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LETTER TO THE EDITOR

Cytogenetic aberrations in multiple myeloma are associated with shifts in serum immunoglobulin isotypes distribution and levels

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Multiple myeloma (MM) is characterized by secretion of monoclonal (M) protein from clonal plasma cells.[1] In most cases, intact immunoglobulins (Igs) consisting of heavy and light chains are secreted whereas in about 20% of cases only free light chains (FLCs) are secreted ('light chain only, LCO, MM').[1] MM is also characterized by a number cytogenetic abnormalities (CAs) categorized into primary (IgH translocations and hyperdiploid trisomies) and secondary events (other CAs). CAs may be used for MM risk stratification in clinical routine.[2-4] One study showed that cases with IgH translocations had higher FLC levels and abnormal FLC ratio.[5] Recently, prognostic significance was shown for isotype matched Ig ratios (e.g. IgGκ/IgGλ).[6-8]

As yet, the possible impact of CAs on Ig and FLC subtypes and their levels is poorly studied in MM. The hypothesis is that CAs are associated with the involved Ig isotypes in MM. We carried out a systematic analysis of Ig and FLC subtypes and their serum levels in relation with common CAs using data on 523 patients recruited in German-Speaking Myeloma Multicenter Group (GMMG)-MM5 trial.[9, 10] For purpose of replication, an independent cohort of 325 cases from GMMG-HD4 trial was obtained.[11, 12]

Blood samples were collected prior to treatment initiation in the above trials, coordinated by University Clinic Heidelberg. Patient characteristics are summarized in Online Supplementary Table S1; see details.[9, 10] Methods used to measure Ig concentrations and subtypes are described elsewhere[13] and in Supplementary material. CAs were identified for available samples using fluorescence in situ hybridization (FISH) techniques.[11] To assign translocation positivity, 10% or more of the tested affected cells had to demonstrate positive FISH test. All statistical analyses were performed using R software (version 3.2.3). Chi-square test of independence and Fisher's exact test were used to test equality of proportions of CA positive cases. Multivariable linear regression models were fitted to test the relationship between clinical variables and CAs. Covariates included in the models were international staging system (ISS), sex, light chain type, bone marrow cell count and secondary CAs.

Collection of patient samples and associated clinical information within both clinical trials was approved by the ethical review board of Heidelberg University, in accordance with the Declaration of Helsinki.

Table 1 depicts the proportion of CA positive cases in three major MM isotypes. In both cohorts, the most frequent CA was hyperdiploidy (57%). Proportions of three CAs varied significantly depending

upon MM type. t(4;14) was significantly higher in IgA MM compared with IgG MM (23% vs 6%; $p=1.1\times10^{-5}$) and LCO MM (23% vs 7%; p=0.02). Hyperdiploidy occurred more frequently in IgG MM and IgA MM compared with LCO MM (66% vs 32%; $p=1.3\times10^{-6}$ and 53% vs 32%; p=0.03, respectively). t(11;14) was most common in LCO MM compared with IgG MM (41% vs 19%; $p=6.0\times10^{-4}$) and IgA MM (41% vs 14%; $p=4.2\times10^{-4}$). In GMMG-HD4 cohort, we replicated association of t(4;14) with IgA MM compared to IgG MM (28% vs 10%; $p=3.3\times10^{-3}$) and association of hyperdiploidy with IgG MM compared to LCO MM (66% vs 35%; $p=6.6\times10^{-4}$). t(14;16) did not influence MM isotype in either cohort. We further analyzed the association between CAs and MM isotypes by considering possible combinations of heavy-light chains. Similar patterns of differences in the proportions were observed across heavy-light chain matched isotypes, as noticed above, for three CAs. Further, isotype matched pairwise comparisons showed significant differences in proportions of three CAs for the IgGκ and IgGλ pair (Online Supplementary Table S2). In GMMG-HD4 cohort, directions of changes remained but the case numbers were small. No such differences were observed in the isotype matched pairwise comparisons for IgA and LCO MM (data not shown). These results indicate that the above noticed association between CAs and Ig isotypes is not influenced by the light chain type.

