Evaluation of the cytogenetic aberration pattern in amyloid light chain amyloidosis as compared to monoclonal gammopathy of undetermined significance reveals common pathways of karyotypic instability

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Abstract:
Chromosomal aberrations (CA) have emerged as important pathogenetic and prognostic factors in plasma cell disorders. Using interphase FISH analysis, we evaluated CA in a series of 75 amyloid light chain amyloidosis (AL) patients as compared to 127 patients with monoclonal gammopathy of unknown significance (MGUS). We investigated IgH translocations t(11;14), t(4;14), t(14;16) as well as gains of 1q21, 11q23, 19q13 and deletions of 8p21, 13q14 and 17p13 detecting at least one CA in 89% of the patients. Translocation t(11;14) was the most frequent aberration in AL with 47% versus 26% in MGUS (p=0.03) and strongly associated with the lack of an intact immunoglobulin (p<0.001), thus contributing to the frequent light chain subtype in AL. Other frequent aberrations in AL included deletion of 13q14 and gain of 1q21, which were shared by MGUS at comparable frequencies. The progression to MM stage I was paralleled by an increased frequency of gain of 1q21 (p=0.001) in both groups. Similar branching patterns were observed in an oncogenetic tree model indicating a common mechanism of underlying karyotypic instability in these plasma cell disorders.
Introduction:
Systemic AL amyloidosis (AL) is characterized by the deposition of immunoglobulin light chains as amyloid fibrils in different organs, where they form toxic protein aggregates. The underlying disease is a plasma cell disorder, overwhelmingly a monoclonal gammopathy 1. The predilection for certain tissues is attributed to some extent to the respective clonality isotype of the light chain 2-5. Whereas the clonal light chain repertoire has been intensively studied in AL, the pathogenetic role of chromosomal aberrations (CA) has only been addressed in a few reports based on small patient cohorts so far 6-9.

This is different in multiple myeloma (MM), a malignant plasma cell disorder, where numerous CA have been recognized as pathogenetic factors. Meanwhile, molecular testing has become a standard in diagnostic evaluation to identify patient subgroups regarding prognosis. Based on ploidy data, hyperdiploid and non-hyperdiploid forms of MM have been delineated as two major pathogenetic pathways 10-13. Hyperdiploid MM is characterized by the accumulation of extra copies of chromosomes. Multiple trisomies most frequently involve chromosomes 3, 5, 7, 9, 15, 19 and 21 14. By a clustering of CA, we recently found non-hyperdiploid MM to separate in three branches: firstly, the translocation t(11;14)(q13;q23), which juxtaposes the immunoglobulin heavy chain locus (IgH) to the oncogene cyclin D1, secondly, deletion of 13q14 – frequently in association with translocation t(4;14)(p16;q23), which involves the oncogenes MMSET and FGFR3 - and thirdly, gain of 1q21 15.

Monoclonal gammopathy of undetermined significance (MGUS) is a pre-malignant disorder characterized by an expansion of monoclonal plasma cells, which can progress to frankly malignant MM 16. Though the interpretation of CA results in MGUS has been complicated by an inferior plasma cell purity due to a lesser degree of bone marrow plasmocytosis and a lower proliferative index 17, chromosome aberrations have been consistently detected by interphase-FISH in a high proportion of patients 17-19. Markedly, CA in MGUS were similar to those in symptomatic MM, with IgH translocations and deletions of 13q14 observed at comparable frequencies.

In this study, we addressed the distribution pattern of CA in AL in comparison to MGUS by interphase FISH analysis.
Patients, Material and Methods

