Clinical Value of Minimal Residual Disease Assessed by Multiparameter Flow Cytometry in Amyloid Light Chain Amyloidosis

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Research Article

Keywords: amyloid light chain amyloidosis, minimal residual disease, multiparameter flow cytometry, organ response, autologous stem cell transplantation

Posted Date: April 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-414519/v1>

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Version of Record: A version of this preprint was published at Journal of Cancer Research and Clinical Oncology on May 8th, 2021. See the published version at <https://doi.org/10.1007/s00432-021-03653-z>.

Abstract

Purpose: To assess the feasibility and prognostic value of minimal residual disease (MRD) evaluated by multiparameter flow cytometry (MFC) in newly diagnosed amyloid light chain (AL) amyloidosis.

Methods: Clinical data from 25 consecutive newly diagnosed AL amyloidosis patients with MRD data tested at 3 months after first-line therapy completion were retrospectively analysed in a single centre from 2012 to 2019. First-line therapy included 8 courses of VD or 4 courses of VD plus sequential autologous stem cell transplantation (ASCT), both without maintenance therapy.

Results: Of 25 patients with very good partial response (VGPR) or better, 19 (76%) achieved MRD negativity. Baseline characteristics were not different between MRD-negative and MRD-positive patients. More ASCT patients than non-ASCT patients (90.0% vs 53.3%, $P=0.043$) achieved MRD negativity. In the MRD-negative and MRD-positive groups, cardiac response was observed in 93% and 25% ($P=0.019$) and any organ response in 94% and 50%, respectively ($P=0.023$). At a median follow-up of 25.1 months, MRD-negative patients showed significantly longer progression-free survival (PFS) from diagnosis than MRD-positive patients (24.52 vs 76.39 months, $P=0.004$).

Conclusions: MRD negativity measured by MFC at 3 months after first-line therapy completion in patients with AL amyloidosis is measurable and associated with improved organ response rates and PFS over a long follow-up.

Introduction

Amyloid light chain (AL) amyloidosis, as a clonal plasma cell disorder, is characterized by organ dysfunction secondary to deposition of misfolded monoclonal light chains and most commonly involves the heart, kidney, and liver (Merlini et al. 2018). AL amyloidosis is typically associated with a lower indolent clonal plasma cell burden within the bone marrow than multiple myeloma (MM) (Merlini and Stone 2006). However, even low levels of unstable light chains secreted by these plasma cells can deposit in organs, resulting in organ dysfunction (Dittrich et al. 2017; Sidana et al. 2018). Thus, the treatment of the disease aims to target the plasma cell clone and completely eliminate toxic light chain production. With the development of novel therapies (Muchtar et al. 2017a), deep responses, as assessed by serum- or urine-based methods such as immunofixation electrophoresis and free light chain (FLC) quantification, can be achieved in a significant proportion of patients with AL amyloidosis (Palladini et al. 2012). However, there are several limitations inherent to FLC assays and immunofixation electrophoresis (Kaufman et al. 2015). Hematologic relapses still occur, and organ function may continue to deteriorate due to small residual clones. The small amounts of light chains produced by these plasma clones may not be detectable by conventional techniques (Kastritis et al. 2020).

In MM, multiparameter flow cytometry (MFC) is being used to detect the presence of minimal residual disease (MRD), and the absence of detectable MRD has been associated with improved survival outcomes across different therapeutic regimens and lines of therapy (Paiva et al. 2016; Munshi et al.

2017). Such data are sparse in patients with AL amyloidosis. Some studies have shown that the absence of MRD detected by MFC is associated with superior outcomes in patients with AL amyloidosis (Jelinek et al. 2018; Muchtar et al. 2020; Sidana et al. 2020), but these studies have limitations given the heterogeneity of patients tested and the lack of predefined time points for MRD assessment. Since 2012, we have utilized MFC to assess MRD in AL amyloidosis patients at the 3rd month after the end of first-line therapy in our centre. The aim of the current study was to evaluate the feasibility and clinical utility of MRD as a surrogate endpoint in these patients with AL amyloidosis after the end of first-line treatment as a substitute for conventional techniques. The results of this study may lead to the generation of MRD risk-adapted clinical trials in AL amyloidosis with interventions to optimize outcomes early in the treatment course.

