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Review

Monitoring Patients with Light Chain (AL) Amyloidosis during and after Therapy: Response Assessment and Identification of Relapse

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Abstract: Light chain amyloidosis is a complex disease where a small B-cell clone produces a monoclonal immunoglobulin light chain that causes deposits and specific organ dysfunction. The available treatment strategies aim to reduce or eliminate amyloidogenic light chain production in order to avoid amyloid deposition and allow the repair of organ damage. An international effort allowed the definition of validated hematologic and organ response criteria based on biomarkers. Recently, new methods for the assessment of minimal residual disease were also proposed but still need international validation. Lastly, a joint effort is also required to accurately define relapse/progression criteria in order to apply timely therapeutic interventions. In this review, we describe the validated response criteria and report on the future direction for the definition of progression criteria in this disease.

Keywords: amyloidosis; response assessment; prognosis

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1. Introduction

Light chain (AL) amyloidosis is a disease where a small B-cell clone produces a monoclonal immunoglobulin light chain that causes deposits and specific organ dysfunction [1]. Organ damage is known to be the result of the amyloid deposition itself or due to the direct cell toxicity of the circulating free light chains (FLC) [2]. The heart and kidney are the most frequently involved organs, with the degree of cardiac involvement being the main prognostic factor [3]. The available treatment strategies aim to reduce or eliminate amyloidogenic light chain production in order to avoid amyloid deposition and allow for the repair of organ damage. The evaluation and monitoring of the hematologic and organ responses to each therapy line and at the time of relapse or progression are essential to adequately manage patients with this disease, both in daily clinical practice and in the setting of clinical trials [3]. For this purpose, it is necessary to perform a complete diagnostic/staging work-up at diagnosis and before every line of therapy focused on establishing the baseline values of main indicators for the hematologic evaluation (serum FLC, serum and/or urine M-protein, and plasma cell infiltration) and also for the organ assessment (24-h protein excretion, cardiac biomarkers, and alkaline phosphatase). In this sense, the measurability of the disease makes this task easier. However, up to 20% of patients will have a difference between involved and uninvolved serum FLC (dFLC) below 50 mg/L, requiring adapted response criteria [4,5]. Moreover, the introduction of newer and more sensitive diagnostic and monitoring tools makes response evaluation an evolving matter, particularly regarding the complete hematologic response, giving rise to the concept of minimal residual disease (MRD) [6,7]. Moreover, the organ response is now a matter of discussion, with an ongoing international attempt of criteria refinement through the inclusion

of different organ response categories [8]. Finally, the definition of relapse/progression criteria has been established, but there is no consensus regarding the right time to start rescue therapy. In this review, we describe the validated response criteria and the future directions of hematologic and organ evaluation in this disease.

2. Hematologic Response Assessment

The first specific report defining hematologic response assessment for patients with AL amyloidosis was reported in 2005 by the International Society of Amyloidosis (ISA) [9]. During the 10th International Symposium on Amyloid and Amyloidosis (Tours, France, April 2004), thirteen leaders in the field were asked to submit their institutional criteria, from which the consensus guidelines were defined. In this report, the diagnostic criteria for organ involvement were also reported, and hematologic and organ response criteria were issued for the first time [9]. In particular, the hematologic response criteria were adapted from those used in multiple myeloma (MM). Densitometry was defined as the best way to assess the concentration of a monoclonal component in serum, and the measurement of serum FLC was recommended for the first time. As reported in Table 1, three levels of response were identified: complete response, partial response, and stable disease. This consensus report did not fully incorporate measurement of serum FLC and cardiac biomarkers for the definition of the hematologic and organ responses. In contrast, several studies subsequently showed that treatments reducing the concentration of amyloidogenic FLC and improving cardiac dysfunction resulted in prolonged survival [10–12]. Accordingly, a second consensus panel was established in 2010, on the occasion of the 12th International Symposium on Amyloidosis (Rome, Italy, April 2010) [13]. The panel explores different predictors of survival by directly gathering data from large patient populations in order to develop better criteria for the management of this complex disease. Therefore, data from seven referral centers in the United States and Europe were assembled. A cohort of 816 patients was composed in order to identify the criteria for the hematologic response to the first line of treatment that best discriminated groups with different survivals. In the same study, the prognostic relevance of traditional (echocardiographic and clinical) and biomarker-based criteria for cardiac response that will be discussed in the next section of this review were assessed [13]. The patient population included only those with AL amyloidosis recorded in the referral center databases who had been evaluated for response at three and/or six months after the initiation of first-line therapy. A validation set was also analyzed from an independent cohort of 374 patients diagnosed and followed at the Pavia Amyloidosis Center (Italy). The final analysis defined four levels of hematologic response: amyloid complete response (aCR, negative serum and urine immunofixation, and normal FLC ratio); very good partial response (VGPR, dFLC < 40 mg/L); partial response (PR, a dFLC decrease > 50%); and no response (NR, all the other categories) (Table 1) [13]. The use of this definition allowed the researchers to identify patients with significantly different overall survival in both the testing and the validation cohorts. In the current validated approach, monoclonal protein concentration evaluation was excluded because it did not provide any additional survival information in any dFLC response group, including the NR subgroup [13]. In addition, it was reported that the evaluation of response according to the proposed criteria could be used at three and/or six months after initiation of therapy.