Table 2 shows median concentrations of serum M-protein and rFLC (κ/λ ratio) in CA positive and negative patients for IgG MM and IgA MM. M-protein levels were systematically increased in t(4;14) positive patients compared with t(4;14) negative patients for both IgG MM and IgA MM. Statistical significance was reached for IgA MM alone (p=0.01; beta=14.36) after adjusting for the covariates (see above). This observation was further supported by a significant positive correlation between percentage of t(4;14) harboring plasma cells and M-protein level for IgA MM (Pearson correlation coefficient, r = 0.46; $p=2.6\times10^{-4}$). There was a consistent negative relationship, of borderline significance, between M-protein level and hyperdiploidy status for IgG MM and IgA MM (p=0.13; beta=-3.86 and p=0.04; beta=-8.23, respectively). Differences in M-protein level were smaller and insignificant for the remaining five CAs. The rFLC values were outside the reference range (0.26–1.65) for all cases and, with one exception, they were above the reference range, i.e., ratios shifted in favor of κ chains. Significant differences in rFLC were found for gain 1q21 (p=0.01; beta=-0.31) in IgG MM but not in IgA MM (p=0.83; beta=0.05).

The median M-protein levels were significantly higher in IgG MM cases compared with IgA MM (36.0 g/l vs 32.3 g/l; $p=2.2\times10^{-3}$; Online Supplementary Table S3). Consistently, in the GMMG-HD4

cohort, the level in IgG MM was also higher compared with IgA MM (42.0 g/l vs 35.8 g/l; $p=4.0\times10^{-4}$). No differences in M-protein levels were observed across the three IgH translocation types ($p>0.05$). For IgA MM, median M-protein levels in patients with any IgH translocations was more than doubled the median M-protein level in hyperdiploidy group (42.4 g/l vs 19.0 g/l; $p=1.1\times10^{-4}$). The difference remained consistent, but smaller and of borderline significant in the GMMG-HD4 cohort (44.9 g/l vs 39.1 g/l; p=0.06).

The individual FLCκ levels were outside the reference range for 80% of cases, and for 63% cases exceeded upper limit (>19.4 mg/l; Online Supplementary Table S4). FLCλ levels were outside the reference range for 63% cases, and for 31% cases surpassed upper limit (>26.3 mg/l). Online Supplementary Table S4 shows median concentration of uninvolved Igs and FLCs by CA type for three MM isotypes. Practically all uninvolved IgG, IgA and IgM were below the reference values, indicating immunoparesis.[14] On the contrary, FLCκ level was above and FLCλ level was within the reference values. For IgG MM, t(11;14) positive cases had suppressed IgA and IgM levels (p=0.03; beta=-0.14 and p=0.02; beta=-0.13, respectively). Differences in FLCλ levels were observed for gain 1q21 in IgG MM (p=0.01; beta=0.20), thus explaining the above noticed differences in rFLC for this CA (Table 2). For IgA MM, IgG levels were significantly suppressed in cases positive for del(13q) or gain 1q21 (p=0.03; beta=-0.11 and $p=3.7\times10^{-3}$; beta=-0.15, respectively), but significantly elevated in hyperdiploidy cases (p=0.01; beta=0.13). Cases with gain 1q21 had IgM levels suppressed (p=0.01; beta=-0.19) while cases with del(13q) had FLCλ levels suppressed (p=0.04; beta=-0.34). For LCO MM, gain 1q21 positive cases had all the Igs levels significantly suppressed. Hyperdiploidy cases had IgA levels elevated (p=0.04; beta=0.21). Cases with t(11;14) had FLCκ levels suppressed (p=0.02; beta=-0.40) while cases with hyperdiploidy or del(17p) had elevated FLCκ levels (p=0.05; beta=0.36 and p=0.01; beta=0.78, respectively).

