Abnormal FISH in patients with immunoglobulin light chain amyloidosis is a risk factor for cardiac involvement and for death

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Importance of interphase fluorescent in situ hybridization (FISH) with cytoplasmic staining of immunoglobulin FISH (clq-FISH) on bone marrow is not well understood in light chain amyloidosis (AL). This is in contrast with multiple myeloma where prognostic and treatment related decisions are dependent on cytogenetic testing. This retrospective study reviewed 401 AL patients with clq-FISH testing performed at our institution between 2004 and 2012. Eighty-one percent of patients had an abnormal clq-FISH. Common abnormalities involved translocations of chromosome 14q32 (52%), specifically: t(11;14) (43%), t(14;16) (3%) and t(4;14) (2%). Other common abnormalities include monosomy 13/deletion 13q (30%), trisomies 9 (20%), 15 (14%), 11 (10%) and 3 (10%). Median overall survival for this cohort of patients is 3.5 years. When plasma cell burden was greater than 10% trisomies predicted for worse survival (44 vs 19 months), and when it was ≤10% t(11;14) predicted for worse survival (53 months vs not reached). Abnormal clq-FISH was significantly associated with advanced cardiac involvement, and remained a prognostic factor on multivariate analysis. This large AL cohort demonstrates that abnormal FISH at diagnosis is prognostic for survival and advanced cardiac disease. Particularly, trisomies and t(11;14) affect survival when degree of plasma cell burden is considered.

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INTRODUCTION

Immunoglobulin light chain amyloidosis (AL) is a rare clonal plasma cell disorder that results in multi-organ dysfunction from deposition of abnormal amyloid fibrils. Although many of the treatment options for AL are extrapolated from data in multiple myeloma (MM), it differs in its biology greatly. We know that in MM, cytogenetic abnormalities have a profound effect on risk stratification, and therefore guide treatment decisions.

Because of the low proliferative nature of conditional AL, using conventional metaphase cytogenetic testing has been inadequate in revealing karyotypic aberrations. The advent of interphase fluorescent in situ hybridization (FISH) with cytoplasmic staining of immunoglobulin FISH (clq-FISH) and an increase in the variety of FISH probes has elucidated more abnormalities in AL. Interphase FISH is an assay that is performed on non-dividing cells and is used to study pre-specified abnormalities. To estimate the prevalence of abnormalities, interphase FISH is best done on purified cells or with the use of simultaneous immunofluorescence.1

This technique was first utilized and reported by Fonseca et al.1 in 1998. Since that study, many chromosomal abnormalities in AL patients using clq-FISH have been described, such as translocations involving 14q32, t(11;14), t(4;14) and monosomy 13/deletion 13q.2-4

This study, which expands upon the prior Mayo Clinic experience, by Bryce et al.5 was intended to determine the frequency of chromosomal abnormalities in the largest cohort of AL patients to date and to assess the prognostic significance and clinical utility of clq-FISH.

MATERIALS AND METHODS

Patients

Of the 1204 patients with a diagnosis of AL seen at Mayo Clinic between 2004 and 2012, 401 patients had clq-FISH performed as a routine clinical test within 90 days of diagnosis and comprised our study population. The diagnosis of AL was based on a positive Congo red stain which demonstrated apple green birefringence under polarized light; 54% of patients had their amyloid typed by immunohistochemistry and 46% by laser capture mass spectrometry. This study was approved by the Mayo Clinic Institutional Review Board, and was done in accordance with the Declaration of Helsinki. All patients had consented to have their medical records reviewed.

Interphase clq-FISH

Bone marrow aspirate samples were acquired and processed at the Mayo Clinic cytogenetics laboratory, and commercially available and in house chromosome-specific FISH probes were used. The clq stain is an immunofluorescent antibody for kappa and lambda to detect plasma cells. FISH enumeration strategies were employed to detect deletion, monosomy or trisomy of chromosomes 13 and 17 by using the 13q14 (R81),13q34 (LAMP1) probe set and the 17p13.1 (TP53) and 17 centromere (D17Z1) probe set. FISH enumeration strategies were also used to detect trisomies of chromosomes 3, 7, 9 and 15 using centromere probes for (D3Z1, D7Z1, D9Z1 and D15Z4). Translocations involving the immunoglobulin heavy chain (IGH) and common partner genes were determined by using the following probes: break-apart IGH at (5′IGH3/IGH), and double-fusion FISH (D-FISH) including 14q32 (IGH-XT) with 4p16.3 (FGFR3), 6p21 (CCND3), 11q13 (CCND1), 16q23 (MAF) or 20q12 (MAFB). Of note, the CCND3 and MAFB probes were not added to the clq-FISH assay until 5/2009. For each probe set ideally 100 plasma cells were scored. To be considered positive at least three abnormal cells had to be identified.
Definitions of risk categories