Materials And Methods

Patients

Since 2012, we have utilized MFC to assess MRD in AL amyloidosis patients who received first-line therapy without maintenance therapy. The first-line therapy in our centre consisted of 4 courses of induction treatment with VD (intravenous bortezomib 1.3 mg/m² and dexamethasone 20 mg/d on days 1, 8, 15, and 22). After four cycles of treatment, patients underwent either autologous stem cell transplantation (ASCT) or another four cycles of treatment with the patient's original regimen according to their condition and request. Up to July 2019, 25 consecutive patients diagnosed with AL amyloidosis according to the standard criteria in our centre were enrolled. These patients had at least very good partial response (VGPR), and MRD testing was performed at the 3rd month after the end of the first-line therapy, including either the initial therapy (8 cycles of VD if the patient selected not to proceed with upfront ASCT) or ASCT. Those patients with MM-associated AL amyloidosis and those with undetectable abnormal plasma cell proliferation at the time of disease diagnosis were excluded. This study was approved by the ethics committee of the First Affiliated Hospital of Sun Yat-sen University, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

MRD testing

MRD testing was performed at the 3rd month after the end of first-line treatment. The bone marrow MRD assessment method used in this analysis was described previously (Gu et al. 2018). EDTA-anticoagulated bone marrow aspirate (2 mL) was washed with PBS three times, and then the cell density was adjusted to $\leq 20 \times 10^6$ /mL. Samples (100 μ L) were then incubated with the following antibodies: anti-CD38, anti-CD56, anti-CD19, anti-CD20, anti-CD54, anti-CD138, anti-CD45, anti-cytoplasmic κ , and anti-cytoplasmic λ antibodies. One to two million nucleated cells were analysed using a BD FACSCanto flow cytometer. Cells were gated based on combinations of the markers CD38, CD138, and CD45 and SSC, and at least 50 plasma cells were analysed. Abnormal plasma cells were differentiated from normal plasma cells by surface markers and cytoplasmic expression of light chain. The results were positive if ≥ 20 plasma cells

with an abnormal phenotype were detected. The sensitivity of our flow cytometry detection of MRD was 5×10^{-5} to 10^{-5} cells.

Response assessment

The basic data of selected patients was retrieved from case data and databases. Follow-up data were collected from previous visits during the treatment process. The deadline for follow-up was August 1, 2020. Hematologic and organ responses and relapse were defined based on standard criteria (Palladini et al. 2012). Complete remission (CR) was defined as the absence of monoclonal protein as assessed by serum and urine immunofixation electrophoresis and a normal serum FLC ratio, and VGPR was defined as a difference between involved and uninvolved FLCs [dFLC] < 40 mg/L. Specifically, 4 patients were MRD negative and showed the absence of monoclonal protein as assessed by immunofixation electrophoresis but did not have a dFLC result because our centre did not have the necessary test at that time; as such, the patient's response was defined as VGPR. Organ response was defined as a > 30% reduction in 24 h proteinuria in the absence of a $\geq 25\%$ decrease in estimated glomerular filtration rate (Jelinek et al. 2018), > 30% reduction in BNP (Staron et al. 2020), and > 50% decrease in alkaline phosphatase for renal, cardiac, and hepatic responses, respectively. Organ and hematologic responses were assessed every 3 months in the first year after the end of first-line therapy and every 6–12 months thereafter. Progression-free survival (PFS) was defined as the time from diagnosis until progression of haematological or organ dysfunction or until death from any cause. Overall survival (OS) was defined as the time from diagnosis until death from any cause.

Statistics

Statistical analysis was conducted using SPSS 22.0 software (SPSS, Chicago, IL) and R. Descriptive statistics included the mean, standard deviation, median, and range for continuous variables and frequency counts and percentages for categorical variables. Chi-square and Fisher's exact tests were used to carry out univariate analysis for categorical variables, and T tests were used for continuous variables. PFS and OS analyses were performed using the Kaplan-Meier method, and the log-rank test was used to compare survival curves. A value of $p < 0.05$ indicated statistical significance.