One main finding of this study was that two CAs showed significant associations with the involved Ig isotypes in both cohorts, hyperdiploidy with IgG MM and t(4;14) with IgA MM. An association was also found for t(11;14) with LCO MM but it did not reached statistical significance in the replication cohort (p=0.09) possibly due to a small sample size. In all these cases CA positivity contributed to higher proportions of the indicated isotypes. What consequences the detected shifts might have remains speculative. t(4;14) positivity was not only related to the increased proportion of IgA isotype but also showed a significant increase in the M-protein level (45.3 g/l vs 28.8 g/l) which was the highest measured median value for this isotype. Whether such high concentration might contribute to

the poor prognosis in t(4;14) remains to be settled. Moreover, future studies should focus on comparing the clinical outcomes of non-IgA and IgA t(4;14) patients.

In a previous study, higher rFLC was found in patients with t(14;16) or del(13).[5] We identified a significant suppression of rFLC in cases with gain 1q21 for IgG MM. M-protein levels in IgG MM were higher compared with those in IgA MM cases. This might be expected as IgG is the principal isotype in the blood and extracellular fluid whereas IgA is the principal isotype in secretions of mucus epithelium of the intestinal and respiratory tracts. The observation that M-protein levels were higher in any IgH translocation group compared with the hyperdiploidy group can possibly be attributed to the active production of M-protein in actively proliferating plasma cells driven by the translocated *cyclin D* genes.[15] Patients lacking hyperdiploidy had higher M-protein level compared with hyperdiploidy patients, although this difference was statistically significant for IgA MM alone.

In summary, our study provides strong evidence for a complex modifying role of at least two CA types on immunophenotype. We showed further that serum Ig and FLC levels were influenced by several CA types. Our study thus sheds new light on the role of CA as important component of MM pathogenesis influencing immunoglobulin isotypes and the serum Ig and FLC levels.

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AUTHOR CONTRIBUTIONS

PY analyzed the data; HG, MM, EKM collected and organized the German patient data; AJ was responsible for cytogenetics; KH, AF and HG designed the study; KH and PY wrote the first manuscript draft; all authors contributed by comments and approved the final manuscript.

CONFLICT OF INTEREST

Authors declare that there are no competing financial interests.

ADDITIONAL INFORMATION

The online version of this article contains a data supplement.

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TABLES

Table 1. Proportion of positivity for cytogenetic abnormalities (CAs) across multiple myeloma (MM) isotypes, IgG, IgA and LCO.

CA	Proportion				P for comparison		
	All cases*	**IgG MM**	**IgA MM**	**LCO MM**	**IgG, IgA**	**IgG, LCO**	**IgA, LCO**
GMMG-MM5	**N=523**	**N=318**	**N=106**	**N=90**			
t(4;14)	0.09 (44/475)	0.06 (17/294)	0.23 (22/97)	0.07 (5/76)	**1.1×10^{-5}**	1.0	**0.02**
t(11;14)	0.22 (100/461)	0.19 (55/288)	0.14 (13/95)	0.41 (29/71)	0.90	**6.0×10^{-4}**	**4.2×10^{-4}**
t(14;16)	0.06 (12/197)	0.06 (7/123)	0.04 (2/45)	0.08 (2/25)	1.0	1.0	1.0
Hyperdiploidy	0.57 (263/458)	0.66 (187/285)	0.53 (50/94)	0.32 (23/72)	0.13	**1.3×10^{-6}**	**0.03**
GMMG-HD4	**N=325**	**N=191**	**N=74**	**N=55**			
t(4;14)	0.14 (41/295)	0.10 (17/173)	0.28 (18/65)	0.11 (6/53)	**3.3×10^{-3}**	1.0	0.15
t(11;14)	0.19 (57/296)	0.19 (33/174)	0.12 (8/65)	0.30 (16/53)	0.93	0.36	0.09
t(14;16)	0.02 (5/284)	0.01 (2/166)	0.02 (1/63)	0.04 (2/51)	1.0	0.72	1.0
Hyperdiploidy	0.57 (162/286)	0.66 (111/169)	0.48 (30/62)	0.35 (18/51)	0.08	**6.6×10^{-4}**	0.69

Proportion: each entry represents proportion of CA positive cases. In parentheses are shown the number of CA positive cases and the number of cases tested for this CA.

N: total number of MM cases with this MM isotype.