To compare risk of different FISH characteristics among the amyloid population, established definitions were used. The mSMART criteria were used for MM FISH risk and are as follows: high-risk, deletion (17p), t(14;16) and t(14;20); intermediate risk, t(4;14); standard risk were all other abnormalities including trisomies, t(11;14) and t (6;14).6 The 1q duplication, which would have been considered as MM mSMART intermediate risk, is underestimated in this data set since it was not part of the standard clinical probe set. The mSMART criteria for smoldering MM (SMM) were used and are as follows: low risk, normal FISH or insufficient plasma cells for analysis; standard risk, t(11;14), maf translocations, other/unknown translocations or monosomy 13/deletion (13q); intermediate risk, trisomies alone; and high risk, t(4;14) or deletion (17p).7

Statistical analysis

The Fisher’s exact test was used to determine the differences in the nominal groups. A two-sided P-value < 0.05 was considered statistically significant. Overall survival (OS) was calculated from the time of diagnosis to death, with patients alive at time of last follow-up censored from analysis. Survival curves were created using the Kaplan–Meier method and compared by log rank test. Univariate and multivariate modeling was performed using Cox proportional hazards. Those parameters significant on univariate analysis were entered into the multivariate model in a stepwise fashion. Analyses to determine specific FISH abnormalities associated with certain demographic parameters were performed, and a P-value < 0.01 was used. FISH groupings for these analyses included: (1) trisomies present; (2) deletion 13/13q; (3) mSMART MM FISH risk categories; and (4) mSMART smoldering myeloma FISH risk categories. The statistical analysis was performed on JMP software package (SAS, Cary, NC, USA).

RESULTS

Patient characteristics

Baseline demographics and clinical characteristics of the 401 patients are shown in Table 1. The median age of the patients was 63 (range: 25–89) and 253 (63%) were male. The majority of patients (92%) had an abnormal free light chain (FLC) ratio. Fifty-one percent of the patients had a measurable monoclonal (M) spike in the serum or urine. There were 198 patients (49%) with bone marrow plasma cells greater than 10%. Patients with abnormal FISH were more likely to have higher serum immunoglobulin FLC level (P < 0.0001), bone marrow plasmacytosis (P < 0.0001), NT-proBNP (P = 0.009) and cardiac stage (P = 0.007).

Distribution of FISH abnormalities

There were 76 patients (19%) that had no abnormalities found by clg-FISH despite sufficient plasma cells for evaluation. The remaining 325 patients (81%) had at least one abnormality demonstrated by clg-FISH. The frequencies of the abnormalities are shown in Table 2. Approximately two-thirds of the patients had abnormalities involving the immunoglobulin heavy chain (IgH), common translocations included the following: t(11;14), t(14;16), t(4;14), t(6;14) and t(14;20) seen in 44, 3, 2, 2 and 1%, respectively. However, there were 39 patients (9%) who had a positive IgH break-apart probe without an identified partner. Monosomy13/deletion (13q) was the second most frequent aberration detected in 119 patients (30%). Trisomies were identified for all chromosomes evaluated (3, 4, 6, 7, 9, 11, 14, 15,

