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Immunoglobulin light chain amyloidosis

Short tutorial in screening, early detection, risk assessment and response evaluation

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Summary Immunoglobulin light chain (AL) amyloidosis is a rare and underdiagnosed life-threatening systemic disease, primarily caused by insoluble depositions of misfolded monoclonal light chains. The monoclonal light chain paraprotein originates from a small clonal B-cell or a clonal plasma cell population. If left undetected the paraprotein can induce a number of complications based on organ damage. The most dangerous and life-threatening organ dysfunction emerges from cardiac involvement. Thus, patients overall survival depends on early detection. Establishing the correct diagnosis and clear characterization of the amyloid-forming protein, staging, risk assessment and treatment are crucial and depend on a highly experienced interdisciplinary, multiprofessional team.

Keywords Albuminuria · Heart failure · Immunoglobulin light-chain amyloidosis · Paraproteinemia · Proteinuria

Abbreviations

AA	Serum amyloid A amyloidosis
AL	Immunoglobulin light-chain amyloidosis
ATTRwt	Transthyretin amyloidosis wild type
CLL	Chronic lymphatic leukemia
cMRI	Cardiac magnetic resonance imaging
dFLC	Difference between involved minus uninvolved free serum light chain level
DPD scan	Diphosphono-1,2-propanodicarboxylic acid scan
ECG	Electrocardiography
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
FL	Follicular lymphoma
HfpEF	Heart failure with preserved ejection fraction
IFE	Immunofixation
iFLC	Involved free light chain
MALT	Mucosa-associated lymphoid tissue
MCL	Mantel cell lymphoma
MGUS	Monoclonal gammopathy with unknown significance
NTproBNP	N-terminal prohormone of brain natriuretic peptide
PEL	Protein electrophoresis
sFLC κ	Serum free light chain kappa
sFLC λ	Serum free light chain lambda
sFLC-R	Serum free light chain ratio
SMM	Smoldering myeloma

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Introduction

Amyloidosis is a general term summarizing a wide range of medical conditions characterized by pathologic extracellular protein depositions. These amyloid depositions are formed by incorrectly structured and misfolded protein fragments. The misfolded protein fragments induce fibril formation leading to insolubility and extracellular depositions [1].

Amyloid depositions can be strictly localized with no need for systemic treatment. However, if various tissues and organs become affected, a systemic and often life-threatening disease may result. During the course of disease, functional organ architecture is more and more devastated by the continuous accumulation of extracellular amyloid depositions leading to complete loss of organ structure and function [1]. At this time, 36 proteins originating from differ-

Table 1 Clinical signs and symptoms in AL

Organs	Symptoms
Cardiac	Cardiac involvement occurs in 60–75% of AL patients and is of highest importance in predicting prognosis and overall survival. Symptoms: dyspnea during exertion, orthopnea, abnormal fatigue and exhaustion, ankle and lower extremity edema, anasarca, pleural effusion, arrhythmia, atrial fibrillation, syncope, and sudden cardiac death
Renal	Renal involvement occurs in 50–70% and is asymptomatic for a long time. The first clinical sign is based on nephrotic range proteinuria (nonselective proteinuria) with predominant albuminuria, ankle and lower extremity edema, eye lid edema, anasarca
Gastrointestinal	Symptomatic gastrointestinal tract involvement is seen in around 10%. Symptoms: diarrhea, constipation, early satiety, nausea, gastroparesis, pseudo-obstructions, vomiting, malabsorption with weight loss
Hepatic	Clinical signs: hepatomegaly, jaundice, pruritus, alkaline phosphatase (ALP) elevation as a sign of sinusoidal infiltration by amyloid
Pulmonal	Symptoms: progressive dyspnea during exertion, pleural effusion, emphysema, thickening of interlobular sept, postcoital hemoptysis [9]
Neuropathic	A) Peripheral symptoms: sensomotoric peripheral neuropathy, small fiber neuropathy, lumbar spinal stenosis. B) Central symptoms: autonomic neuropathy with orthostatic hypotension and frequent collapse-tendency as well as postural hypotension, gastroparesis
Coagulopathic	Clinical signs are asymptomatic abnormal coagulation tests, like acquired factor X deficiency or other acquired factor deficiencies (factors II, V, VII, X, XI, XII) leading to postinterventional bleedings as well as periorbital ecchymosis, face and neck purpura from subcutaneous vascular disruption
Soft tissue	Bilateral carpal tunnel syndrome, macroglossia (17%), hoarseness as sign of vocal cord infiltration, subcutaneous nodules, shoulder pad sign (glenohumeral arthropathia)

