Bucharest.

Our study aligns with the international trend of increasing incidence of amyloidosis. This increase is explained by improved knowledge of this pathology, increased accessibility of patients to hematologists, and the identification campaign for patients with hereditary transthyretin amyloidosis ("ATTRh Caravan").

We presented the variety of types of amyloidosis identified and described the phenotype of rare and specific Romanian hereditary amyloidosis (ATTRh Glu54Gln and ALys Asp67Gly). We identified the origin areas of ATTRh patients and demonstrated the common origin of patients with ATTRh Glu54Gln in Northeastern Romania.

Considering the differences in prognosis based on organ involvement, we defined and characterized the organ dominance for AL patients. We monitored the specific characteristics of each organ predominance for early warning signs. Differentiating between organ dominances is useful for evaluating immediate risk in case of cardiac dominance or the risk of progression to dialysis (loss of kidney function) for dominant renal involvement.

Our study highlights the importance of typing for the diagnosis and prognosis of amyloidosis patients and the importance of access to adequate typing methods for a definitive diagnosis. The existence of a specialized center with a multidisciplinary team and specialized laboratory for the diagnosis, typing and management of amyloidosis patients is essential.

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1. **Jercan A**, Bădeliţă S, Drăghici M, Stoica E, Iacob S, Coriu D. **Clinical manifestations in hereditary amyloidosis with the variant Glu54Gln transthyretin.** Amyloid. 2019;26(sup1):31-32. two: 10.1080/13506129.2019.1582501. PMID: 31343281. Indexed PubMed, Impact Factor 6.571 (Chapter 5),

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