| Table 1. Baseline and demographic data of patients upon first evaluation at the Mayo Clinic |
|------------------------------------------|------------------------------------------|
| **Characteristic** | **Patients with abnormal clg-FISH** | **Patients with normal clg-FISH** |
| **N (%)** | **Median (range)** | **N (%)** | **Median (range)** |
| N | 325 (81) | 63 (25–89) | 76 (19) | 62 (38–85) |
| Age, years | 210 (65) | | 43 (57) | |
| Males | 210 (65) | 2009 (2004, 2012) | 43 (57) | 2009 (2004, 2012) |
| Year of diagnosis | | | | |
| Serum M spike g/dl | 168 (51) | 0.6 (0–6) | 39 (51) | 0.6 (0–3.4) |
| Involved FLC, mg/dl | 300 (92) | 27.4 (1.1–2330) | 71 (93) | 9.0 (1.3–1050) |
| Lambda restricted | 205 (63) | 20 (77) | 39 (52) | |
| BM PC > 10 | 177 (54) | 20 (77) | 39 (52) | |
| Urine total protein, g/24 h | 294 (90) | 1.0 (0.02, 34.9) | 65 (86) | 0.8 (0.02, 16.8) |
| Serum creatinine | 309 (95) | 1.1 (0.5, 7.1) | 73 (96) | 1.0 (0.4, 5.9) |
| Alkaline phosphatase | 225 (69) | 83 (24, 3434) | 54 (71) | 91 (34, 761) |
| Troponin, ng/ml (nl < 0.01) | 200 (61) | 0.03 (< 0.01–1.6) | 48 (63) | 0.02 ( < 0.01–0.46) |
| NT-proBNP pg/ml (nl < 138) | 203 (62) | 2839 (15–53278) | 49 (64) | 1211 (50–18161) |
| NT-proBNP > 8500 pg/ml | 44 (22) | 4 (8) | | |
| Mayo stage (2004) | 191 (59) | 45 (59) | | |
| I | 37 (11) | 9 (12) | | |
| II | 77 (24) | 19 (25) | | |
| III | 77 (24) | 17 (22) | | |
| Missing | 134 (41) | 31 (41) | | |
| Mayo stage (2012) | 178 (55) | 41 (53) | | |
| I | 30 (9) | 16 (21) | | |
| II | 43 (13) | 7 (9) | | |
| III | 47 (15) | 11 (15) | | |
| IV | 58 (18) | 7 (9) | | |
| Missing | 147 (45) | 31 (46) | | |
| Received ASCT | 118 (36) | 30 (39) | | |

Abbreviations: ASCT, autologous stem cell transplant; BM, bone marrow; PC, plasma cells; nl, normal; FISH, fluorescent in situ hybridization; FLC, free light chain. *Kappa nl 0.33–1.94 mg/dl; Lambda nl 0.57–2.63 mg/dl. **< 0.05. *Stage I neither troponin > 0.03 or NT-ProBNP > 332: if one elevated then Stage II; and if both are elevated then Stage III. *Stage I: none the following are elevated: troponin > 0.025 ng/ml and NT-ProBNP > 1800 pg/ml and serum immunoglobulin free light chain difference > 18 mg/dl; if any one parameter is high, then Stage II; if two parameters are high then Stage III; and if all three are elevated, then Stage IV.
16 and 17) except for 13. The most common trisomy observed was trisomy 9 (20%), followed by trisomies: 15 (14%), 7 (10%), 3 (10%) and 11 (9%). There were very few tetrasomies observed, and the most common was tetrasomy 11 (2%). Only nine patients (2%) were identified with a deletion (17p).

| Abnormality | Number of patients (%) |
|-------------|------------------------|
| Normal      | 76 (19)                |
| Abnormal    | 325 (81)               |
| t(11;14)    | 175 (44)               |
| t(14;16)    | 14 (3)                 |
| t(4;14)     | 9 (2)                  |
| t(6;14)     | 7 (2)                  |
| t(14;20)    | 4 (1)                  |
| IgH without a partner | 39 (9) |
| Monosomy 13/Del 13q | 119 (30) |
| Monosomy 14  | 24 (6)                 |
| Monosomy 16  | 7 (2)                  |
| Del17p      | 9 (2)                  |
| Any trisomy | 87 (27)                |
| Trisomy 9   | 66 (20)                |
| Trisomy 15  | 45 (14)                |
| Trisomy 7   | 34 (10)                |
| Trisomy 3   | 33 (10)                |
| Trisomy 11  | 31 (9)                 |
| Trisomy 17  | 16 (5)                 |
| Trisomy 14  | 2 (0.1)                |
| Trisomy 4   | 1 (0.03)               |
| Trisomy 6   | 1 (0.03)               |
| Trisomy 16  | 1 (0.03)               |
| Hyper diploid | 40 (12)                |
| Any tetrasomy | 15 (5)                 |
| Tetrasomy 11 | 8 (2)                  |
| Tetrasomy 9  | 3 (0.9)                |
| Tetrasomy 15 | 2 (0.1)                |
| Tetrasomy 3  | 1 (0.03)               |
| Tetrasomy 7  | 1 (0.03)               |