ent sources have been identified [2]. Three entities constitute the most common forms of systemic amyloidosis: AL (light chain amyloidosis), ATTRwt (wild type transthyretin amyloidosis) and AA (serum amyloid A amyloidosis).

AL typically occurs in patients with a premalignant clonal B-cell or plasma cell population secreting a pathologic-structured paraprotein. In contrast, in ATTRwt the amyloid deposits are build up by transthyretin. Transthyretin is a common plasma protein responsible for binding and transporting thyroid hormones and originates in the liver and the choroid plexus. ATTRwt affects mostly the older and male population with pre-existing systemic diseases like chronic kidney disease (CKD) chronic lung disease (chronic obstructive pulmonary disease [COPD], emphysema). ATTRwt is usually diagnosed by cardiologists as it affects primarily the myocardium being one possible cause of heart failure with preserved ejection fraction (HFpEF). In AA the protein depositions are formed by serum amyloid A (SAA) functioning as a common acute phase protein secreted during inflammation and further associated with chronic inflammation (familiar Mediterranean fever, inflammatory bowel disease, rheumatic diseases, and hyperinflammation syndromes).

The main focus of this article is immunoglobulin light chain amyloidosis (AL). In AL the fibrillary protein fragments are composed by misfolded clonal immunoglobulin-free light-chains produced and secreted by clonal plasma cells, or by a monoclonal B-cell population [3]. All premalignant entities associated with secretion of monoclonal paraprotein MGUS, SMM, CLL, FL, MCL, MALT-lymphoma and others, harbor the risk of paraprotein-triggered tissue damage [4]. The mechanisms of paraprotein-driven tissue damage and systemic deterioration are multifarious and complex and a new and rapidly growing field of interest. A new group of rare and specialized para-protein-initiated and driven diseases was established termed monoclonal gammopathy of clinical significance (MGCS) [5]. Based on the multiple mechanisms of tissue damage, varying categories of MGCSs are defined: organized depositions like fibrils or crystals versus nonorganized depositions; paraprotein-triggered autoimmune disorders; paraprotein-associated activation of alternative complement pathway; absorption of active molecules, or uncontrolled secretion of cytokines. The organs and systems involved within MGCS include kidney, peripheral nerves, skin, eyes, and hemostasis resulting in bleeding disorders [4]. Based on this new knowledge the regular screening of MGUS patients should not be focused exclusively on progression to multiple myeloma but also on detection of paraprotein-associated organ damage [4, 5].

AL is one of the best analyzed entities within the group of MGCS. In AL two different modes of organ damage are induced by toxic light chains: one such mechanism includes the step-by-step disruption of tissue architecture and slowly evolving loss of organ function due to progressive formation of extracellular depositions disrupting the mechanical and structural tissue properties [6]. The other mechanism of damage is a direct toxic effect of the misfolded circulating light-chain prefibrils on cardiomyocytes termed proteotoxicity [7] in AL. In vitro tests with human cardiac fibroblasts demonstrated that the cardiotropic light-chain prefibrils were internalized and interact with specific mitochondrial proteins. These complex and not fully clarified intracellular processes are suspected of being responsible for impaired cardiomyocyte viability, increased reactive oxygen species production and mitochondria dysfunction leading to rapid and progressive cell death of cardiomyocytes [7]. Early diagnosis and correct differentiation between the different forms of systemic amyloidosis (AL versus ATTR versus AA) are thus paramount before determining the most suitable type of therapy.