P: Bonferroni corrected p value from a Chi-square test of pairwise comparisons of proportions in the three groups, namely, IgG, IgA and LCO for each CA. Fisher's exact test was used when the expected count was less than 5.

Bold types: significant differences ($P \leq 0.05$) are shown in bold.

*All cases included additional 9 and 5 cases in GMMG-MM5 and GMMG-HD4, respectively, with involved Ig isotypes as IgM or IgD.

LCO: light chain only.

Table 2. Association between cytogenetic abnormalities (CA) and concentration of serum M-protein and rFLCs (κ/λ ratio) in IgG MM and IgA MM.

CA	Laboratory parameter	Reference range*	Median (m_1, m_2)	Cases (n_1, n_2)	BETA	P
IgG MM (N=318)						
t(4;14)	M-protein (g/l)	-	50.5, 35.3	17, 277	7.68	0.13
	rFLC	0.26–1.65	21.8, 14.8	17, 274	0.04	0.88
t(11;14)	M-protein (g/l)	-	40.2, 35.6	55, 233	2.24	0.48
	rFLC	0.26–1.65	3.2, 17.2	54, 231	0.04	0.81
t(14;16)	M-protein (g/l)	-	34.5, 36.1	7, 116	-3.27	0.74
	rFLC	0.26–1.65	0.01, 30.2	7, 116	-0.49	0.25
gain 1q21	M-protein (g/l)	-	37.4, 34.5	100, 189	2.49	0.34
	rFLC	0.26–1.65	**7.2, 21.2**	100, 186	-0.31	**0.01**
Hyperdiploidy	M-protein (g/l)	-	34.0, 40.2	187, 98	-3.86	0.13
	rFLC	0.26–1.65	19.3, 7.3	186, 96	0.08	0.52
del(13q)	M-protein (g/l)	-	35.4, 35.9	123, 172	-2.97	0.25
	rFLC	0.26–1.65	9.6, 20.3	122, 170	0.04	0.75
del(17p)	M-protein (g/l)	-	29.2, 36.4	30, 266	-4.57	0.25
	rFLC	0.26–1.65	98.2, 13.0	30, 263	0.26	0.16
IgA MM (N=106)						
t(4;14)	M-protein (g/l)	-	**45.3, 28.8**	22, 75	14.36	**0.01**
	rFLC	0.26–1.65	23.2, 5.02	22, 74	0.10	0.75
t(11;14)	M-protein (g/l)	-	28.3, 33.8	13, 82	-1.64	0.80
	rFLC	0.26–1.65	9.8, 6.9	13, 81	-0.27	0.45
t(14;16)	M-protein (g/l)	-	3.7, 35.1	2, 43	-22.64	0.16
	rFLC	0.26–1.65	9.9, 7.2	2, 43	-1.17	0.14
gain 1q21	M-protein (g/l)	-	35.3, 30.4	54, 41	0.45	0.92
	rFLC	0.26–1.65	3.8, 13.5	53, 41	0.05	0.83
Hyperdiploidy	M-protein (g/l)	-	**29.2, 36.5**	50, 44	-8.23	**0.04**
	rFLC	0.26–1.65	9.8, 4.6	49, 44	-0.05	0.83
del(13q)	M-protein (g/l)	-	35.6, 27.8	53, 44	4.68	0.27
	rFLC	0.26–1.65	9.7, 5.0	52, 44	0.30	0.21
del(17p)	M-protein (g/l)	-	34.6, 32.3	13, 84	7.44	0.23
	rFLC	0.26–1.65	10.8, 6.9	13, 83	0.08	0.82

m_1, m_2: median concentration of serum M-protein and rFLC in CA positive and negative cases, respectively.
n_1, n_2: number of CA positive and negative cases, respectively.
BETA, P: estimate of coefficient and p value for CA in a multiple linear model involving laboratory parameter (e.g. M-protein and rFLC) as dependent variable and CA, international staging system (ISS), sex, light chain as independent variables. Logarithmic transformation was performed for rFLC.
Bold types: median concentrations shown in bold for $P \leq 0.05$.
* Reference range as reported by the International Myeloma Foundation.
- implies not applicable.