More recently, the ISA Board of Directors proposed that the 2012 response criteria should be expanded to define complete hematologic response as the absence of amyloidogenic light chains (either free or part of a complete immunoglobulin), which was defined as negative serum and urine immunofixation and either a FLC ratio within the reference range or an abnormal FLC ratio as long as the uninvolved-FLC concentration was greater than the involved-FLC (iFLC) concentration (Table 1) [14]. This clarification was required by the clinical introduction of different treatment regimens that are now able to deeply reduce uninvolved-FLC due to severe immunoparesis with a subsequent modification of the FLC ratio.

Table 1. Validated hematologic response criteria for AL amyloidosis.

Response Categories	2005 Criteria [9]	2012 Criteria [13]
Complete response (aCR)	Both criteria must be met: • Serum and urine negative for a monoclonal protein by immunofixation, • Free light chain ratio normal.	Both criteria must be met: • Absence of amyloidogenic light chains (either free and/or as part of a complete immunoglobulin) defined by negative immunofixation electrophoresis of both serum and urine. • Either a FLC ratio within the reference range or an uninvolved FLC concentration greater than the involved FLC concentration, with or without an abnormal FLC ratio [14].
Very good partial response (VGPR)	Not reported	dFLC concentration < 40 mg/L
Partial response (PR)	• If serum M component > 0.5 g/dL, a 50% reduction. • If light chain in the urine with a visible peak and >100 mg/day and 50% reduction. • If free light chain > 10 mg/dL (100 mg/L) and 50% reduction.	dFLC decrease > 50% compared to baseline
Stable/No response	No CR, no PR, no progression *.	All other patients.

dFLC, difference between amyloidogenic (involved) and non-amyloidogenic (uninvolved) free light chain concentrations; FLC, free light chains; M component, monoclonal component. * Progression [9]: from CR, any detectable monoclonal protein or abnormal free light chain ratio (light chain 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; a visible peak must be present; free light chain increase of 50% to >10 mg/dL (100 mg/L).

Finally, the emergence of oligoclonal bands should also be considered in patients in hematologic VGPR or CR in order to avoid equivocal diagnosis of relapse. This phenomenon, well recognized in the setting of MM [15], was observed in 60% of patients with AL amyloidosis included in a small study, more frequently in those who achieved CR, and was associated with favorable outcomes [16].

3. Hematologic Response Assessment for Patients with Non-Measurable Disease

The application of the ISA validated response criteria in clinical practice has changed the approach to AL amyloidosis. In all the subsequent prospective clinical trials, the current definition of hematologic response was incorporated and used as a valid end-point [17–19]. In order to allow the assessment of VGPR, considering the imprecision of the FLC assay [20], it was agreed by the consensus panel that the baseline level of dFLC should be at least 50 mg/L to be considered measurable disease. This definition excluded all patients with a low dFLC burden from all clinical trials [21]. Subsequently, different groups elucidated the clinical characteristics of patients with a low dFLC burden and defined specific hematologic response criteria for this subgroup [4,5,22]. In particular, two parallel studies from the Pavia group [4] and the Heidelberg referral center [5] described and validated the so called low-dFLC response criterion, defined as a dFLC < 10 mg/L in patients with a baseline dFLC between 20 and 50 mg/L. Achievement of a dFLC < 10 mg/L after first-line therapy in this population was associated with a significant survival advantage and significantly reduced risk of dialysis in both cohorts. The proposed criterion was also explored in other series of patients, with the same results [22–24].