Patients

This study evaluated a series of 231 consecutive patients with plasma cell disorders from a single institution, who were tested for cytogenetic (FISH) abnormalities from May 2003 to February 2007. The AL cohort consisted of 85 patients, all of them with a histological confirmed diagnosis. Among them 76 were untreated and only 9 had received prior therapy without achieving a remission of their underlying plasma cell disorder. A series of 146 patients with MGUS or MM I not requiring therapy were used as control groups. Patients with an MM stage II or III were not considered in this analysis, also in case of a concomitant AL. As recommended by a consensus workshop report 19 patients with an IgM heavy chain subtype were also not eligible for this study. For the classification of patients into the MGUS and MM I groups we applied standard diagnostic criteria 20, however with a bone marrow plasmocytosis of 30% instead of 10% as cut-off value between MGUS and MM I, according to the Boston group and Mayo Clinic criteria for AL 21-22 and our own practice 23. Thus we obtained four patient groups: group 1 (n=75) included AL patients without concomitant MM, group 2 (n = 10) included AL patients with concomitant MM I, group 3 (n = 127) included patients with MGUS and group 4 (n = 19) with MM I. Groups 1+2 were combined for the diagnosis of AL versus group 3+4. Groups 1+3 were combined from the point of view of monoclonal gammopathy versus MM I (group 2+4). The characteristics of the patient groups are given in detail in Table 1. As expected, lack of an intact immunoglobulin and lambda light chain restriction prevailed among AL patients. However, age and gender were not statistically differently distributed among patient groups.

Informed consent was obtained from the patients. The study was approved by the Ethics Committee of the University of Heidelberg.

Cytogenetic testing:

Density gradient centrifugation of bone marrow aspirates over Ficoll Hypaque (Biochrom, Berlin, Germany) was performed to separate mononuclear cells by standard protocol. CD138 positive plasma cells were isolated by magnetic activated cell sorting using anti CD138 immunobeads and an auto MACS separation system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s protocol. Purity was confirmed by the CD38+ and CD138+ phenotype in flow cytometric analysis.
FISH was carried out using a panel of commercial two-colour probe sets for the detection of numerical chromosome changes for the following loci: 1q21/8p21, 11q23/13q14, 17p13/19q13, and for the identification of translocations t(11;14), t(4;14) and t(14;16). From May 2003 to November 2005, the first 94 patients were assessed for t(11;14) and t(4;14) only. Starting with the 95th patient of this study in November 2005, the strategy was switched to a determination of t(11;14) and the IgH breakapart probe first. In the event that t(11;14) turned out to be negative and the IgH breakapart probe yielded a positive result, the search of the translocation partner was pursued with probes for t(4;14) and t(14;16). Hybridization was performed according to the manufacturer’s instructions. One hundred interphase nuclei per probe were evaluated using a DM RXA fluorescence microscope (Leica, Wetzlar, Germany). Hybridization efficiency was validated on metaphase spreads and interphase nuclei obtained from the peripheral blood and bone marrow of a healthy donor. The thresholds for gains, deletions and translocations were set as 10%.

Statistical analysis

The frequencies of CA of the above mentioned patient groups were compared by Fisher's exact test. Of note, for each parameter, p values resulting from all tests were adjusted by Holm's method to control the family wise error rate at 0.0524.

Oncogenetic tree models derived by maximum likelihood estimation were described by von Heydebreck et al.25 using the R package oncomodel. The root of the tree represents the state of normal cell. The marginal probabilities of the observed events are encoded by the length of the paths between the root and the corresponding nodes representing the following CA: gain of 1q21 and 11q23, deletion of 13q14, translocations t(11;14) and t(4;14). The length (horizontal distance) from the event (CA) to the root or the next inner node is the negative logarithm of the conditional probability that this aberration occurs at this node, given that the hidden events corresponding to this node happened. Independent events are represented by non-overlapping paths to the root. To assess the uncertainty of the obtained tree models, a nonparametric bootstrap by Felsenstein et al.26 is used. In Figure 1, confidence values for the internal edges of the maximum likelihood trees (based on 500 bootstrap data sets) are given. Therefore, the proposed tree structure has to be interpreted with caution, still allowing us to formulate hypotheses about the association between the above-mentioned CA.
To analyse the association between CA and hematological factors, Fisher's exact test and exact Wilcoxon rank sum test were performed, respectively, with Bonferroni-Holm correction for resulting p values.

Clinical factors and patient characteristics were compared by Fisher's exact test for categorical variables and by the exact Wilcoxon rank sum test for quantitative variables, respectively. All statistical tests were two-sided.

