Prognosis and Staging of AL Amyloidosis

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Abstract

The treatment options for systemic light chain amyloidosis (AL) are currently widening in an unprecedented way, brought about by an expanding arsenal of anti-myeloma therapy as well as by novel approaches to target toxic light chains and, most recently, deposited amyloid directly. In this context, accurate estimates of prognosis in AL, which allow for reliable patient advice and for example comparison of different therapies, are particularly important to clinicians. Some biomarkers and especially the genetic background of the underlying clonal disease as evaluated by interphase fluorescence in situ hybridization even have predictive value, enabling an appropriate treatment selection. Derived from the most frequently involved organs in AL, heart and kidney, this review focuses on overall survival and renal survival. A comprehensive overview and summary of reported prognostic factors and biomarkers in AL is given and the most important and validated factors are highlighted. Finally, established staging systems in AL as well as validated and perspective response criteria are presented.

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Introduction

Light chain amyloidosis (AL) is a heterogeneous and life-threatening disease caused by misfolded free light chains (FLC) that typically originate from small plasma cell clones and in rare cases B-cell clones. Although less than 10% of AL patients qualify for CRAB criteria [1], the majority of these patients have a significant impairment of vital organs, such as the heart, kidney, and liver [2]. This implies that myeloma risk factors are usually not applicable in AL patients. Due to the complexity of AL, several factors determine the prognosis and have to be considered. Although this review does not claim to be exhaustive, biomarkers and factors that were of prognostic value for AL patients in large clinical studies are presented in Tables 1 and 2. The most important factors and biomarkers are discussed in greater detail within the text.

We discriminate plasma cellular factors on the one hand, which correspond to the pathogenic light chains and underlying clonal bone marrow disorder and, on the other hand, organ biomarkers, which reflect the end-organ damage caused by the toxic FLC and the deposited amyloid fibrils. The (early) prognosis of AL patients is almost entirely determined by the amyloidogenic end-organ damage, with severe heart involvement conferring

Table 1. Prognostic significance of plasma cellular factors/biomarkers at time of baseline assessment

Factor/biomarker	Medium	Threshold	Prognostic significance	Ref. No.
Plasmacytosis	BM cytology or histology	>10%	Adverse OS and PFS in MVA	1, 3, 4
		≥20%	Adverse OS in MVA	5
	BM MFC	>1%; ≥2.5%	Adverse OS and PFS in MVA	6, 7
	PBPC	>5 × 10 ⁵ /L	Adverse OS (limited MVA)	8
M protein	Urine	Cont. or >1 g/24 h	Adverse OS in MVA	9, 10
FLC	Serum: iFLC	>125/152 mg/L	Adverse OS in MVA	11, 12
	Serum: dFLC	Cont. or >50/180/196/λ 182/κ 294 mg/L	Adverse OS in MVA, more frequent and severe heart involvement	2, 13–15
Immunoparesis ¹	All uninvolved Ig in serum	Below lower limit of normal	Adverse OS in MVA and in patients with cardiac involvement, poor response to first-line treatment	16, 17
Any chromosomal aberration	FISH/BM	Presence	Increased plasmacytosis, cardiac involvement, adverse OS in MVA	18, 19
t(11;14)	FISH/BM	Presence	Adverse OS and EFS in MVA (bortezomib-based regimens or if ≤10% BM plasmacytosis) Favorable OS after HDM, better EFS with daratumumab	19–23
Gain of 1q21	FISH/BM	Presence	Adverse OS and EFS in MVA or UVA (M-Dex, daratumumab)	23, 24
Deletion of 17p	FISH/BM	>50% cells	Trend towards shorter OS	25
Trisomies	FISH/BM	Presence	Adverse OS in MVA (generally or if >10% BM plasmacytosis)	19, 21

BM, bone marrow; MFC, multiparametric flow cytometry; PBPC, circulating peripheral blood plasma cells; Cont., continuous; OS, overall survival; PFS, progression-free survival; MVA, multivariate analysis; UVA, univariate analysis; FLC, free light chains; iFLC, involved FLC; dFLC, difference of involved minus uninvolved FLC; FISH/BM, fluorescence in situ hybridization of plasma cells from bone marrow aspiration; EFS, event-free survival; HDM, high-dose melphalan + autologous stem cell transplantation.