Results

The relationship between clinical features and MRD status

A total of 25 patients with AL amyloidosis diagnosed between 2012 and 2019 met the inclusion criteria of this study. Their clinical characteristics are reported in Table 1. Fifteen patients were men (40%), and the median age was 59 years (range, 42–80). Eighty percent of patients had lambda light chain AL, 40% had Mayo stage-I disease, 28% had Mayo stage-II disease, and 32% had Mayo stage-III disease. Of the 25 patients, 18 (72%) had cardiac involvement, 25 (100%) had renal involvement, 7 (28%) had liver involvement, and 15 (60%) had peripheral involvement at the time of diagnosis. Infiltrating clonal plasma

cells made up a median of 3% of bone marrow cells. Primary treatment was bortezomib-based for all patients, while 10 patients (40%) received ASCT.

Table 1
Baseline and treatment characteristics.

	All patients, N = 25	MRD positive, N = 8	MRD negative, N = 19	P-value
Age, median (range) years	59 (42–80)	62 (55–71)	58 (42–80)	0.148
Sex, male, n (%)	15 (40.0%)	4 (26.7%)	11 (73.3%)	0.667
Lambda involved light chain, n (%)	20 (80.0%)	5 (25.0%)	15 (75.0%)	0.283
Baseline BMPCs, median (range) %	3 (1–10)	3 (1–7)	4(1–10)	0.604
2004 Mayo stage, n (%)				
I	10 (40.0%)	4 (40.0%)	6 (60.0%)	0.355
II	7 (28.0%)	3 (42.9%)	4 (57.2%)	
III	8 (32.0%)	1 (12.5%)	7 (87.5%)	
ALB, median (range) g/L	23.2 (10.7–37.0)	25.1 (12.6–36.0)	24.5 (10.7–37.0)	0.866
Proteinuria, median (range), g/24 h	4.89 (0.18–19.24)	6.78 (0.18–16.93)	6.80 (0.14–19.24)	0.990
Creatinine, median (range), µmol/L	8 2(42–522)	186 (49–522)	111 (42–273)	0.324
NTProBNP, median (range) pg/mL	413.00 (49.3–19,638.0)	329.47 (59.90–1,475.00)	2,816.17 (49.30–19,638.00)	0.292
Troponin-T, median (range) ng/mL	0.022 (0.007–0.333)	0.059 (0.007–0.340)	0.043 (0.008–0.157)	0.606
Alkaline phosphatase, median (range), U/L	78 (42–257)	77 (42–150)	106 (49–257)	0.254
Organ involvement				
Heart	18 (72.0%)	4 (22.2%)	14 (77.8%)	0.156
Kidney	25 (100%)	8 (32.0%)	17 (68.0%)	NA
Liver	7 (28.0%)	2 (28.6%)	5 (71.4%)	0.607
Peripheral nerve	15 (60.0%)	5 (33.3%)	10 (66.7%)	0.607
First line treatment				
ASCT	10 (40.0%)	1 (10.0%)	9 (90.0%)	0.043
No ASCT	15 (60.0%)	7 (46.7%)	8 (53.3%)	

Of the 25 patients, 19 (76%) patients were MRD negative at the time of MRD testing (at the 3rd month either after initial therapy (8 cycles of VD if the patient elected not to proceed with upfront ASCT) or after ASCT). In Table 1, the baseline characteristics of MRD-negative vs. MRD-positive patients are shown, and there were no statistically significant differences between the two groups in terms of characteristics such as age, sex, bone marrow infiltration by plasma cells, light chain type, biomarkers, organ involvement or Mayo stage (all p values > 0.5), but there was a significant difference between the two groups in terms of ASCT. In patients undergoing MRD evaluation after the end of first-line therapy at the 3rd month, MRD-negative rates were higher in those who underwent ASCT than in those who did not [90% (9/10) vs. 53.3% (8/15), $p = 0.043$].

The relationship between MRD and response

At the 3rd month after the end of first-line therapy, the hematologic response was as follows: CR was achieved in 68% of patients ($n = 17$), VGPR was achieved in 32% of patients ($n = 8$), and the MRD negative rate among patients in CR was 82.4% (14/17). At MRD assessment, 18/25 (72%) patients had achieved a renal response, 14/18 (77.8%) patients with cardiac involvement had a cardiac response, and 6/7 (85.7%) had a liver response. Response in any organ was recorded in 80% (20/25) of patients: 94% (16/17) of those patients who were MRD negative showed a response, while 50% (4/8) of those patients with detectable MRD showed a response. ($p = 0.023$). MRD negativity was associated with a higher likelihood of cardiac response at the time of MRD assessment than MRD positivity (93% (13/14) vs. 25% (1/4), $p = 0.019$). For renal response assessment, the renal response rates at the time of MRD assessment were similar in MRD-negative vs. MRD-positive patients (50% (4/8) vs. 82% (14/17), $p = 0.116$). Among those with liver involvement, we found no significant differences in the liver response rate (100% (5/5) in MRD-negative patients vs 50% (1/2) in MRD-positive patients, $P = 0.286$) (Fig. 1).