Fig. 1 Typical perioral and periorbital ecchymosis. A 50-year-old woman with amyloid purpura. **a** July 2018—at diagnosis, **b** November 2018—after 4 × BCD. (Photographs reproduced with permission from Univ. Prof. Dr. Christian Sillaber). *BCD* bortezomib/cyclophosphamide/dexamethasone

Clinical signs and symptoms

The most commonly involved organs in AL are heart and kidney, while also liver, gastrointestinal tract, lungs, the peripheral and autonomic nervous systems might be affected. The pattern and intensity of organ involvement determines the clinical presentation. Symptoms are often very unspecific, especially at the beginning and may include the following: fatigue, dyspnea, ankle and leg edema, weight loss, diarrhea, obstipation and orthostatic hypotension [8]. However, there are some specific but very rare signs suggesting the presence of amyloidosis like periorbital ecchymosis, enlargement of the tongue, bilateral carpal tunnel syndrome and a typical form of nail dystrophy (Table 1; Fig. 1).

Biomarker-based screening and diagnostic approach

Screening and early suspicion of AL in patients with monoclonal gammopathy

If the diagnosis of a monoclonal gammopathy is verified by the recommended serum and urine tests [4], the regular use of biomarkers during follow-up are of utmost importance. In particular, patients with an abnormal free light-chain ratio should undergo careful evaluation regarding paraprotein-induced organ damage by use of organ-specific biomarkers. The most appropriate biomarkers for cardiac tissue damage are elevation of NTproBNP and cardiac troponin T in absence of renal failure or atrial fibrillation [10]. Proteinuria and albuminuria in the absence of long-lasting diabetes or hypertension are often associated with renal damage [4]. These easily accessible serum and urine-based biomarkers increase during the early phases of disease months before clinical symptoms become evident [4]. NTproBNP, albuminuria and proteinuria are useful for raising early suspicion of tissue damage. If NTproBNP is elevated, further cardiac tests

are necessary: noninvasive imaging techniques like ECG, echocardiography, cardiac MRI and DPD scan should be performed. Proteinuria and/or albuminuria are very effective indicators for renal involvement [11].

Verification of AL

To avoid misdiagnosis and consecutive mistreatment, patients with suspected AL have to undergo a complex multistep diagnostic procedure. The detection of Congo red positivity in the tissue biopsy is not sufficient for diagnosis and treatment of AL. Fibri -characterization is mandatory [12].

Tissue biopsy and Congo red staining

Congo red staining and Congo red positivity is the first step in the diagnostic procedure for all forms of amyloidosis. For the staining procedure different tissues can be used [13]:

- Deep subcutaneous fat aspiration (is frequently used), but further fibril characterization is very difficult and often not feasible.
- Silent site biopsies with low risk of additional procedural organ damage as salivary gland biopsies or deep rectum biopsy.
- Bone marrow biopsy can be used for Congo red staining in AL diagnosis. Bone marrow biopsy is useful in AL for further characterization of the B-cell or plasma cell clone, and for cytogenetic evaluation.
- Affected organ biopsy (kidney, heart, liver, gastrointestinal tract) should be performed if there is a high-grade suspicion of AL and Congo red amyloid depositions could not be detected within the low-risk tissue biopsies. In order to minimize the risk of additional organ damage (bleeding complications, perforation, complete loss of organ function) affected organ biopsies should be performed by a highly specialized and experienced team.

It is of major importance to keep in mind the risks and pitfalls regarding the Congo red staining procedure and affected organ biopsies. As mentioned above tissue can be taken from sites with low risk of procedural damage (silent site biopsies). Deep abdominal subcutaneous fat aspiration is frequently used for Congo red staining and but further amyloid characterization by immunohistochemistry is very difficult and often not feasible. The reported positivity for Congo red staining of deep abdominal fat aspiration varies between >70% and 90% according to the center's experience [12].