¹ Defined as the suppression of the uninvolved immunoglobulins (IgG/IgA/IgD/IgM).

the worst prognosis. However, some plasma cellular factors are associated with a specific organ involvement pattern. The most important association, although not causatively clarified, is that the severity of heart involvement seems to depend on the serum FLC levels at initial diagnosis, while renal involvement is frequently found with lower circulating FLC [2, 14, 39]. When it comes to chemotherapy, plasma cellular factors gain prognostic value independent of or in addition to organ biomarkers. In specific cases, the mutational background of the clonal plasma cell is even predictive for the response to different treatment regimens [20, 21, 24].

Prognostic Value of Plasma Cellular Factors in AL Amyloidosis

Free Light Chains

The diagnosis and monitoring of AL patients were revolutionized by the introduction of a polyclonal antibody-based nephelometric assay for quantification of serum FLC (Freelite, Binding Site, UK) [40, 41]. One decade lat-

er, another commercial assay based on monoclonal antibodies became available. Both assays are currently used in diagnostic laboratories. It is important to note that the assays perform very differently in clinical practice and cannot be used interchangeably [42]. All work presented in this review and the validated thresholds used by the AL community refer to the initially introduced Freelite assay.

The FLC detection provided the possibility to quantify the amyloid-precursor protein itself, which obviously makes such an assay advantageous in AL. Moreover, an FLC test is also applicable in patients who lack an intact immunoglobulin, which is the majority of cases in AL [2, 27]. Last but not least, the FLC tests are quite sensitive and as such more suitable for the usually small plasma cell clones in AL compared to other plasma cellular factors. Several studies proved and validated the prognostic significance of serum FLC for overall survival (OS) in AL [2, 11–14], and the FLC test soon became regarded as essential for the diagnosis and monitoring of treatment response in the international consensus guidelines for AL amyloidosis care [43]. Various thresholds for the difference between involved and uninvolved FLC (dFLC) were

Table 2. Prognostic significance of individual organ factors/biomarkers at time of baseline

Factor/biomarker	Medium	Threshold	Prognostic significance	Ref. No.
NT-proBNP	Serum	≥152/>332/>1,800/>2,736 pg/mL >8,500 pg/mL	Adverse OS in MVA Adverse OS in MVA (within high-risk subgroup of patients)	10, 12, 15, 26, 27 28
BNP	Serum	>81 pg/mL >700 pg/mL	Equivalent to NT-proBNP >332 pg/mL Equivalent to NT-proBNP >8,500 pg/mL	29 29
cTNT	Plasma or serum	Cont. or >0.03/≥0.035 µg/L	Adverse OS in MVA	9, 10, 15
cTNI	Plasma or serum	Cont. or >70/>100 ng/L	Adverse OS in MVA	9, 10, 12
hs-cTNT	Plasma or serum	Cont. or ≥14/≥50/≥54/>77 ng/L	Adverse OS in MVA	12, 30, 31
Ejection fraction	Echo (LVEF) MRI (MCF)	EF Cont. or <45%/<50% MCF ≤56.6%	Adverse OS in MVA	9, 10, 31, 32
LV longitudinal function	Echo (GLS) MRI (LAS)	GLS <-11.8 % LAS <-7%	Adverse OS in MVA	32–34
Septum thickness	Echo	Cont. or >15 mm	Adverse OS in MVA	9, 10
NYHA class	Clinical examination	>2	Adverse OS in MVA	12
Systolic blood pressure	clinical examination	<100 mm Hg	Adverse OS in MVA (within high-risk subgroup of patients)	28
Atrial arrhythmia	ECG/clinical examination	presence	Adverse OS in MVA	35, 36
EGFR	From serum creatinine	<50 mL/min	Favorable OS in MVA, adverse RS in MVA	35, 37
Uric acid	Serum	>8 mg/dL	Adverse OS in MVA	38
Albumin	Serum	Cont. or ≤30 g/L	Adverse OS in MVA, adverse RS in UVA	10, 37
Proteinuria	Urine	>5 g/24 h	Adverse RS in MVA	37
Albumin-to-creatinine ratio	Urine	>220 mg/mmoL	Adverse EFS in MVA (daratumumab-based regimens)	23