The relationship between MRD and survival

For all patients ($n = 25$), at a median follow-up of 25.1 months (12.58–93.04 months) after diagnosis, the median PFS was 55.1 months, and the median OS was not reached. As shown in Fig. 2, the PFS was significantly longer in MRD-negative than MRD-positive patients. (24.52 vs 76.39 months, $p = 0.004$), while there was no difference in the OS, and the median OS was not reached in either group ($p = 0.2$). Of the 25 patients, 7 patients relapsed. Of these 7 patients, one patient, who was in the MRD-positive group, received ASCT, and 6 patients received therapy without ASCT. Progression of organ or hematologic dysfunction occurred in 2/17 (11.8%) MRD-negative patients and in 5/8 (62.5%) MRD-positive patients. The two patients in the MRD-negative group both received therapy without ASCT.

Discussion

AL amyloidosis is a rare disease caused by the tissue deposition of misfolded immunoglobulin light chains produced by clonal plasma cells, and these deposits can cause organ dysfunction and death (Muchtart et al. 2020). The clinical relevance of flow cytometry-based MRD assessment and its predictive value in AL amyloidosis are of growing interest (Muchtart et al. 2017b; Kastiris et al. 2020; Muchtar et al.

2020; Sidana et al. 2020; Staron et al. 2020). However, current studies have limitations given the heterogeneity of patient treatment regimens and the lack of predefined time points for MRD assessment (Sidana et al. 2020). We retrospectively reviewed 25 AL amyloidosis patients from our centre who received MRD testing and achieved VGPR or better at the 3rd month after the end of first-line therapy from 2012 to 2019. The first-line therapy in our centre consisted of 8 courses of VD or 4 courses of VD and sequential ASCT, both without maintenance therapy.

Our data support the feasibility and clinical applicability of assessing MRD using MFC in patients with AL amyloidosis. First, we observed that the majority of the patients with VGPR or better and very low tumour burden still had detectable MRD. In our study, among patients with AL amyloidosis with a conventional VGPR or CR, 25% were found to be MRD positive according to MFC. In our series, 82.4% of patients in CR were MRD negative. Similar to our results, Shaji K. Kumar demonstrated that the MRD-negative rate after one line of therapy was 71% (Sidana et al. 2020). Some prior studies have shown that approximately 40–50% of AL amyloidosis patients in CR are MRD negative by EuroFlow-based MRD assessment, which is lower than our result (Lee et al. 2017; Kastiris et al. 2018). This difference might be because prior studies included both newly diagnosed and relapsed patients with AL amyloidosis, and MRD testing was conducted 32 to 71 months after a response was achieved. Our study only included newly diagnosed patients. The timepoint of MRD assessment was fixed in the current study and significantly shorter than that used in previous studies. This result indicates that MRD negativity rates are higher after first-line therapy and MRD assessment has prognostic value even early in the treatment course. Similar findings have also been seen in MM (Gu et al. 2018).

Second, undetectable MRD is associated with excellent outcome, with a very high probability of organ response and a very low risk of hematologic or organ relapse. Organ response is a crucial outcome for patients with AL amyloidosis. Generally, a deep hematologic response (VGPR or better) is required for organ response, but even those who achieve CR can endure persistent organ dysfunction. It is believed that incomplete elimination of clonal PCs after treatment may hinder organ recovery (Staron et al. 2020; Szalat et al. 2020). In our study, patients with a deep hematologic response who achieved MRD negativity had a higher rate of response in any organ, especially cardiac response (93% (13/14) vs 25% (1/4), $p = 0.019$), than those who had MRD positivity. Similar findings have also been seen in other studies (Sidana et al. 2020). Although MRD negativity correlated with a higher frequency of renal and liver responses, statistical significance was not achieved. This may be because in our study, the sample size was relatively small. In addition, Staron et al. (2020) demonstrated that patients who achieve MRD negativity have a higher likelihood of developing a future organ response than those who remain MRD positive. These results indicate that achievement of MRD negativity as assessed by MFC provides an added organ response advantage for patients with AL amyloidosis.