4. New Proposed Hematologic Response Criteria Based on FLC Measurement

Different studies have explored new possible definitions of hematologic response in AL amyloidosis. In particular, Manwani and coworkers showed that a dFLC < 10 mg/L was a meaningful end-point in all patients and, importantly, was associated with prolonged survival in a large series of patients in aCR after a bortezomib-based treatment [25]. Furthermore, Mayo Clinic investigators, in two different studies, explored the use of iFLC concentration as a possible end-point. In one of these studies, the authors reported that patients who obtained an iFLC concentration < 20 mg/L had the better outcome [26]; in a further report [27], normalization of iFLC was associated with a higher rate of organ response and longer progression-free survival. In addition, the Boston researchers noticed that patients achieving an iFLC < 10 mg/L for any period of time post-therapy had over 90% survival at ten years [28]. Lastly, two companion papers published in 2020 explored the ability of different endpoints in two retrospective series, one from the Pavia Center [29] composed of patients who received a non-transplant first line approach and one from the Boston Amyloidosis Unit [30], mainly treated with autologous stem cell transplants (ASCT). In both cohorts, aCR defined according to the ISA validated criteria was the best predictor of outcome compared to different low FLC endpoints. In addition, among patients who reached aCR, no significant differences were seen in the outcomes of those who also obtained a low FLC concentration.

5. Hematologic Response Based on New Tools for Assessment

The achievement of aCR has been defined as the best treatment goal [29,30]. However, aCR does not translate to a deeper organ response in all patients. This could be partially explained by irreparable organ damage, permanence of the amyloid deposits or the persistence of non-detectable amyloid-light chains [6]. Different methods now available for the detection and possible quantification of MRD have been studied in AL amyloidosis patients, as this is an emerging area of interest [6,7,31–37].

High-sensitive next-generation flow (NGF) cytometry is used to detect MRD in MM and could be used as a robust surrogate endpoint for clinical trials and guidance for treatment [38–42]. Accordingly, it has been suggested as the new treatment end-point for MM [43]. In AL amyloidosis, the Mayo Clinic researchers showed that lack of clonal bone marrow plasma cells by standard-sensitivity multiparameter flow cytometry was associated with improved progression-free survival [35,36]. Moreover, the Boston group showed a trend for a higher probability of organ response in patients in aCR and undetectable MRD [44]. More recently, an international collaborative study detected MRD by NGF in approximately half of 92 patients in aCR and, more importantly, persistence of MRD was significantly associated with not only persistent organ dysfunction, but also both cardiac and renal damage [6].

Next generation sequencing (NGS) was also used as a possible tool for MRD assessment in AL amyloidosis by the Boston group [45] and further studies in larger patient populations are warranted.

Lastly, new tools for the assessment of monoclonal proteins in serum and urine were introduced into routine clinical practice using mass spectrometry applications [46–48]. The use of this method showed a comparable diagnostic sensitivity compared to the current standard techniques [48] and allows the identification of possible post-translational modifications related to the amyloid light chains [49,50]. Subsequently, different studies explored this application as a possible MRD assessment method and revealed a possible use in AL amyloidosis [51,52] and MM [53].

6. Organ Response Assessment

The organ response categories in AL amyloidosis were established for the first time in 2005, with their definition being found in the consensus response categories after the Tours International conference [9]. Heart response was defined as a mean interventricular septal thickness decrease of 2 mm, 20% improvement in ejection fraction (EF), improvement by

two New York Association Classes (NYHA) without modification in diuretic use, and no increase in wall thickness. This criterion was defined before the introduction of cardiac biomarkers in the routine assessment of AL amyloidosis patients. For renal involvement, a 50% decrease of 24-h urine protein excretion (mainly albumin) without a creatinine or creatinine clearance worsening by 25% over the baseline was required. In order to avoid coding a response due to variations in the urinary protein collections, a minimum variation of at least 0.5 g/24 h was required and a patient was considered evaluable for renal response only if the baseline proteinuria was >0.5 g/24 h. Liver response was defined as a 50% decrease in the abnormal alkaline phosphatase value or a decrease in the liver size radiographically (by computed tomographic or ultrasonographic craniocaudal liver scan) of at least 2 cm. The peripheral nervous system response was defined as an improvement in electromyogram nerve conduction velocity [9].