OS, overall survival; Cont., continuous; MVA, multivariate analysis; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain; ECG, electrocardiogram; EGFR, estimated glomerular filtration rate; RS, renal survival; MCF, myocardial contraction fraction; LAS, long axis strain.

shown to be prognostic (Tables 1, 2), indicating that dFLC could also be regarded as a continuous risk factor. The threshold of 180 mg/L is well validated and used as part of an AL staging system [15, 35]. Serum FLC levels are closely correlated with amyloidogenic end-organ damage. A high dFLC is associated with more frequent and more severe organ involvement and dysfunction, especially heart, translating into an unfavorable prognosis independent of other factors [2, 13, 41]. On the other hand, AL patients with a low dFLC represent a distinct clinical subgroup with a favorable prognosis and preferentially kidney involvement [2, 14].

The nephelometric FLC test does not distinguish between the clonal light chains and unspecific polyclonal background. Unspecific accumulation of serum FLC frequently occurs with impaired renal function and might limit the significance of FLC blood levels [44]. This po-

tentially impedes the identification of the amyloidogenic light chain and the prognostic assessment in many patients with AL amyloidosis [45]. The FLC K/L ratio is less dependent on kidney function and therefore used for diagnosis of the disease rather than absolute FLC levels alone [44]. Moreover, the dFLC is routinely used for monitoring of the disease [46]. Nevertheless, the detection of very small clones and detection of early relapse in AL is challenging with the current standard techniques. Mass spectrometry-based analyses have shown superiority in detecting clonal disease and should further improve prognostication and response evaluation in AL [47].

Plasma Cellular Burden

The bone marrow diagnostic is important for making the initial diagnosis of systemic AL and characterizing the underlying clonal disease. Although a high plasma cell

burden in AL usually translates into higher levels of amyloidogenic FLC [3, 41], this association depends on the one hand on the secretory activity of individual plasma cell clones and on the other hand on the elimination from the serum; therefore, this assumption does not always hold true. However, a higher plasma cellular burden in bone marrow cytology or histology, or even the detection in peripheral blood, reflects a higher disease activity and is prognostically adverse for OS in AL [1, 3–5], while smaller clones commonly have a deeper and longer response to treatment [2, 14]. Multiparametric flow cytometry emerged as a reliable tool for the detection of small plasma cell clones in bone marrow aspirates and the prognostic value of this analysis in AL could be demonstrated by several groups [6, 7, 48]. It was also shown that immunoparesis, an adverse prognostic marker in plasma cell proliferative disorders, predicts the response to first-line therapy and confers an independent prognostic marker in AL [16, 17].

Before the establishment of the FLC test, M protein was used to assess the clonal size. Urine M protein and not serum M protein was reported to be prognostic in multivariate analyses [9, 10]. This again indicates that light chains, which often concentrate in the urine of AL patients with renal involvement or excessive serum light chain burden, have a higher prognostic value compared to the clonal heavy chains. The absence of the heavy chain is an independently negative prognostic factor for OS, suggesting that unbound light chains may be inherently more prone to undergo misfolding into an amyloid configuration [13].

Genetics of Plasma Cell Clones

The prognostic value of fluorescence in situ hybridization (FISH) abnormalities has been well established in MM for decades [49]. Soon it became evident that FISH abnormalities were also independent prognostic factors in AL [24, 50]. Most of the AL patients (70–90%, depending on the cohort and sensitivity of the analysis) have detectable cytogenetic abnormalities with iFISH [19, 21, 50, 51]. However, the genetic landscape of plasma cell clones from AL patients differs compared to myeloma patients. Hyperdiploidy and features regarded as high risk in MM are less common in AL. In contrast, there is a much higher prevalence of translocations involving the immunoglobulin heavy chain locus, which is suspected to be an early event in the clonal evolution of gammopathies [52]. Translocation $t(11;14)$ is a hallmark of AL amyloidosis and is associated with the Bence Jones phenotype as well as less complex clonal landscape [20, 53].