| MM mSMART riskb | Number of patients (%) |
|-----------------|------------------------|
| Standard        | 366 (91.3)             |
| Intermediate    | 9 (2.2)                |
| High            | 26 (6.5)               |

| SMM mSMART riskc | Number of patients (%) |
|-----------------|------------------------|
| Low             | 76 (19)                |
| Standard        | 273 (68)               |
| Intermediate    | 34 (8.5)               |
| High            | 18 (4.5)               |

Abbreviations: FISH, fluorescent in situ hybridization; SMM, smoldering multiple myeloma. *Hyperdiploidy—trisomies of at least two of the three chromosomes 5, 9 and 15.15 MM mSMART: high risk, deletion 17p, t(14;16) and t(14;20); intermediate risk t(4;14); standard risk are all other abnormalities. SMM mSMART: low, normal FISH or insufficient plasma cells for analysis; standard, t(11;14), maf translocations, other/unknown translocations, or deletion 13/13q; intermediate, trisomies alone; and high, t(4;14) or deletion 17p.

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Figure 1. Distribution of FISH abnormalities. (a) Overlap of common FISH abnormalities, high risk is defined as deletion of 17p, t(14;16) and t(14;20). (b) Overlap of patients with t(4;14), deletion 13/13q, trisomies, and deletion 17p. (c) Overlap of FISH abnormalities for patients with high-risk cytogenetics.

26 patients with high-risk MM mSMART cytogenetics, and only one patient more than one high-risk MM FISH aberrations (Figure 1c). While considering individual types of FISH abnormalities, the presence of monosomy 13/deletion (13q) was associated with
high dFLC, NT-proBNP levels (Figure 2) and bone marrow plasmacytosis (Figure 3). On multivariate analysis, monosomy 13/deletion (13q) was independently associated with high NT-proBNP ($P = 0.0009$) even when either dFLC or bone marrow plasmacytosis was included in the model (data not shown). In contrast, the presence of trisomies was associated with high dFLC and bone marrow plasmacytosis, but not with high cardiac biomarkers (Figures 2 and 3). Translocation (11;14) was not associated with high dFLC, NT-proBNP levels (Figure 2) or bone marrow plasmacytosis (Figure 3).

We next turned to the existing FISH risk stratification models trying to better elucidate the relationship between FISH and advanced amyloid-related cardiac disease. The MM mSMART stratification for FISH did not predict for high NT-proBNP, nor did it predict for high FLC, or bone marrow plasmacytosis (data not shown). In contrast, the SMM mSMART risk groups also had a significant association with dFLC, with the lowest levels in the low-risk group (7.6%) and the highest levels in the high-risk group cytogenetics (51%) with a $P$-value $< 0.0001$ (Figure 4). The SMM mSMART risk grouping was also associated with NT-proBNP, with the lowest NT-proBNP values (NT-proBNP = 1211 pg/ml) in patients with normal FISH and the highest values in the patients with high-risk SMM FISH (NT-proBNP = 4228 pg/ml) (Figure 4).