It is important to keep in mind that false-positive staining results of deep abdominal fat aspirates from diabetic patients (localized AL amyloidosis from frequent subcutaneous insulin administration) might occur [14]. On the other hand as mentioned above, Congo red negativity is not uncommon in abdominal fat aspirations or silent site biopsies and does not exclude systemic AL [15].

Amyloid fibril characterization

A crucial process within the diagnostic work flow is the definitive characterization of the amyloid-forming fibrils. This can be done by different techniques:

i) Immunohistochemistry: 75–80% sensitivity, 80% specificity. It is challenging and requires a highly specialized and properly trained pathology lab [16].
ii) Immunoelectron microscopy: 70–80% sensitivity, 100% specificity. It is a very efficient technique but not widely available [17].
iii) Laser capture microdissection with mass spectrometry: the gold standard in amyloid characterization with 95% sensitivity, 100% specificity, but not routinely available [18].

The differentiation between the two most common forms of amyloidosis, AL and ATTRwt is of utmost importance, as the incidence of both MGUS and ATTRwt increases with age. Therefore, the precise definition of the underlying amyloid-forming protein is crucial in this patient population. In recent years new results have been published. In 2017 the first data were published describing a higher frequency of monoclonal gammopathies (10–25% up to 49%) in patients suffering from ATTRwt [19]. In 2018, these findings were confirmed [20]. In November 2019, the first report describing four patients, each one with two different types of amyloid depositions was published [21]. These new and very interesting results need further validation since they will significantly impact AL and ATTR work up.

In the light of these findings, bone scintigraphy with a 99mTc-labelled pyrophosphate or DPD tracer may be of future interest for definitive exclusion of ATTRwt or suspicion of coexisting ATTRwt in patients with AL. These new insights will clearly influence the diagnostic work up for AL and ATTR in the near future [8].

Cardiac work up

The cardiac workup is described in detail in the manuscript of Duca and Binder in this issue [22]. The cardiac work up is of major importance particularly since cardiac impairment directly influences treatment tolerability and survival. All patients with cardiac amyloidosis (AL, ATTR and all other categories of amyloidosis with cardiac involvement) exhibit heart failure with preserved ejection fraction (HFpEF) and normal left ventricular function parameter. The typical pathologic findings in the ECG, cardiac ultrasound and cardiac MRI are listed and described in the article of our cardiology team mentioned above.

The importance of AL amyloid-associated cardiac impairment is reflected by the Mayo staging systems established in 2004 and revised in 2012. High levels of cardiac biomarkers reflecting high-grade cardiac impairment are associated with short overall survival.

In our center, all patients with newly diagnosed AL undergo a DPD scan for exclusion of coexisting ATTR. This is not recommended by international guidelines at this time, but in our opinion these data will be of increasing interest in the near future, especially due to the latest findings specifying 10–25% ATTRwt patients with coexisting monoclonal gammopathy and in addition to the first report regarding four patients with two different types of amyloid deposits [21].

Renal work up

In order to minimize the risk of additional organ damage (bleeding complications, perforation, complete loss of organ function) organ biopsies should only be performed by a highly specialized and experienced team. Hence, proteinuria and albuminuria can readily be checked on a routine basis by spot urine examination using protein/creatinine ratio and albumin/creatinine ratio. For quantification of proteinuria, albuminuria and quantitative free light chain excretion 24 h urine collection is highly recommended.

Primary staging and risk assessment

Although AL is a primary hematologic disorder, the course of AL is significantly determined by the severity of organ dysfunction. Cardiac dysfunction is the most important factor with impact in morbidity, mortality and overall survival.