It is understandable that the genetic background of the plasma cell clone provides additional prognostic information to the mere secretory capacity. One reason is that the efficacy of chemotherapeutic regimens seems to be closely linked to the mutational landscape of the plasma cell [20, 24, 54]. Therefore, the FISH diagnostic is potentially more important for long-term outcome of AL patients and should mostly be judged in combination with the therapeutic regimen.

Myeloma high-risk cytogenetics ($t(4;14)$, $t(14;16)$, $del17$) are rare in AL and not an unfavorable prognostic factor in AL patients treated with bortezomib [20], while more complex karyotype clones and higher rates of $del17$ have a slightly worse prognosis [19, 21, 25]. Gain of 1q21 is an independent adverse prognostic factor in AL amyloidosis patients treated with melphalan and dexamethasone standard chemotherapy as well as with daratumumab [23, 24]. In contrast, $t(11;14)$ is an unfavorable independent prognostic factor in AL patients treated with bortezomib [20, 21]. However, ASCT and daratumumab at least overcome this negative impact [21–23, 55].

These findings strongly suggest that a risk-adapted treatment in AL amyloidosis has to recognize FISH diagnostic [56]. For example, patients with $t(11;14)$ preferentially receive high-dose therapy with stem cell transplant or melphalan-based chemotherapy [55]. Recently, it was found that myeloma cells with $t(11;14)$ show a particularly high expression of the anti-apoptotic protein Bcl-2 [54], indicating susceptibility to Bcl-2 inhibition by venetoclax and supporting the predictive value of FISH diagnostic in AL patients. A series of case reports presented excellent results with venetoclax for treatment of myeloma and AL amyloidosis with $t(11;14)$ [57–59].

IgM-Related AL Amyloidosis

In 5–10% of cases, AL is caused by an IgM paraprotein, often secreted by a B-cell clone [60, 61] and rarely by a plasma cell clone. IgM AL is regarded as a distinct clinical entity with several clinical features and organ involvement patterns, which distinguish it from non-IgM AL amyloidosis. Therefore, the prognostic values of the aforementioned factors can only be transferred with caution to this clinical entity of AL amyloidosis. A revised staging system combining liver involvement and the presence of neuropathy with the established factors elevated NT-proBNP and troponin was proposed for this disease [61], but requires further validation. It is critical for the prognosis of IgM-AL that diagnosis and treatment is directed at the underlying clonal disease [60, 61].

Fig. 1. OS according to cardiac staging systems. Kaplan-Meier plots; shaded areas indicate the 95% CI (own data, published in Dittrich et al. [35]).

Prognostic Value of Organ Biomarkers in AL Amyloidosis and Staging Systems

A wide range of organ biomarkers are available that reflect the severity of organ dysfunction and amyloid load in AL patients (Table 2).

Heart

The morbidity and mortality of AL patients is strongly linked to the severity of heart involvement. Therefore, most of the organ biomarkers with prognostic relevance to OS in AL are either representing the cardiac amyloid load (septum thickness), destruction of cardiomyocytes (troponins), or the cardiac dysfunction (LVEF, 2D-GLS, NT-proBNP/BNP, parameters of clinical examination).

Minor myocardial damage can be quantified by the highly specific and sensitive cardiac troponins T (cTnT) and I (cTnI) [10]. The sensitivity is even increased with high-sensitivity assays (hsTnT) [31]. In response to distension and stretching caused by volume expansion and/or pressure overload, the prohormone preproBNP is secreted by cardiac myocytes. PreproBNP is then cleaved to proBNP, which is again cleaved to the active hormone BNP and the biologically inactive NT-proBNP with a longer half-life [29]. The negative prognostic value of elevated cardiac troponins and NT-proBNP for survival in AL has been identified and validated in multiple studies, using different cut-offs (Table 2). Both biomarkers were therefore consequentially incorporated into a first widely

used staging system for AL [10]. The elevation of either no, one, or both biomarkers forms 3 stages that are associated with tremendous differences in survival (Fig. 1; Table 3). Recently, thresholds for BNP instead of NT-proBNP were proposed by the Amyloidosis Center of the Boston University to use the established AL staging systems with BNP [29].