SUPPLEMENTARY DATA

Cytogenetic aberrations in multiple myeloma are associated with shifts in serum immunoglobulin isotypes distribution and levels

Supplementary Methods

Serum Immunoglobulin measurements

The concentrations of serum intact immunoglobulins and free light chains (FLCs) were measured by a latex enhanced immunoassay on a Behring 2 nephelometer, except for IgG which was measured by electrophoresis. The reference ranges were used according to the manufacturer's instructions as follows: 3.3–19.4 mg/L for kappa light chain, 5.7–26.3 mg/L for lambda light chain and 0.3–1.6 for kappa/lambda ratio. The FLC test was considered positive when the criteria of both an abnormal FLC ratio and an elevation of the involved light chain above the respective upper range were met. Immunofixation of serum was performed using the Paragon Electrophoresis System kit by Beckman Coulter. In case of an ambiguous result, the immunofixation was repeated after prior denaturation with 2-mercaptoethanol and kits by Dako, Binding Site or Technoclon were applied additionally.

Supplementary Tables

Supplementary Table S1. Patient characteristics from GMMG-MM5 and GMMG-HD4 clinical trials.

	GMMG-MM5 (Total =523)	**GMMG-HD4 (Total=325)**
Male	312 (60%)	191 (59%)
Age (years)	58±8 (32–70)	55±7 (27–65)
ISS[a] (Stage 1/2/3)	197 (38%)/180 (34%)/146 (28%)	125 (41%)/105 (34%)/76 (25%)
Myeloma with involved heavy/light chain		
IgGκ	230 (44%)	154 (48%)
IgGλ	88 (17%)	37 (11%)
IgAκ	64 (12%)	36 (11%)
IgAλ	42 (8%)	38 (12%)
LCO-κ	56 (11%)	28 (9%)
LCO-λ	34 (6%)	27 (8%)
Others[b]	9 (2%)	4 (1%)

Age is presented as mean± standard deviation (range). LCO: light chain only disease; ISS: international staging system. [a] GMMG-HD4 had 19 missing values.

[b] included additional 9 patients (1 IgMλ, 1 IgDκ and 7 IgDλ) in GMMG-MM5 and 5 patients (1 IgMκ, 1 IgDκ, 2 IgDλ and 1 misssing) in GMMG-HD4.

Supplementary Table S2. Proportion of positivity for cytogenetic abnormalities (CAs) in IgG isotypes.

CA	Proportion		P
	IgGκ	IgGλ	
GMMG-MM5	**(N=230)**	**(N=88)**	
t(4;14)	0.06 (13/215)	0.05 (4/79)	1.0
t(11;14)	0.15 (31/210)	0.31 (24/78)	**3.7×10^{-3}**
t(14;16)	0.02 (2/96)	0.19 (5/27)	**5.6×10^{-3}**
Hyperdiploidy	0.70 (145/208)	0.55 (42/77)	**0.02**
GMMG-HD4	**(N=154)**	**(N=37)**	
t(4;14)	0.10 (14/142)	0.10 (3/31)	1.0
t(11;14)	0.18 (25/142)	0.25 (8/32)	0.48
t(14;16)	0.01 (1/135)	0.03 (1/31)	0.34
Hyperdiploidy	0.68 (94/138)	0.55 (17/31)	0.23

Proportion: each entry represents proportion of CA positive cases. In parentheses are shown the number of CA positive cases and the number of cases tested for this CA.
N: total number of cases with this MM isotype.
P: P value from a Chi-square test for comparison between isotype machted Igs (e.g. IgGκ vs IgGλ). Fisher's exact test was used when the expected count was less than 5.
Bold types: significant differences (P≤0.05) are shown in bold.

Supplementary Table S3. Concentrations of serum M-protein in IgG MM and IgA MM cases based on cytogenetic abnormalities (CAs), showing median values together with ranges.