In 2012, the international study conducted by Palladini et al. reported for the first time the use of N-terminal natriuretic peptide type B (NT-proBNP) as a marker for cardiac response in AL amyloidosis [13]. As per the hematologic response criteria, the cardiac response criterion was obtained from a survival analysis and validated in an independent dataset. For patients with cardiac measurable disease (with baseline NT-proBNP of at least 650 ng/L), a cardiac response was defined as a decrease in NT-proBNP from baseline >300 ng/L and >30%. In addition, an NYHA class response, defined as a $\geq$two-class decrease if baseline NYHA is class 3 or 4, was associated with a survival advantage. More recently, the Boston group proposed that BNP could be used instead of NT-proBNP for the assessment of cardiac response [54]. In this study, a reduction of BNP concentration > 30% and >50 ng/L resulted in a significant survival advantage.

Renal response assessment was evaluated in retrospective datasets from two referral centers. Palladini et al. reported that obtaining a >30% reduction of proteinuria or a reduction of proteinuria below 0.5 g/24 h was associated with a significant reduction of the risk for end stage renal disease [55]. This criterion was tested in the Italian dataset and validated in a cohort of patients from Heidelberg. In order to avoid coding a reduction of proteinuria related to a worsening of kidney function as a response, renal response was defined as evaluable only in patients who did not reach a decrease in estimated glomerular filtration rate > 25% (defined as renal progression). This definition of renal response was subsequently validated in two different datasets reported by Kastritis et al. [56] and Drosou et al. [57]. Kastritis and colleagues also proposed a modification of the renal response criteria based on the reduction in the ratio of 24 h proteinuria to eGFR (24 h UPr/eGFR) by at least 25% or below 100 (if initially > 100), and this criterion was also tested in the Mayo cohort [57].

A composite hematologic and organ response (CHOR) model was validated to identify those patients with a better outcome after treatment. The CHOR model was designed using combined scores of 0–3 for hematologic response (0—CR, 1—VGPR, 2—PR, 3—no response) and 0–2 for organ response (0—response in all organs, 1—response in some organs, 2—no organ response). According to this model, patients who achieve a CHOR score of 0–3 after treatment have a longer survival compared with those who reached a score of 4–5 [58].

The use of other possible biomarkers for the definition of organ response has also been explored. In particular, the growth differentiation factor 15 (GDF-15) was evaluated in an international collaboration between the Athens Myeloma Unit and the Pavia Amyloidosis Center [59]. In this study, a reduction in GDF-15 concentration > 25% was associated with a significant survival advantage and a level of GDF-15 > 4000 ng/L resulted in a significant risk of progression to dialysis [59].

Lastly, it has been proposed that depth of organ response should be assessed in a similar way to the hematologic response. Four categories for cardiac, renal and liver response have been identified: complete organ response (nadir NT-proBNP $\leq$ 400 ng/L; nadir proteinuria $\leq$ 0.2 g/24 h; nadir alkaline phosphatase $\leq$ 2 times institutional lower limit of normal); very good partial organ response (target biomarker reduction > 60% from

baseline, not meeting complete organ response definition); partial organ response (target biomarker reduction 31–60% from baseline); and no response (target biomarker reduction $\leq$ 30% from baseline). The first data on these graded organ response criteria showed that the deeper the organ response, the better the outcome [8]. An international effort is ongoing to better define this specific grading of organ responses, and the final results are pending.

7. Hematologic and Organ Progression Criteria

The hematologic and organ progression criteria in AL amyloidosis were proposed by a consensus panel within the ISA [9]. Specifically, hematologic relapse from aCR was defined by the reappearance of the original monoclonal protein in serum and/or urine, or an increase in the iFLC (at least doubling), resulting in an abnormal FLC ratio. From PR or stable disease, hematologic progression required a 50% increase in the serum M protein (to >5 g/L) or in urine M protein (to >200 mg/day), with a concomitant 50% increase in the iFLC concentration (to >100 mg/L). Regarding organ progression, criteria based on New York Heart Association class and interventricular septum thickness (for heart involvement), 24-h urine excretion, serum creatinine or creatinine clearance (for renal involvement), serum alkaline phosphatase (for liver involvement), and electromyography or nerve conduction studies (for peripheral nerve involvement) were also established [9]. Cardiac progression criteria were subsequently updated with the introduction of cardiac biomarker assessment [13].