It was later shown that a second, higher threshold for NT-proBNP at 8,500 pg/mL as well as low systolic blood pressure can identify a very high-risk group with a median OS of less than 5 months among the patients with both troponins and NT-proBNP elevated, resulting in a system of 4 stages [28, 62]. Concurrently, plasma cell factors were incorporated in a revised staging system by utilizing serum FLC (threshold ≥ 180 mg/L) in combination with NT-proBNP and troponins at different thresholds (Fig. 1; Table 3) [10, 15]. The performance and accuracy of these three widely accepted and used staging systems for AL were recently compared by several retrospective analyses. The 4-stage system using two thresholds for NT-proBNP emerged as superior, especially in comparison to the initial 3-stage system [35, 63]. The main advantage of this staging system is the identification of patients with very high risk for early death after diagnosis. However, based on the comparison data it can be assumed that adding dFLC to a cardiac biomarker-based model increases the ability to predict for long-term survival [63]. In case of relapse, restaging before initiation of the sec-

Table 3. Cardiac staging systems in AL

Definition	Stage	HR for OS	Median OS, months	Ref. No.
<i>Mayo Clinic, 2004 [10]</i>				
One higher stage each for elevated troponin and elevated NT-proBNP ¹	I	Reference	130	35
	II	2.3	54	
	III	6.4	10	
<i>Mayo Clinic, 2012 [15]</i>				
One higher stage each for elevated troponin, elevated NT-proBNP, and elevated dFLC ²	I	Reference	130	35
	II	1.8	72	
	III	3.7	24	
	IV	7.1	6	
<i>European modification [26, 60]</i>				
Derived from Mayo 2004: stage I–II correspond, stage III separated by additional NT-proBNP >8,500 ng/L	I	Reference	130	35
	II	2.4	54	
	IIIa	4.2	24	
	IIIb	11.3	4	
Thresholds for biomarkers are reported based on proposed modifications of the staging systems [29].				
¹ cTnT >0.035 µg/L or cTnI >0.1 µg/L or hsTnT >54 pg/mL; NT-proBNP >332 ng/L.				
² cTnT >0.025 µg/L or cTnI >0.1 µg/L or hsTnT >54 pg/mL; NT-proBNP >1,800 ng/L; dFLC >180 mg/L.				

ond-line therapy increases the accuracy of the staging systems. The integration of dFLC into the staging system has even more priority in this case, as very poor responders with severe heart involvement confer a particularly poor prognosis [27].

Frequent clinical complications of cardiac AL are moderate to severe kidney impairment as well as atrial fibrillation. Both conditions are known to increase NT-proBNP. This does, however, not decrease the prognostic value of elevated NT-proBNP in these patients [35]. Indeed, atrial fibrillation and kidney impairment are independent negative prognostic factors in AL [35, 36]. It has also been shown that serum uric acid levels added to the prognostic value of cTnT and NT-proBNP in multivariate analysis [38]. However, the underlying mechanisms are unknown and elevated uric acid was also strongly associated with impaired kidney function, potentially suggesting uric acid as additional surrogate parameter for impaired kidney function and the use of diuretics in AL patients [64].

Although not included in current AL staging systems, advanced cardiac imaging modalities such as echocardiography, cardiovascular MRI, radionuclide bone scintigraphy, and PET provide valuable additional prognostic information and are also important for the precise diagnosis and monitoring of the disease [65]. The combinations of multiple echocardiographic parameters are routinely used for

Table 4. Renal staging systems in AL

Definition	Stage	Patients on dialysis after 3 years, %
<i>Palladini et al. [37], 2014</i>		
One higher stage each for elevated 24-h UPr and eGFR <50	I	0/4 ^a
	II	7/30 ^a
	III	60/85 ^a
<i>Kastritis et al. [69], 2017</i>		
24-h UPr/eGFR ratio <30	I	0
24-h UPr/eGFR ratio 30–99	II	11
24-h UPr/eGFR ratio ≥100	III	46

^a Testing cohort/validation cohort.

confirming amyloidogenic organ involvement [66]. Septum thickness, left longitudinal function, and ejection fraction, either measured by echocardiography or MRI, are each prognostic for short-term OS [9, 10, 31, 32]. Furthermore, the presence of low peripheral voltages by ECG is associated with a more severe cardiac involvement [67].