Statistical analysis was carried out using the software package R, version 2.5.1.

Results:

Frequencies of chromosomal aberrations
In the AL without concomitant MM cohort (group 1), a CA could be detected in 95%. Translocation involving the IgH heavy chain locus on chromosome 14q32 was found in 73% of patients. The most frequent translocation partner was chromosome 11. The resulting translocation t(11;14) was detected in 47%, whereas other known translocations of the IgH heavy chain locus, t(4;14) and t(14;16), were rarely found in AL, accounting only for 3% and 4%, respectively. Other frequent aberrations in AL included gain of 11q23 (33%), deletion of 13q14 (31%), gain of 1q21 (19%) and gain of 19q13 (15%), whereas deletion of 8p21 was a rare event and deletion of 17p13 could not be detected. The frequencies of all CA tested are specified according to the four patient subgroups in Table 2. The frequency of 47% for t(11;14) in AL was higher than the incidence of 26% in MGUS (patient group 3) (p = 0.03).

In AL and MGUS, disease progression to MM I was associated with an increased frequency of gain of 1q21 (16% versus 52%, p=0.001) and t(4;14) (7% versus 21%), however statistical significance for t(4;14) was lost after correction for multiple testing (p=0.23).

Clustering of cytogenetic aberrations
In order to detect clustering of CA we applied an oncogenetic tree model 15,25 which was based on the whole study population, including also patients with MM I. Translocations of t(11;14), t(4;14), gains of 11q23 and 1q21 as well as deletion of 13q14 were chosen for this model. For these five major CA the data set was complete in 210/231 (91%) patients. Seventy-two out of 84 AL patients (86%) and 97/126 MGUS patients (77%) displayed at least one of the five aberrations and could thus be included into the cluster analysis.
In the AL group we could discern two major independent branches (see Figure 1). The first branch was characterized by t(11;14) together with gain of 11q23, the second by deletion of 13q14, gain of 1q21 and t(4;14). In the first branch, t(11;14) and gain of 11q23 separated early from each other. In the second branch, t(4;14) was placed at the node farthest from the root, whereas gain of 1q21 separated earlier from this pathway. The major difference between the AL and the MGUS groups was the grouping of gain of 11q23. Whereas it was linked with the t(11;14) branch in AL, it was placed on the opposite side of the root in the MGUS group. The significance of this finding in cluster analysis was confirmed by Fisher’s exact test, where the combination of t(11;14) and gain of 11q23 proved more frequent in AL than in MGUS (20% versus 7%, p = 0.005). As a minor difference between the respective AL and MGUS trees, gain of 1q21 separated earlier from the deletion of 13q14 branch in AL.

In spite of these two differences, the trees for AL and MGUS shared common features: they both showed a t(11;14) branch independent of deletion of 13q14 pathway. Also in both tree models, t(4;14) and gain of 1q21 were grouped together with deletion of 13q14.

Association of cytogenetic aberrations with hematological parameters

We analyzed the association of CA with hematological parameters, including the detection of an intact immunoglobulin and – if detected - its subtype, kappa versus lambda light chain restriction and the bone marrow plasma cell content.

In the overall study population the detection of t(11;14) was associated with a lack of intact immunoglobulin in immunofixation (p<0.001, see Table 3). Gain of 19q13 was associated with the detection of an intact immunoglobulin (94% versus 72%, p=0.05). The immunoglobulin heavy chain subtype retained no statistical significance following p value adjustment for multiple testing (data not shown) for all analysed CA. Gain of 1q21 turned out to be the only CA which was associated with a lambda light chain restriction (see Table 4). Gain of 1q21 and 11q23 as well as deletion 13q14 were associated with a higher plasma cell content (median of 11% versus 9%, p=0.002, 12% versus 9%, p=0.03 and 12% versus 9%, p=0.04, respectively).