Organ-specific biomarkers reflecting organ damage

Based on the results of GP Merlini et al. regarding NTproBNP and the results from the Mayo Clinic scientists regarding cardiac troponin T, the first staging and risk assessment system in AL was introduced in 2004

[10]. Based on the two cardiac biomarkers, elevated NTproBNP and elevated cardiac troponin T, three categories of cardiac dysfunction, i.e., of disease severity, were defined. In 2012, a revised version was published [23]. A third parameter was introduced additional to cardiac troponin T (≥ 0.025 ng/mL) and NTproBNP (≥ 1800 pg/mL). The new parameter was defined as the difference between involved and uninvolved free serum light chain levels (dFLC ≥ 180 mg/L) reflecting the monoclonal light chain burden. With this additional parameter the revised staging system was extended to four categories of disease severity. This staging system was validated in multiple trials and is currently used as revised Mayo Cardiac Staging System. Based on the primary staging system from 2004, two European collaborative biomarker trials were conducted. The European protocols were also based on NTproBNP and cardiac troponin T. But, stage III was divided into IIIA with NTproBNP < 8500 mg/L and IIIB with NTproBNP > 8500 mg/L. The results were published in 2015 [24]. Stage IIIB defines patients with an advanced stage of cardiac involvement and very dismal prognosis with a median OS < 6 month and 1-year survival rate < 40%.

Proteinuria and especially albuminuria are hallmarks of renal AL Two staging systems for renal involvement were introduced, while only the system defined by Palladini et al. is currently validated [11]. Both systems are primarily based on eGFR and proteinuria. If there is no proteinuria and no reduction of eGFR < 50 mL/min/70 kg body weight at the time of diagnosis, the risk for ESRD is very low. If both parameters are affected, the probability of developing ESRD within 3 years after diagnosis is > 50%. Based on eGFR and proteinuria, the risk of progression to ESRD requiring dialysis can be estimated independently from hematologic response.

Hematologic biomarkers

Introduction of free light chain measurement revolutionized AL primary diagnosis and disease monitoring In 2001, the first commercial test system for free light chain measurement became available. The quantification of the serum free light chain concentration was performed with a polyclonal antibody-based nephelometric assay (Freelite™, Binding Site Group Ltd, Birmingham, UK) [25]. Ten years later a second test system for free light chain measurement was launched (N Latex™ sFLCκ and λ, Siemens Healthcare Diagnostics GmbH, Germany) [26]. In contrast, the Siemens test is based on a monoclonal detection system for measurement of the serum light chain concentration. Both systems are in use but act differently and are not interchangeable. All published and validated staging, risk assessment and response parameters within the international consensus guidelines for

MGUS, SMM, MM and AL [27] are based on the use of the Freelite™ test from Binding Site.

Bone marrow biopsy provides important insight into documentation of plasma cell infiltration and genetic testing A higher plasma cell infiltration rate reflects higher disease activity and clearly corresponds with impaired overall survival [28].

Similar to multiple myeloma, genetic abnormalities are of high importance [29] Translocation t [12, 15] is detected in > 50% of AL.

Additional prognostic factors including age, performance status, number of involved organs, systolic blood pressure and presence of immunoparesis [30] at diagnosis, as well as new biomarkers (growth differentiation factor 15, von Willebrand factor) are under evaluation [31, 32].

Response assessment

Two different categories of responses have to be evaluated in AL: hematologic as well as organ response.

Hematologic response

Hematologic response is assessed by serum free light chain measurement. Reduction of the serum free light chain level is the primary requirement for organ response and overall survival benefit [33]. Serum free light chain concentrations are measured by the use of the Freelite™ assay. Again, it has to be noted that currently only the Binding Site Freelite™ test system is validated for clonal response evaluation. For hematologic response assessment the serum free light chain difference (dFLC), protein electrophoresis (PEL) and immunofixation (IFE) in serum and urine are validated response criteria.