In addition, in our study, patients who achieved VGPR or better according to the conventional response criteria and were MRD positive had a trend toward a shorter PFS compared with those who were MRD negative. Therefore, even if the level of the AL is significantly reduced, the bone marrow tumour burden may remain elevated and serve as a source for relapse. Kumar (Muchtar et al. 2020; Sidana et al. 2020)

and Dimopoulos (Kastritis et al. 2020) observed that MRD negativity was associated with improved PFS in AL amyloidosis, even with a short follow-up. Our study showed that early MRD assessment has great significance for predicting the prognosis of AL amyloidosis. And our study includes the longest follow-up period yet reported.

Third, there were no differences in the baseline characteristics of patients who ultimately achieved MRD negativity vs those who remained MRD positive. In our data, it was impossible to determine which group of patients were more likely to be MRD negative with any of the baseline characteristics except for first-line treatment type. Although the sample size was small, patients in our study who underwent ASCT as part of first-line therapy had higher MRD-negative rates than those receiving nontransplant therapy (90% vs. 53.3%, $p = 0.043$). Similar findings have also been seen in MM (Rawstron et al. 2013; Attal et al. 2017). Patients undergoing ASCT had higher MRD-negative rates than those receiving chemotherapy alone. In contrast, a recent study reported that ASCT does not increase the rate of MRD negativity (Kastritis et al. 2020; Staron et al. 2020). However, in their study, ASCT was not always used as a first-line treatment. According to our results, ASCT as a first-line treatment is better than nontransplantation therapy, and a higher rate of MRD negativity was obtained for patients who received ASCT than for those who did not.

During the follow-up period of our study, of the 25 patients, 7 relapsed. Only 1 patient in the transplantation group relapsed, and the MRD test was positive. All the patients who relapsed in the MRD-negative group did not receive ASCT. Another finding underscored by our results is the relatively high incidence of MRD negativity among patients who did not receive ASCT, which highlights concerns about the need for further consolidation or maintenance therapy after chemotherapy alone in patients with AL.

Some of the limitations of this study are its retrospective nature; it also had a highly specific patient cohort, as patients with advanced cardiac involvement may not have lived long enough to obtain a deep response. Moreover, outcomes were evaluated based on a one-time MRD assessment. However, our study is one of the only studies reported to date to include MRD assessment with MFC for patients with AL amyloidosis, and it has the longest follow-up duration and a fixed the time point for MRD assessment. In conclusion, MRD negativity measured by MFC at the 3rd month after first-line therapy in patients with AL amyloidosis is measurable and associated with superior organ response rates and improved PFS over a long follow-up. Our study is valuable because it reports the clinical outcomes of patients with AL amyloidosis and uses advanced flow cytometry MRD detection to predict prognosis and monitor patients as part of a standard of care response assessment tool. Our results can inform future MRD assessment strategies in clinical practice and help incorporate MRD endpoints into clinical trials. MRD should be explored as a surrogate endpoint in clinical trials, and MRD-based risk-adapted trials may help optimize treatment for AL amyloidosis.

Declarations

Acknowledgments

We thank the research staff for recruiting patients to the study and for their involvement in patient recruitment, data collection, and data management.

Funding

No funding

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Beihui Huang, Junru Liu, Meilan Chen and Jingli Gu. The first draft of the manuscript was written by Juan Li and Xiaozhe Li and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline and treatment characteristics.

	All patients, N=25	MRD positive, N=8	MRD negative, N=19	P- value
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Liver	7 (28.0%)	2 (28.6%)	5 (71.4%)	0.607
Peripheral nerve	15 (60.0%)	5 (33.3%)	10 (66.7%)	0.607
First line treatment				
ASCT	10 (40.0%)	1 (10.0%)	9 (90.0%)	0.043
No ASCT	15 (60.0%)	7 (46.7%)	8 (53.3%)	

Figures

Figure 1

Frequency of organ response at the time of MRD assessment among patients in VGPR or better

Figure 2

A PFS according to MRD status following first-line treatment; B OS according to MRD status following first-line treatment