In AL (groups 1+2) the association of t(11;14) with lacking intact immunoglobulin could also be shown (p<0.001). Gain of 1q21 was more frequent in AL patients who displayed an intact immunoglobulin (p=0.03). Detection of gain of 11q23 was associated with kappa light chain restriction (p=0.03). CA were not associated with serum free light chain levels.
Delineation of characteristic features of AL by multivariate analysis
A multivariate analysis was performed in order to identify the parameters which are characteristic for AL from the point of view of genetics and monoclonal gammopathy and which set it apart from other plasma cell dyscrasias. The following variables were included as possible explanatory factors in the multivariate logistic regression model for development of an AL phenotype: the five major CA, namely gain of 11q23 and 1q21, deletion of 13q14, t(11;14) and t(4;14), and the haematological parameters bone marrow plasmocytosis, light chain restriction and detection of intact immunoglobulin. The lack of an intact immunoglobulin and the preponderance of lambda light chain restriction maintained themselves as hallmarks of AL (p<0.001 and p<0.001, respectively). Neither the higher incidence of t(11;14) nor the lower frequency of t(4;14) in AL reached statistical significance in this model (p=0.91 and p=0.11, respectively). Thus none of the CA by itself could explain the phenotype of AL.

Lacking association of cytogenetic aberrations with clinical parameters in the amyloidosis group
Next we tested the association of CA of the AL cohort with clinical characteristics. The following clinical parameters were included: gender; age; number of affected organs; NT-proBNP values; involvement of heart, kidney, GI tract, liver, soft tissue; peripheral neuropathy, Karnofsky index and eligibility for high dose chemotherapy, the latter only being evaluated in patients younger than 70 years. Markedly, none of these clinical parameters showed a significant association with any of the CA (data not shown). CA therefore do not seem to influence the pattern of organ involvement in AL.

Discussion:
Our analysis of cytogenetic aberrations in AL revealed a strikingly high frequency of 47% for the translocation t(11;14), which was significantly higher than the 26% for our MGUS group and also exceeded by far the frequencies reported for symptomatic MM patients in previous studies, which range from 13% to 21%28-32. This high t(11;14) positivity in AL in our analysis is in tune with previous reports, which – though based on a lower patient number – detected this translocation in 16 / 29 (55%) and 9 / 24 (38%) of AL patients, respectively8,7. The overrepresentation of the t(11;14) translocation among patients with AL suggests that cyclin D1 up-regulation33,34 and disruption of the heavy chain locus might also be important pathogenetic mechanisms in AL. Indeed, 82% of patients with a
t(11;14) lacked an intact immunoglobulin in serum immunofixation. This finding in our study is reminiscent of the higher t(11;14) frequencies of 31%, 79% and 88% reported for light chain only, non-secretory and IgM myeloma, respectively, as compared to the lower frequencies in myelomas with an intact rearranged immunoglobulin, namely 15%, 10% and 22% for IgG, IgA and IgD in studies by Avet-Loiseau et al. 35,36. Whereas the high frequency of t(11;14) in AL accounts for the high rate of intact immunoglobulin deficiency, it is probably unrelated to the lambda light chain predilection in AL, which is another hallmark of AL from the point of view of monoclonal gammopathy. In AL, the kappa patients even had a slightly higher rate of t(11;14) than the lambda patients (57% vs. 41%).

Next we evaluated the association of t(11;14) with other CA. As shown in tree model and Fisher testing, t(11;14) in AL was frequently detected in combination with gain of 11q23, which has been identified as a critical genomic region for CA in MM 29 and other B-cell disorders 37. Since this could only rarely be detected in the MGUS group, the combination of t(11;14) and gain of 11q23 appears to be a specific feature of AL.

Translocation t(4;14), another IgH translocation, on which conflicting frequencies have been reported in AL so far 7,9, could only be detected in 4% of AL patients in our cohort. This was lower than that of our MGUS group with 12% and also inferior to frequencies reported for MM, which range from 11% to 17% 15,28,31,32,38.