CA	IgG MM		IgA MM	
	M-protein (g/l)	Number of cases	M-protein (g/l)	Number of cases
GMMG-MM5				
All cases	36.0 (1.5, 125.6)	318	32.3 (1.1, 81.8)	106
t(4;14)	50.5 (5.7, 125.6)	17	45.3 (7.8, 81.8)	22
t(11;14)	40.2 (3.5, 82.1)	55	28.3 (10.9, 60.1)	13
t(14;16)	34.5 (3.1, 102.9)	7	3.7 (2.4, 4.9)	2
any IgH	42.6 (3.5, 125.6)	67	42.4 (2.4, 81.8)	28
Hyperdiploidy	34.0 (1.5, 95.6)	84	19.0 (1.5, 79.2)	18
GMMG-HD4				
All cases	42.0 (0.6, 106.1)	191	35.8 (0, 73.0)	74
t(4;14)	54.1 (20.9, 90.5)	17	53.9 (0, 67.9)	18
t(11;14)	45.1 (3.1, 86.8)	33	44.9 (29.2, 54.5)	8
t(14;16)	37.0 (24.5, 49.5)	2	54.6 (54.6, 54.6)	1
any IgH	46.1 (3.1, 90.5)	39	44.9 (0, 73.0)	21
Hyperdiploidy	41.7 (3.5, 91.1)	93	39.1 (13.1, 72.7)	24

Supplementary Table S4. Concentration of serum uninvolved immunoglobulins and free light chains (FLCs) depending upon cytogenetic abnormalities (CAs) in IgG MM, IgA MM and LCO MM cases.

CA	Laboratory parameter	Reference range*	Median (m_1, m_2)	Cases (n_1, n_2)	BETA	P
IgG MM (N=318)						
t(4;14)	IgA (g/l)	0.7–3.8	0.4, 0.3	17, 275	0.14	0.17
	IgM (g/l)	0.4–2.8	0.2, 0.2	17, 275	0.07	0.47
	FLCκ (mg/l)	3.3–19.4	31.0, 89.5	17, 274	0.13	0.51
	FLCλ (mg/l)	5.7–26.3	8.6, 8.2	17, 274	0.10	0.52
t(11;14)	IgA (g/l)	0.7–3.8	**0.25, 0.33**	54, 232	-0.14	**0.03**
	IgM (g/l)	0.4–2.8	**0.16, 0.20**	54, 232	-0.13	**0.02**
	FLCκ (mg/l)	3.3–19.4	37.9, 99.3	54, 231	0.01	0.95
	FLCλ (mg/l)	5.7–26.3	9.9, 8.0	54, 231	-0.03	0.77
t(14;16)	IgA (g/l)	0.7–3.8	0.27, 0.34	7, 116	0.11	0.57
	IgM (g/l)	0.4–2.8	0.08, 0.20	7, 116	-0.14	0.38
	FLCκ (mg/l)	3.3–19.4	13.0, 124.5	7, 116	-0.13	0.74
	FLCλ (mg/l)	5.7–26.3	218.0, 6.9	7, 116	0.37	0.16
gain 1q21	IgA (g/l)	0.7–3.8	0.28, 0.33	99, 188	0.04	0.45
	IgM (g/l)	0.4–2.8	0.2, 0.2	99, 188	0.00	0.99
	FLCκ (mg/l)	3.3–19.4	23, 118	100, 186	-0.12	0.27
	FLCλ (mg/l)	5.7–26.3	**9.3, 7.4**	100, 186	0.20	**0.01**
Hyperdiploidy	IgA (g/l)	0.7–3.8	0.33, 0.26	187, 96	0.05	0.40
	IgM (g/l)	0.4–2.8	0.2, 0.2	187, 96	0.01	0.77
	FLCκ (mg/l)	3.3–19.4	107.5, 52.2	186, 96	-0.01	0.93
	FLCλ (mg/l)	5.7–26.3	7.4, 9.3	186, 96	-0.09	0.27
del (13q)	IgA (g/l)	0.7–3.8	0.28, 0.33	123, 170	-0.03	0.57
	IgM (g/l)	0.4–2.8	0.21, 0.18	123, 170	0.03	0.50
	FLCκ (mg/l)	3.3–19.4	43.9, 102.1	122, 170	-0.02	0.83
	FLCλ (mg/l)	5.7–26.3	8.5, 7.4	122, 170	-0.06	0.43
del (17p)	IgA (g/l)	0.7–3.8	0.4, 0.3	30, 264	-0.01	0.92
	IgM (g/l)	0.4–2.8	0.2, 0.2	30, 264	-0.02	0.79
	FLCκ (mg/l)	3.3–19.4	276.5, 62.6	30, 263	0.07	0.64
	FLCλ (mg/l)	5.7–26.3	5.6, 8.5	30, 263	-0.19	0.12
IgA MM (N=106)						
t(4;14)	IgG (g/l)	7.0–16.0	3.0, 3.6	22, 75	-0.10	0.10
	IgM (g/l)	0.4–2.8	0.17, 0.18	21, 75	-0.05	0.58
	FLCκ (mg/l)	3.3–19.4	31.4, 26.0	22, 75	-0.05	0.83
	FLCλ (mg/l)	5.7–26.3	8.0, 9.0	22, 74	-0.15	0.46
t(11;14)	IgG (g/l)	7.0–16.0	3.1, 3.5	13, 82	-0.08	0.26
	IgM (g/l)	0.4–2.8	0.1, 0.2	13, 81	-0.13	0.24
	FLCκ (mg/l)	3.3–19.4	24.6, 30.8	13, 82	-0.40	0.16
	FLCλ (mg/l)	5.7–26.3	6.4, 8.7	13, 81	-0.13	0.62
t(14;16)	IgG (g/l)	7.0–16.0	3.5, 3.3	2, 43	-0.09	0.65
	IgM (g/l)	0.4–2.8	0.1, 0.2	2, 42	-0.28	0.12
	FLCκ (mg/l)	3.3–19.4	16.5, 31.4	2, 43	-0.59	0.37
	FLCλ (mg/l)	5.7–26.3	3005.8, 8.4	2, 43	0.58	0.30
gain 1q21	IgG (g/l)	7.0–16.0	**3.0, 4.1**	54, 41	-0.15	**3.7×10^{-3}**
	IgM (g/l)	0.4–2.8	**0.17, 0.20**	53, 41	-0.19	**0.01**
	FLCκ (mg/l)	3.3–19.4	21.1, 31.7	54, 41	-0.13	0.51