Kidney

The kidney is the second most frequently involved organ in AL patients [68]. For many patients with primarily kidney involvement, renal survival, i.e., the estimated

Table 5. Hematologic response criteria in AL

Criterion	Definition	Timepoint	Outcome	Ref. No.
aCR	No evidence of involved M component by serum and urine IFE; FLC ratio normal	3 months 6 months	Reference for HR	43, 72
VGPR	If initial dFLC ≥ 50 mg/L: dFLC < 40 mg/L	3 months 6 months	HR 2.67	37, 72
PR	If initial dFLC ≥ 50 mg/L: 50% reduction	3 months 6 months	HR 6.24	43, 72
No response	All other	3 months 6 months	HR 12.34	72
Low-dFLC PR	If initial dFLC 20–50 mg/L: dFLC < 10 mg/L	3 months 6 months	Renal/cardiac response as good as with aCR	2, 14, 73–75
Progression/relapse	From CR, any detectable monoclonal protein or abnormal FLC ratio (iFLC must double) From PR or stable response, 50% increase in serum M protein to > 0.5 g/dL or 50% increase in urine M protein to > 200 mg/day (visible peak) or dFLC increase of 50% to > 100 mg/L		Expert opinion	43
<i>Perspective</i>				
Stringent dFLC response	If initial dFLC ≥ 20 mg/L: dFLC < 10 mg/L	6 months	Favorable OS, TNT and cardiac response compared to CR or VGPR	76
Stringent CR ¹ /MRD negative	aCR + no evidence of clonal plasma cells in BM by multiparametric flow cytometry ²	100 days post-ASCT	Longer PFS, better organ response compared to aCR (statistically non-significant)	77–80

CR, complete response; aCR, CR criterium for AL; VGPR, very good partial response; PR, partial response; OS, overall survival; TNT, time to next treatment; PFS, progression-free survival; HR, hazard ratio for death (hematologic and cardiac response) or dialysis (renal response); IFE, immunofixation electrophoresis; FLC, free light chains; iFLC, involved FLC; BM, bone marrow; dFLC, difference of involved minus uninvolved FLC; MRD minimal residual disease.

¹ Monoclonality established by immunohistochemistry, morphology, and flow cytometry.

² Currently reported sensitivity: 1 in 1×10^4 to 2×10^5 .

living time without end-stage renal disease (ESRD), is the most relevant outcome. Proteinuria or more specifically albuminuria is the hallmark of kidney involvement in AL amyloidosis, while a moderately decreased eGFR can also be caused by advanced cardiac involvement [35].

Two staging systems based on the combination of proteinuria and eGFR have been proposed to estimate the risk of ESRD (Table 4). AL patients with both parameters unaffected have a very low probability of suffering from ESRD, while patients with both parameters affected have a risk of 50% or higher to develop ESRD within 3 years from the initial diagnosis. Of note, the staging system proposed by Palladini et al. [37] has been independently validated and was also used for comparison by Kastritis et al. [69], while this later proposed staging system has not yet been validated. ESRD rates have varied considerably

among the different cohorts evaluated by Palladini et al. [37] and Kastritis et al. [69], which makes comparisons of the results difficult.

Recently, a severe nephrotic range albuminuria has been reported as adverse for outcome (hematologic event-free survival) with daratumumab. Especially in combination with a dFLC > 180 mg/L, satisfying hematologic responses were rare. The suggested mechanism was a constant renal loss of monoclonal antibody in nephrotic patients with consecutive lower daratumumab serum concentrations and insufficient renal target saturation [23].