Alike MGUS and MM, karyotypic instability was a recurring feature of AL. With our testing panel, 95% of AL patients displayed at least one CA and all major CA identified in MGUS were also detected in our AL group. Aberrations like t(14;16), gains of chromosomes 1q21, 11q23, and 19q13 and deletions of chromosomes 8p21, 13q14 and 17p13 were found at comparable frequencies in the AL and the MGUS group. Further similarities between the AL and the MGUS group were revealed by the clustering of CA based on the oncogenetic tree model 15. The association of t(11;14) with gain of 11q23 was more frequent in AL, but apart from this finding the tree models for the AL and the MGUS / MM I group widely overlapped. In addition to the t(11;14) and 11q23 branches, deletion of 13q14, t(4;14) and gain of 1q21 clustered together as a common branch. This association of deletion of 13q14 with t(4;14) and gain of 1q21 previously described in MM 15,39,40 could thus be confirmed in our AL and MGUS groups.

Markedly, gain of 1q21, which has been recognized as a prognostically unfavourable marker 39,40, was found at higher frequencies in the MM I groups both with and without AL (50% and 53%, respectively) as compared to the monoclonal gammopathy groups (19% and 15%, respectively). Our data therefore suggest that the concept of gain of 1q21 as a
progression marker characteristic of cytogenetic evolution during disease progression Applies to AL and MGUS alike. The similarities of cytogenetic patterns between AL and MGUS were also highlighted by our multivariate analysis, where none of the major CA proved statistically significantly associated with the AL phenotype in competition with hematological parameters. Furthermore, none of the CA had an influence on the tissue affinity of amyloid fibrils as also shown by Bryce et al or determined the pattern and the severity of organ involvement. This also suggests that the CA do not directly mediate amyloidogenicity.

In conclusion, the concept of distinct pathogenetic CA and karyotypic instability, which was developed for MM, is also applicable to AL. Accordingly, the clinical picture of AL is rather dictated by the amyloidogenic properties of the light chain than by karyotypic instability itself. This concept has also been referred to AL as “MGUS with an unlucky protein”. In this context, the low frequencies of t(4;14) and deletion of 17p13, which are considered to bring about a rapid progression of the plasma cell disorder, fit to the clinical course of patients; since complications in AL arise rather from the amyloidogenic potential of the secreted light chains than from progression into symptomatic MM. Vice versa the high frequency of t(11;14) in AL suggests that this translocation – though less aggressive – nevertheless sustains the proliferation of the aberrant plasma cell clone so that amyloidosis related symptoms prompt the detection of this “more benign” gammopathy which otherwise may not have become symptomatic so soon. In tune with this model, Fonseca et al – based on a higher frequency of t(11;14) in MGUS than in MM - have suggested that t(11;14) is negatively selected for progression from an early-stage plasma cell disorder to symptomatic MM. It will be very interesting to evaluate in future studies whether cytogenetic aberrations in AL are of prognostic value for achieving hematological remission after melphalan-based chemotherapy regimens.
Acknowledgement:

Contribution:

TB, SOS, UH, FC, AJ, HG designed research,
AJ, DH, FC, JB performed research,
UH, SOS, TB, AJ, FC collected data,
UH, SOS, TB, FC, AJ, MM, CB, ADH, HG analyzed and interpreted data,
CH, AB performed statistical analysis
UH; TB, SOS, CH drafted the manuscript.
All co-authors revised the manuscript.
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Conflict of interest disclosure: The authors declare no competing financial interests.
References:


32. Gutierrez NC, Castellanos MV, Martin ML, et al. Prognostic and biological implications of genetic abnormalities in multiple myeloma undergoing autologous stem cell transplantation: t(4;14) is the most relevant adverse prognostic factor, whereas RB deletion as a unique abnormality is not associated with adverse prognosis. Leukemia. 2007;21:143-150.