	FLCλ (mg/l)	5.7–26.3	9.1, 8.5	53, 41	-0.17	0.34
Hyperdiploidy	IgG (g/l)	7.0–16.0	**4.2, 3.0**	50, 44	0.13	**0.01**
	IgM (g/l)	0.4–2.8	0.20, 0.17	49, 44	0.08	0.28
	FLCκ (mg/l)	3.3–19.4	32.8, 13.2	50, 44	0.19	0.30
	FLCλ (mg/l)	5.7–26.3	7.2, 8.8	49, 44	0.26	0.10
del (13q)	IgG (g/l)	7.0–16.0	**3.0, 4.2**	53, 44	-0.11	**0.03**
	IgM (g/l)	0.4–2.8	0.17, 0.20	52, 44	-0.09	0.24
	FLCκ (mg/l)	3.3–19.4	30.9, 21.2	53, 44	-0.07	0.74
	FLCλ (mg/l)	5.7–26.3	**7.8, 9.0**	52, 44	-0.34	**0.04**
del (17p)	IgG (g/l)	7.0–16.0	2.6, 3.5	13, 84	-0.02	0.80
	IgM (g/l)	0.4–2.8	0.1, 0.2	12, 84	-0.13	0.23
	FLCκ (mg/l)	3.3–19.4	10.3, 28.4	13, 84	0.03	0.93
	FLCλ (mg/l)	5.7–26.3	24.7, 8.3	13, 83	-0.08	0.74