Liver

It can be assumed that severe AL liver involvement results in adverse OS [70]. However, the quality and quan-

Table 6. Organ response criteria in AL

Criterion	Definition	Timepoint	Outcome	Ref. No.
<i>Cardiac response</i>				
NT-proBNP response	If baseline NT-proBNP ≥ 650 ng/L: decrease of $>30\%$ and >300 ng/L	3 months 6 months	HR 0.23	12, 72
NT-proBNP stable	No increase or decrease of NT-proBNP	3 months 6 months	Reference for HR	72
NT-proBNP progression	NT-proBNP increase of $>30\%$ and >300 ng/L	3 months 6 months	HR 4.36	12, 72, 81
cTn progression	$>33\%$ increase in cTn	6 months	HR 2.27	72
NYHA class response	If baseline NYHA class 3 or 4: $\geq$ two-class decrease	6 months	HR 0.28	72
EF progression	$\geq 10\%$ decrease in EF	6 months	HR 1.95	72
<i>Renal response</i>				
Renal response (Palladini et al. [37])	$\geq 30\%$ decrease in proteinuria or drop of proteinuria below 0.5 g/24 h in the absence of renal progression	3 months 6 months	HR 0.25	37
Renal progression (Palladini et al. [37])	$\geq 25\%$ decrease in eGFR	3 months 6 months	HR 4.74	37, 43
Renal response (Kastritis et al. [69]) ¹	Reduction of 24-h UPr/eGFR ratio by at least 25% from baseline and below 100 (if ≥ 100)	3 months 6 months	ESRD within 2 years: 0 vs 24% 0 vs. 22%	69
Renal progression (Kastritis et al. [69]) ¹	Increase of 24-h UPr/eGFR ratio by at least 25% from baseline or ≥ 100	3 months	ESRD within 2 years: 22 vs 2.5%	69
<i>Hepatic response</i>				
Hepatic response	50% decrease in abnormal alkaline phosphatase (AP) value Decrease in liver size radiographically at least 2 cm		Expert opinion	43

HR, hazard ratio for death (hematologic and cardiac response) or dialysis (renal response); cTn, cardiac troponin; ESRD, end-stage renal disease.
¹ Validation pending.

tity of available biomarkers to quantify liver involvement (serum gamma-GT, AP, Fibroscan) are limited compared to heart and kidney. For the subgroup of AL patients with an underlying IgM, which more frequently have liver involvement, the presence of liver involvement is an independent risk factor [61].

Novel Organ Biomarkers

As reviewed recently, there is an increasing pool of novel organ biomarkers with potential prognostic value: growth differentiation factor-15, proadrenomedullin, osteopontin, hepatocyte growth factor, soluble suppression of tumorigenicity 2, von Willebrand factor antigen, and osteoprotegerin [71]. The link between amyloidogenic organ damage and biomarker elevation is not yet fully resolved for many of these novel biomarkers and validation of the prognostic value in large case series is warranted. Therefore, these biomarkers have not been incorporated into routine practice yet.

Response to Plasma Cell-Directed Therapy

Many of the factors with upfront prognostic value in AL were confirmed to be useful for monitoring the success of plasma cell-directed therapy and estimate the prognosis of AL patients based on biomarkers after several months of treatment (landmark analysis). These parameters were incorporated in combined hematologic and organ response criteria (Tables 5, 6).

Hematologic Response

Anti-plasmacellular therapy is the cornerstone of treatment in AL amyloidosis, since the light chain is the precursor of the amyloid protein. The goal of anti-plasmacellular therapy in AL, which is required for an optimal organ response, is the virtual complete eradication of the amyloidogenic light chain [72]. In one retrospective analysis of 313 patients who responded to chemotherapy with a normalization of FLC levels, higher values of the

FLC at diagnosis (dFLC >180 mg/L) predicted the organ response, and an early normalization of FLC levels predicted an improved OS [82].