These are the only criteria reported and approved to date by the ISA but there is no consensus among the experts on the correct definition of relapse/progression routinely used in the clinic in order to start rescue therapy in a timely manner. In 2017, an international survey demonstrated that there is not a worldwide agreement on which elements prompt the decision to resume treatment [60]. In this study, 50 expert physicians were asked to describe their clinical decision making and possible progression criteria. The decision to start second line therapy was mainly influenced by three main factors: baseline dFLC values (35%), disease severity at presentation (24%), and the time between response to frontline therapy and the subsequent rise of FLCs (18%), and many possible dFLC cut-points were reported [60]. At present, the international debate on this topic is still open and two different positions have been proposed: starting rescue treatment as early as dFLC increase is detected and before organ damage worsens, or delaying treatment initiation until organ progression occurs [61,62]. The first strategy is supported by the observation that even a slight increase of dFLC (defined as high risk dFLC progression: a dFLC > 20 mg/L, a >20% increase from baseline, and a >50% increase from the value at the best response) might precede cardiac progression by months [63]. This criterion was reported in a retrospective study of patients with AL amyloidosis mainly treated with non-transplant therapy approach at the Pavia Amyloidosis Center. The second strategy is supported by clinical and socio-economic observations. First, two studies reported that relapse after ASCT may be asymptomatic and that patients in VGPR may tolerate a mild increase in dFLC [64,65]. Secondly, rescue treatment may result in impaired quality of life and represents an important cost for the healthcare system [66,67]. Unfortunately, there are not enough data to definitely determine which of these two different approaches is the best in relapsed AL amyloidosis, and further international studies are needed to clarify this issue. Meanwhile, an individualized approach is recommended, taking into account patient and disease-dependent factors when considering a hematologic and/or organ progression deserving rescue therapy.

8. Monitoring Response Assessment during and off Treatment

Assessments of hematologic and organ response should be performed on a regular basis, both during treatment and off treatment [3]. During therapy, the frequency of hematologic assessments will depend on the type of treatment approach: short-term (e.g., bortezomib-based combinations) [25,68,69], long-term (e.g., anti-CD38 antibody-based combinations) [19,70,71], or autologous transplant. Accordingly, serum FLC and serum

and urine M-protein (by electrophoresis) should be measured after every cycle or, once in response, every two cycles. When aCR is suspected, serum and urine immunofixation are mandatory according to the ISA definition, and once aCR is confirmed, a bone marrow aspirate with MRD evaluation by NGF cytometry should be performed. In patients with a detectable MRD, closer monitoring should be suggested. As for the organ response, although it is usually delayed with respect to the hematologic response, the use of new treatment strategies with faster mechanisms of action could make it necessary for this evaluation to be performed on a monthly or bimonthly basis. It should also be always adapted to the organ involvement profile of every particular patient, although considering the rare possibility of addition of newly involved organs. Therefore, assessment of symptoms, physical examination, laboratory studies including serum creatinine and eGFR, 24 h protein excretion, and alkaline phosphatase should be performed every 2–3 months [3]. Other assessments, such as echocardiography, liver imaging, gastrointestinal endoscopy, and nerve conduction studies should be performed only if clinically indicated. Hematologic and organ response after ASCT is usually first assessed at 3 months post-infusion and every 3 months thereafter. However, more frequent hematologic evaluations might be needed if relapse/progression is suspected. Similarly, follow-up off treatment, after non-transplant strategies, will consist of hematologic assessments performed every 2–3 months during the first 2 years, every 3–4 months during the next 2 years, and every 6 months thereafter. In this setting, organ evaluation will be assessed every one or two visits. Since these assessments aim to detect early progression in order to start prompt therapeutic intervention if clinically indicated, the frequency and type of assessments will depend on patient factors (age, performance status, willingness to be treated), disease characteristics at diagnosis (low or high iFLC values, organs involved, aggressiveness of disease), and real options of rescue therapy.

9. Conclusions

Response to therapy in AL amyloidosis should be evaluated with the use of validated hematologic and organ response criteria. This is a major step forward for the combination of a clinical and biomarker-based approach, and only those criteria should be used into the routine clinical practice and for clinical trial management. The use of non-validated end-points or combined modified progression-free survival approaches should be carefully evaluated and compared with the standard validated criteria. In addition, the introduction of new possible tools for the assessment of response, such as different MRD definitions, should be introduced to the clinic only after rigorous validation in different clinical settings. Finally, international collaborations are warranted in order to define new time-points for response assessment and to validate the progression criteria.

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