Since 2012 the international amyloidosis consensus criteria for hematologic response evaluation are in use [34, 35]. Four levels of response (determined after 3 and 6 months) were defined:

- **aCR** (amyloid complete response): negative serum and urine IFE and normalization of the free light chain ratio (FLC ratio)
- **VGPR** (very good partial response): decrease of the difference between involved minus uninvolved light chains (dFLC) < 40 mg/L
- **PR** (partial response): reduction of the difference between involved minus uninvolved light chains (dFLC) > 50%
- **NR** (no response).

Limitations of biomarker-based hematologic response evaluation

Approximately 13–20% of all AL patients exhibit low FLC concentrations and dFLC levels < 50 mg/L at time of diagnosis. This patient population is excluded from all trials since the response assessment according to

the criteria from 2012 is not applicable. However, it is clearly documented that this group of patients exhibit an excellent prognosis with prolonged survival compared with AL patients with a detectable dFLC >50 mg/L [36, 37]. Sidana et al. presented OS data with OS not reached vs. 69 months in AL patients achieving a dFLC <10 mg/L in this special group of patients [38].

Patients in a CR as defined in 2012 with negative serum and urine immunofixation and normalization of the FLC ratio that continue demonstrating a persisting progressive organ deterioration comprise another relevant population subset. This continuous organ damage can be provoked by non-amyloidogenic tissue-stress factors or by further production of minimal concentrations of toxic light chains not captured by conventional techniques. "False" normalization of the FLC ratio through discordant suppression of FLC levels can mask the persistent production of minimal concentrations of toxic free light. To avoid "false" aCR results, minimal residual disease (MRD) detection is under intensive investigation.

Aiming minimal false negative amyloidogenic serum free light chain levels new concepts of very low involved free light chains (iFLC) levels are under investigation. iFLC <20 mg/L compared with consistent higher levels of iFLC within a normal FLC ratio is of major interest. An additional serologic parameter of a very low dFLC level (<10 mg/L) for optimization of deep response assessment is being considered. Other forms of MRD measurements are assessed by new generation multiparametric flow cytometry, but are limited to highly specialized centers and are currently under investigation [39].

Organ response

Severity of cardiac dysfunction, cardiac response and organ recovery are crucial for overall survival.

If severe cardiac dysfunction is present at diagnosis according to stage IIIB or stage IV, median survival is limited to 3–6 months. Significant organ response results in direct improvement of survival. For sufficient organ recovery, toxic light chain concentrations have to be reduced to VGPR or better. The most frequently used marker for cardiac involvement and treatment response is NTproBNP (NTproBNP reduction >30% and >300 ng/L decrease if baseline NTproBNP >650 ng/L). The severity of renal involvement does not directly affect survival but is of major importance in risk assessment concerning progression to end stage renal disease and dialysis. There is a high risk of progression to ESRD and dialysis independent of the hematologic response if proteinuria is >5 g/24 h and eGFR is <50 mL/min at the time of diagnosis [11].

Limitations of biomarker-based organ response assessment

Organ response evaluation based on biomarker measurement has certain limitations since biomarker levels are influenced by many unrelated parameters, including comorbidities, comedication, and cardiac arrhythmias. It is also of note that organ responses might appear months after reduction of the toxic light chain concentration.

Currently, great efforts are being made to establish an organ response grading system including functional tests such as the 6-minute walk test for assessing response improvement.

Conclusion

Early diagnosis of systemic AL and all entities of MGCS require a high level of awareness and the frequent use of established biomarkers during the regular visits from time of diagnosis. Ultimately, successful treatment depends on the collaboration of a highly skilled team, experienced in managing the complex diagnostic work up and monitoring ongoing therapeutic progress.

Take home message

- Paraprotein might be dangerous.
- Early detection of paraprotein-associated organ damage is of tremendous importance.
- Early detection is easy by the use of serum biomarkers and spot urine at the regular appointments.
- Management of AL patients should be limited to centers with a high degree of interdisciplinary, multiprofessional cooperation and a high level of expertise.

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