The criteria for hematologic response were initially proposed by AL consensus guidelines that were broadly derived from myeloma [43]. However, in contrast to myeloma, less than half the AL patients have a detectable circulating intact Ig paraprotein and in many others the concentration is so low that accurate quantification is not possible. Immunofixation electrophoresis (IFE) is more sensitive than serum or urine protein electrophoresis and can detect free monoclonal immunoglobulin light chains in the urine of 80–90% of patients, but IFE results are not quantitative. Consequently, conventional immunochemical and electrophoretic techniques generally provide only qualitative measures of hematologic response to treatment in AL amyloidosis [83]. Moreover, in AL, the quality of FLC response is strictly and directly associated with survival [72]. Therefore, the quantitative serum FLC measurement plays a paramount role for response evaluation and normalization of serum FLC ratio and levels were incorporated into the complete response criterium for AL (aCR) early on [43, 83]. Of note, the prognostic value of the dFLC response is superior compared to the M protein response [84]. Indeed, the M protein response does not improve survival in those patients without an FLC response [72]. A 50% decrease of iFLC as well as dFLC upon therapy was found to be associated with significantly improved OS (partial response; PR) [85, 86]. To achieve organ response in a fraction of patients, a decrease of dFLC below 40 mg/L should be the minimal goal of therapy (very good PR; VGPR) [72].

Previously, a baseline dFLC of below 50 mg/L was regarded as “not measurable” for hematologic response [46, 87]. However, not all dFLC changes in AL patients with baseline dFLC between 20 and 50 mg/L turned out to be meaningful, and based on the renal organ response a threshold of 10 mg/L was established as an additional hematologic response parameter besides aCR for this specific clinical subgroup (“low dFLC PR”) [2, 14, 73–75]. Later, this dFLC response threshold of 10 mg/L was proposed for all patients with baseline dFLC >20 mg/L. It has been shown in one large prospective observational study – but not yet validated – that this “stringent dFLC response” translated into a significantly longer time to next treatment and a benefit for OS additional to CR or VGPR [76].

More stringent response criteria will eventually reduce the present number of patients with an apparently satisfying hematologic response who, however, do not achieve a sufficient organ response. Even very small residual plasma

cell clones are sufficient to impede organ response in some AL patients. A stringent complete response was recently proposed as the optimal endpoint after stem cell transplantation, defined as undetectable bone marrow clonal plasma cells by flow cytometry. Progression-free survival was significantly shorter in patients failing to achieve a stringent complete response compared to aCR. A bone marrow examination post-transplant is important because it can identify patients who fail to achieve a stringent complete response and thereby predicts earlier progression [77]. Minimal residual disease (MRD) assessment in AL by next-generation flow cytometry is currently being validated by several groups and the implementation of MRD assessment will further improve response evaluation [78–80].

Organ Response

Principally, organ response in AL is evaluated by the monitoring of biomarkers used for diagnosis and grading of organ involvement (Tables 5, 6). The first organ response criteria was proposed by the ISA in 2005 [43]. Subsequently, outcome-driven criteria were developed – for heart based on OS [72] and for kidney based on renal survival, i.e., time to ESRD [37, 69]. Recently, it could be demonstrated that graded response criteria (<30%, 31–60%, >60% reduction of biomarker from baseline or complete normalization), are able to predict significantly different OS of respective patients [70, 82]. Validation of these novel criteria within the broader AL community is in progress. Different organ response criteria for subgroup of patients with advanced cardiac AL might be needed with the success of novel fast-acting therapies [23, 28].

Conclusion

The prognosis of AL patients is determined by the biology of the: (1) underlying clonal disease, (2) pattern and extent of organ involvement at baseline, as well as (3) response to treatment. Multiple biomarkers were integrated into validated staging systems. The OS is most appropriately estimated by cardiac staging systems, of which the European modification of the original Mayo staging system by a second threshold for NT-proBNP showed the best performance (Fig. 1). Renal staging systems are powerful in estimating the time to ESRD. Organ response rates and OS were used to further develop hematologic response criteria. The ultimate goal of therapy in AL is to decrease the amyloidogenic-involved light chain as fast and deep as possible without causing significant damage. iFISH is widely used to evaluate cytogenetic aberrations

in AL plasma cells and even able to predict treatment response. The international AL community is constantly improving staging systems and response criteria. The prognostic value of biomarkers and the performance of staging systems are significantly influenced by currently available treatment options. Therefore, potential novel approaches to directly target the toxic light chains and deposited amyloid might also change the applicability of the current validated AL staging systems.

Disclosure Statement

The authors have no conflicts of interest to declare.

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