Table 1: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group 1 AL without MM I (n = 75)</th>
<th>Group 2 AL plus MM I (n = 10)</th>
<th>Group 3 MGUS (n = 127 *)</th>
<th>Group 4 MM I (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>62 (39 – 81)</td>
<td>52 (45 – 70)</td>
<td>59 (30 – 84)</td>
<td>65 (41 – 77)</td>
</tr>
<tr>
<td>Gender (male / female)</td>
<td>44 / 31</td>
<td>2 / 8</td>
<td>58 / 69</td>
<td>10 / 9</td>
</tr>
<tr>
<td>Light Chain (kappa / lambda)</td>
<td>18 / 57</td>
<td>3 / 7</td>
<td>80 / 46</td>
<td>8 / 11</td>
</tr>
<tr>
<td>Intact immunoglob. (yes / no)</td>
<td>34 / 41</td>
<td>7 / 3</td>
<td>122 / 5</td>
<td>19 / 0</td>
</tr>
<tr>
<td>Plasma Cell Content (median, range)</td>
<td>10% (2% - 26%)</td>
<td>17% (5% - 42%)</td>
<td>9% (1% - 28%)</td>
<td>28% (3% - 50%)</td>
</tr>
<tr>
<td>LC urine mg/d (median, range)</td>
<td>99 (&lt;5 – 850)</td>
<td>1368 (&lt;5 – 2457)</td>
<td>&lt;5 (&lt;5 – 871)</td>
<td>37 (&lt;5 – 1960)</td>
</tr>
<tr>
<td>MM stage I as defined by the value of serum M protein</td>
<td>0 / 75</td>
<td>0 / 10</td>
<td>0 / 127</td>
<td>11 / 19</td>
</tr>
</tbody>
</table>

Table 1 shows the patient characteristics specified according to the four groups: 1. AL amyloidosis without a concomitant multiple myeloma stage I, 2. AL amyloidosis plus a concomitant multiple myeloma stage I, 3. MGUS and 4. multiple myeloma stage I. Age and gender were statistically equally distributed between AL and MGUS, whereas lambda light chain restriction and intact immunoglobulin deficiency prevailed among AL patients.

*This group includes one patient with a gammopathy biclonal for kappa and lambda.
Table 2: Frequencies of the respective chromosomal aberrations

<table>
<thead>
<tr>
<th></th>
<th>Group 1 AL without MM</th>
<th>Group 2 AL plus MM</th>
<th>Group 3 MGUS</th>
<th>Group 4 MM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 75</td>
<td>n = 10</td>
<td>n = 127</td>
<td>n = 19</td>
</tr>
<tr>
<td><strong>IgH Translocations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgH breakapart</td>
<td>37 / 51 (73%)</td>
<td>4 / 7 (57%)</td>
<td>39 / 66 (59%)</td>
<td>10 / 12 (83%)</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>35 / 75 (47%)</td>
<td>3 / 10 (30%)</td>
<td>33 / 127 (26%)</td>
<td>5 / 19 (26%)</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>2 / 75 (3%)</td>
<td>1 / 10 (10%)</td>
<td>12 / 127 (9%)</td>
<td>5 / 19 (26%)</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>2 / 54 (4%)</td>
<td>0 / 7 (0%)</td>
<td>2 / 74 (3%)</td>
<td>0 / 12 (0%)</td>
</tr>
<tr>
<td>IgH rearrangement with unknown translocation partner</td>
<td>11 / 51 (22%)</td>
<td>2 / 7 (29%)</td>
<td>13 / 66 (20%)</td>
<td>1 / 11 (9%)</td>
</tr>
<tr>
<td><strong>Gains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1q21</td>
<td>14 / 74 (19%)</td>
<td>5 / 10 (50%)</td>
<td>16 / 109 (15%)</td>
<td>9 / 17 (53%)</td>
</tr>
<tr>
<td>11q23</td>
<td>25 / 75 (33%)</td>
<td>4 / 10 (40%)</td>
<td>42 / 126 (33%)</td>
<td>6 / 19 (32%)</td>
</tr>
<tr>
<td>19q13</td>
<td>8 / 55 (15%)</td>
<td>3 / 8 (38%)</td>
<td>20 / 79 (25%)</td>
<td>4 / 13 (31%)</td>
</tr>
<tr>
<td><strong>Deletions</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>8p21</td>
<td>4 / 55 (7%)</td>
<td>1 / 8 (13%)</td>
<td>5 / 77 (6%)</td>
<td>3 / 12 (25%)</td>
</tr>
<tr>
<td>13q14</td>
<td>23 / 75 (31%)</td>
<td>4 / 10 (40%)</td>
<td>39 / 127 (31%)</td>
<td>10 / 19 (53%)</td>
</tr>
<tr>
<td>17p13</td>
<td>0 / 74 (0%)</td>
<td>1 / 10 (10%)</td>
<td>5 / 124 (4%)</td>
<td>0 / 19 (0%)</td>
</tr>
</tbody>
</table>

Table 2 shows the frequencies of the respective CA specified according to the four patient groups. Whereas the AL amyloidosis group displays the highest frequency of t(11;14), the MM I group is characterized by a higher frequency of gain 1q21.