The SMM mSMART risk grouping also was associated with bone marrow plasmacytosis with a $P < 0.0001$ (data not shown). The nine patients with deletion 17p had the highest level of bone marrow plasmacytosis (25 vs 9%, $P = 0.004$).
Survival and prognosis
At a median follow-up of 53.8 months, 202 patients have died, 21 from the normal FISH group and 181 from the abnormal FISH group. Median OS from diagnosis was 55 months (range: 0.1–119 months), with a significantly different survival between patients with abnormal cIg-FISH and no FISH abnormalities (43 months vs not reached $P=0.0001$) (Figure 5a). On univariate analysis, having an abnormal cIg-FISH was associated with a worse survival (Table 3). A number of other factors were found to be prognostic on univariate analysis, including those that related to treatment, plasma cell burden (bone marrow plasmacytosis and serum immunoglobulin FLC), cardiac risk factors and age. On multivariate analysis, however, only three factors remained prognostic: whether or not ASCT was a treatment, abnormal FISH, and NT proBNP $\geqslant 1800$ (Table 3). Given that eligibility for a transplant is an excellent prognostic factor, we also performed the multivariate analysis with ASCT excluded, and abnormal FISH persisted as an independent prognostic factor with NT-proBNP $>1800$, and age (Table 3). The multivariate analyses were performed as well excluding the 76 patients with normal results on FISH, and the results were virtually the same (data not shown).

No specific abnormality found on cIg-FISH was found to be of independent prognostic significance, including t(11;14), which had a risk ratio of death of 1.17 (0.87, 1.58, $P=0.2$) on univariate. Even when patients were clustered into FISH risk groups recognized for MM or smoldering myeloma, there was no prognostic differential among the abnormal FISH subcategories (data not shown). Overall, the presence of any FISH abnormality—rather than a specific abnormality—was associated with risk of death. However, if patients were divided into those with bone marrow plasmacytosis $\leqslant 10\%$ and those $>10\%$; we found a median OS of 81 months vs 31 months, respectively. Further subset analyses of these two groups of patients showed, that amongst the lower tumor burden patients ($\leqslant 10\%$ bone marrow plasmacytosis), t(11;14) was the only significantly negative prognostic factor (Figure 5b, median OS 53 months vs not reached, $P=0.02$). Among the higher tumor burden patients ($>10\%$ bone marrow plasmacytosis) presence of any trisomy was noted to be the only adverse prognostic factor (Figure 5c, median OS 19 months vs 44 months, $P=0.01$).
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TABLE 3. Univariate and multivariate analysis of prognostic factors for survival

| Prognostic factor | Univariate | Multivariate A | Multivariate B (without ASCT) |
|-------------------|------------|----------------|-------------------------------|
|                    | Risk ratio (95% CI) | P       | Risk ratio (95% CI) | P       | Risk ratio (95% CI) | P       |
| No ASCT            | 4.37 (3.07, 6.41) | < 0.0001 | 3.73 (2.27, 6.46) | < 0.0001 | excluded            |         |
| NT-proBNP ≥ 1800 (pg/ml) | 3.51 (2.38, 5.31) | < 0.0001 | 2.28 (1.52, 3.50) | < 0.0001 | 2.33 (1.47–3.88) | 0.0002 |
| Abnormal clg-FISH  | 2.54 (1.58–4.35) | < 0.0001 | 2.82 (1.57, 5.60) | 0.0002   | 2.13 (1.18–4.26) | 0.01   |
| Age               | 1.03 (1.01–1.04) | 0.0002   | NS                | 1.02 (1.01–1.04) | 0.004 |
| Troponin ≥ 0.03 (mcg/ml) | 2.42 (1.69, 3.51) | < 0.0001 | NS                | NS       | NS                 |         |
| NTproBNP ≥ 332    | 2.31 (1.76, 3.07) | < 0.0001 | NS                | NS       | NS                 |         |
| dFLC ≥ 18 (mg/dL) | 1.79 (1.47–2.20) | < 0.0001 | NS                | NS       | NS                 |         |
| Bone marrow plasma cell > 10 (%) | 1.77 (1.30–2.42) | 0.0007 | NS                | NS       | NS                 |         |
| Mayo 2012 Staging | 1.79 (1.47–2.20) | < 0.0001 | NS                | NS       | NS                 |         |
| Mayo 2004 Staging | 2.31 (1.76, 3.07) | < 0.0001 | NS                | NS       | NS                 |         |

Abbreviations: ASCT, autologous stem cell transplant; CI, confidence interval; FISH, fluorecent in situ hybridization; FLC, free light chains; NS, not significant.