LCO MM (N=90)						
t(4;14)	IgA (g/l)	0.7–3.8	0.3, 0.4	5, 71	-0.15	0.45
	IgG (g/l)	7.0–16.0	4.3, 4.8	5, 71	-0.18	0.07
	IgM (g/l)	0.4–2.8	0.12, 0.20	5, 70	-0.23	0.16
	FLCκ (mg/l)	3.3–19.4	2810, 486	5, 71	0.26	0.48
	FLCλ (mg/l)	5.7–26.3	11.2, 13.3	5, 71	0.08	0.80
t(11;14)	IgA (g/l)	0.7–3.8	0.4, 0.3	29, 42	-0.08	0.37
	IgG (g/l)	7.0–16.0	4.8, 4.6	29, 42	0.02	0.63
	IgM (g/l)	0.4–2.8	0.18, 0.19	28, 42	-0.08	0.31
	FLCκ (mg/l)	3.3–19.4	**437, 719**	29, 42	-0.40	**0.02**
	FLCλ (mg/l)	5.7–26.3	16.3, 11.4	29, 42	-0.11	0.45
t(14;16)	IgA (g/l)	0.7–3.8	2.0, 0.3	2, 23	-0.08	0.79
	IgG (g/l)	7.0–16.0	2.4, 4.9	2, 23	0.21	0.39
	IgM (g/l)	0.4–2.8	0.14, 0.21	2, 23	0.07	0.81
	FLCκ (mg/l)	3.3–19.4	6503, 633	2, 23	0.87	0.38
	FLCλ (mg/l)	5.7–26.3	2213.7, 11.2	2, 23	0.73	0.25
gain 1q21	IgA (g/l)	0.7–3.8	**0.2, 0.5**	25, 50	-0.29	**0.01**
	IgG (g/l)	7.0–16.0	**4.1, 5.0**	25, 50	-0.12	**0.03**
	IgM (g/l)	0.4–2.8	**0.1, 0.2**	25, 49	-0.21	**0.02**
	FLCκ (mg/l)	3.3–19.4	7, 733	25, 50	-0.22	0.25
	FLCλ (mg/l)	5.7–26.3	250, 11	25, 50	-0.18	0.25
Hyperdiploidy	IgA (g/l)	0.7–3.8	**0.3, 0.4**	23, 49	0.21	**0.04**
	IgG (g/l)	7.0–16.0	4.9, 4.3	23, 49	0.06	0.23
	IgM (g/l)	0.4–2.8	0.20, 0.18	23, 48	0.09	0.30
	FLCκ (mg/l)	3.3–19.4	**506, 534**	23, 49	0.36	**0.05**
	FLCλ (mg/l)	5.7–26.3	13.5, 11.2	23, 49	0.23	0.14
del (13q)	IgA (g/l)	0.7–3.8	0.4, 0.4	48, 27	-0.05	0.62
	IgG (g/l)	7.0–16.0	4.2, 6.1	48, 27	-0.09	0.11
	IgM (g/l)	0.4–2.8	0.17, 0.25	47, 27	-0.13	0.14
	FLCκ (mg/l)	3.3–19.4	496, 633	48, 27	0.22	0.27
	FLCλ (mg/l)	5.7–26.3	12.4, 11.5	48, 27	-0.07	0.65
del (17p)	IgA (g/l)	0.7–3.8	0.3, 0.4	7, 70	0.02	0.91
	IgG (g/l)	7.0–16.0	4.0, 4.9	7, 70	0.02	0.81
	IgM (g/l)	0.4–2.8	0.1, 0.2	6, 70	-0.12	0.43
	FLCκ (mg/l)	3.3–19.4	**2130, 464**	7, 70	0.78	**0.01**
	FLCλ (mg/l)	5.7–26.3	8.0, 13.4	7, 70	-0.11	0.64

m1, m2: median concentration of serum immunoglobulins and FLC in CA positive and CA negative cases, respectively.

n1, n2: number of CA positive and CA negative cases, respectively.
BETA, P: estimate of coefficient and p value for CA in a multiple linear model involving logarithmic transformed laboratory parameter as dependent variable and international staging system (ISS), sex, light chain type, bone marrow cell count and secondary CAs as independent variables.
Bold types: median concentrations shown in bold for $P \leq 0.05$.
* Reference ranges were as reported at the http://www.laborlexikon.de/Vision.htm or by the International Myeloma Foundation.
LCO: light chain only