The determination of the IgH breakapart frequency is based on 136 patients, whereas the subgroups t(11;14), t(4;14) and t(14;16) are based on a larger cohort of 231, 231 and 147 patients, respectively. Due to the different size of the patients groups, the sum of the single translocations does not correspond exactly to the overall IgH breakapart frequency.
Table 3: Association of t(11;14) with hematologic parameters

<table>
<thead>
<tr>
<th>t (11;14)</th>
<th>Overall study population</th>
<th>AL amyloidosis series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient groups 1 – 4 (n = 231)</td>
<td>Patient groups 1 – 2 (n = 85)</td>
</tr>
<tr>
<td></td>
<td>t(11;14)</td>
<td>no t(11;14)</td>
</tr>
<tr>
<td>Intact Immunoglob. (immunofixation)</td>
<td>45 / 76 pts (59%)</td>
<td>137 / 155 pts (88%)</td>
</tr>
<tr>
<td>Light Chain Restriction (lambda)</td>
<td>43 / 75 pts (57%)</td>
<td>78 / 155 pts (50%)</td>
</tr>
<tr>
<td>Involved FLC concentration (median)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Plasma Cell Content (median)</td>
<td>10 %</td>
<td>10 %</td>
</tr>
</tbody>
</table>
Table 4: Association of gain of 1q21 with hematologic parameters

<table>
<thead>
<tr>
<th>Gain 1q21</th>
<th>Overall study population Patient groups 1 – 4 (n = 231)</th>
<th>AL amyloidosis series Patient groups 1 – 2 (n = 85)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gain 1q21</td>
<td>No gain 1q21</td>
</tr>
<tr>
<td>Intact Immunoglob. (immunofixation)</td>
<td>40 / 44 pts (91%)</td>
<td>122 / 166 pts (73%)</td>
</tr>
<tr>
<td>Light Chain Restriction (lambda)</td>
<td>34 / 44 (77%)</td>
<td>81 / 165 pts (49%)</td>
</tr>
<tr>
<td>Involved FLC concentration (median)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Plasma Cell Content (median)</td>
<td>11%</td>
<td>9%</td>
</tr>
</tbody>
</table>

Table 3 and 4 show the association of the CA t(11;14) and gain 1q21 with hematological parameters. Effects could be shown for the detection of an intact immunoglobulin, light chain restriction and the bone marrow plasmocytosis. Markedly, the strongest association was detected between the detection of t(11;14) and the lack of an intact immunoglobulin.

Legend to Figure 1

Figure 1: Clustering of chromosomal aberrations in the oncogenetic tree model:

Maximum likelihood tree models for the AL group (group 1+2) and MGUS group (group 3+4), based on the CA observed in 210 of the cases. The length of each horizontal edges $e$ is proportional to $-\log(p_e)$ [for example in group 1+2, the distance of deletion 13q14 to the next inner node is 0, which means that this aberration has always occurred at this node and precedes the translocation t(4;14)].

Bootstrap confidence values (in percent) for the inner edges are given. In group 1+2 the translocation t(11;14) was often observed together with gain of 11q23 (88.6%) in contrast to only 16% in group 3+4. Also, in 46.4% of bootstrap samples t(11;14) was individually separated from the other four aberrations in group 3+4.
Figure 1

AL (patient groups 1 + 2)  MGUS (patient groups 3 + 4)
Evaluation of the cytogenetic aberration pattern in amyloid light chain amyloidosis as compared to monoclonal gammopathy of undetermined significance reveals common pathways of karyotypic instability

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