DISCUSSION

In our cohort of 401 AL patients who had clg-FISH using a standard MM panel at diagnosis, 81% of patients harbored chromosomal abnormalities. No specific clg-FISH abnormality was more prognostic than another; simply having any abnormal clg-FISH finding resulted in a worse overall survival. Abnormal FISH may be present in almost every patient with a clonal plasma cell disorder. More results are ordered, or more sensitive techniques are employed. Our results may simply indicate there are favorable abnormal FISH changes that we have yet to discover. However, when the patients were evaluated according to those with higher tumor burden vs lower tumor burden, the prognostic impact of trisomies and t(11;14) was unmasked. In addition, for all patients there was also an intriguing association between abnormal clg-FISH, higher plasma cell burden and a more advanced cardiac stage at diagnosis. Despite these associations abnormal clg-FISH had a negative prognostic impact independent of NT-proBNP and other adverse features on multivariate analysis. Even high-dose chemotherapy with stem cell support did not abrogate the risk imparted by abnormal FISH.

The connection between abnormal FISH and more advanced cardiac disease has been described previously by our group, but this finding is substantiated in this study of a larger cohort of patients. Moreover, monosomy 13/deletion (13q) does seem to correlate with more extensive cardiac involvement irrespective of plasma cell burden, that is, bone marrow plasmacytosis or dFLC. Our current work brings us no closer to the underlying mechanism of these findings. Cardiac biomarker stage and serum immunoglobulin FLC. In their patients, was an unfavorable prognostic factor in AL patients demonstrated that the gain of 1q21, which was present in 23% of series, patients with 1q21 gain appeared to have more of a myeloma phenotype with 61% of the 1q21 gain patients having significantly higher bone marrow FLCs and bone marrow plasmacytosis but also higher rates of intact heavy chain. Since this probe was not available at our institution for testing, we can neither validate nor challenge this important observation.

Our study confirms that having aberrant clg-FISH does predict for inferior OS. In our series, no particular abnormality was more prognostic than another, except when applied to patients with bone marrow plasmacytosis >10% or ≤10%. In addition, there was an intriguing association between advanced cardiac disease and abnormal FISH, especially monosomy 13/deletion 13q. This study exemplifies the complexity of AL, with the mortality of patients dependent not only on the burden and behavior of MM points to their shared pathologic pathway but the differential frequency of their presence may point to part of their differences.

Recently it has been reported that the presence of any trisomy in MM is a good prognostic factor and suggests an improved overall survival. In contrast, in our AL cohort, trisomies occurred in twenty-one percent of patients and did not predict for superior OS despite the fact that the relative rates and overlap of individual trisomies were similar to other studies in MM. Rather, trisomies in patients with more than 10% plasmacytosis in the bone marrow had an inferior OS if trisomies were present. Another possibility for this difference might be that the MM observation was made in the context of patients primarily treated with lenalidomide as first line, which is a treatment that is not preferred in patients with AL.

Boyd et al demonstrated that grouping of high-risk cytogenetic factors together such as t(4;14), t(14;16), t(14;16, +q21) and deletion (17p) were associated with a worse prognosis than each independently. Only one of our patients had more than one high-risk feature albeit our study does not include chromosome 1q data. In contrast, in our data set there were seven patients who had both high-risk cytogenetics and trisomies, the latter of which has been reported to attenuate risk in MM. In our study no significant difference in OS from the high-risk FISH features could be documented but we are limited by very small numbers of high-risk abnormalities.

AL risk stratification and prognostication is based primarily on the severity of organ involvement—cardiac involvement especially—and FLC levels. The Mayo AL staging systems incorporate troponin T, NT pro-BNP, and more recently serum immunoglobulin FLCs. Unlike MM which relies heavily on cytogenetic aberrations for risk stratification, until recently there were not any established cytogenetic prognostic markers for AL. Bochtler et al demonstrated that the gain of 1q21, which was present in 23% of their patients, was an unfavorable prognostic factor in AL patients treated with melphalan and dexamethasone independent of cardiac biomarker stage and serum immunoglobulin FLC. In their series, patients with 1q21 gain appeared to have more of a myeloma phenotype with 61% of the 1q21 gain patients having not only significantly higher bone marrow FLCs and bone marrow plasmacytosis but also higher rates of intact heavy chain. Since this probe was not available at our institution for testing, we can neither validate nor challenge this important observation.
of the plasma cell clone but also on the toxicity of the immunoglobulin FLCs.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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