Abnormal heavy/light chain ratio after treatment is associated with shorter survival in patients with IgA myeloma

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Immunoglobulin (Ig) heavy/light chain (HLC) assays enable the separate quantification of the different light chain types of each Ig class. We retrospectively analyzed the correlation of heavy/light chain ratio (HLCR) with clinical status and its impact on outcome in 120 patients with multiple myeloma (MM). Abnormal HLCR was seen more frequently in patients with poorer myeloma response, and it appeared to be more sensitive for detecting clonality in IgA myeloma compared to IgG myeloma after treatment. Among the 85 patients who achieved ≥VGPR, the patients remained HLCR abnormal were showed significantly shorter overall survival (OS) compared to those achieving a normal HLCR (not reached vs 55.5 months, $P = 0.032$). This correlation was seen in IgA myeloma patients (not reached vs 30.1 months, $P = 0.014$), but not in IgG myeloma patients when patients were analyzed separately. Univariate and multivariate analysis of factors that may affect survival identified abnormal HLCR at the best response as the only independent risk factor (hazard ratio, 4.7; 95% confidence interval, 1.4 – 15.26; $P = 0.012$) for shorter OS in this subset of patients. This study highlighted the HLC assay as a prognostic predictor in patients with IgA myeloma.

The serum immunoglobulin (Ig) heavy/light chain (HLC) assay is a newer method that enables separate quantification of the different light chain types of each Ig class, i.e., IgGκ, IgGλ, IgAκ, and IgAλ.[1] Heavy/light chain ratio (HLCR) is calculated with Ig κ as the numerator. Use of heavy/light chain assay could quantify the amount of M-protein more accurately compared to serum protein electrophoresis (SPEP) or nephelometry. In addition, heavy/light chain assay allows assessment of uninvolved Ig suppression of the same Ig class. This Ig matched pair suppression (HLC pair suppression) has been reported to be associated with poor prognosis of patients with monoclonal gammopathy of undetermined significance (MGUS),[2,3] newly diagnosed MM and relapse refractory MM.[4] However, the sensitivity for detecting clonality appeared different between IgA and IgG HLC assay.[1,5] Usability of HLC assay for monitoring of treatment response in MM patients remains to be explored. Although normalization of free light chain (FLC) κ and λ ratio has been reported to be associated with superior outcome among patients with MM after treatment,[6,7] such an association in HLCR has not been explored to date. In addition, data concerning the association between different IMWG response categories, FLC ratio, and HLC ratio after treatment are scarce. To investigate the clinical utility of HLC assay, we measured HLC ratio (HLCR) and FLC in relation to the IMWG responses in patients with IgG and IgA myeloma after treatment. We also analyzed the prognostic relevance of the normalization of HLCR at the best response in patients with MM.

Materials and Methods

A total of 304 frozen serum samples from 120 patients with MM treated at Kameda Medical Center (Kamogawa-shi, Chiba, Japan) and Kanazawa University Hospital (Kanazawa-shi, Ishikawa, Japan) at the times of various IMWG responses were collected. Among them, diagnostic samples were available for 97 patients. Because post-treatment samples were stored systematically since January 2010, only patients diagnosed MM after April 2009 and obtained the best response after 2010 were included in the survival analysis. Samples were analyzed using the HLC assay and the results were compared with SPEP, free light chain ratio (FLCR), immunofixation, total IgG, and total IgA results. SPEP and immunofixation electrophoresis (IFx) were performed on agarose media with densitometry scanning. Serum Ig and serum FLCκ and FLCλ were quantified by nephelometry (BN II; Dade Behring, Deerfield, IL, USA). Immunoglobulin HLC pairs (IgGκ/IgGλ and IgAκ/IgAλ) were measured by HLC immunoassays (Hevylite; Binding Site, Birmingham, UK) on an SPAPLUS turbidimeter (The Binding Site). Heavy/light chain ratio (HLCR) was calculated with Ig κ (G or A) as the

numerator and compared to 95% ranges for IgG and IgA (IgGκ: 3.84 – 12.07 g/L; IgGλ: 1.91 – 6.74 g/L; IgGκ/IgGλ: 1.12 – 3.21 and IgAκ: 0.57 – 2.08 g/L; IgAλ: 0.44 – 2.04 g/L; IgAκ/IgAλ: 0.78 – 1.94). HLC immunoassays were run either in The Binding Site (TBS, Birmingham, UK) or Medical and Biological Laboratory (MBL, Nagoya, Japan).

Uninvolved Ig suppression (systemic immunoparesis) was defined as a decrease of at least one uninvolved Ig polyclonal isotype below the lower limit of the normal range (Total IgG<7 g/L, IgA<0.7 g/L, and IgM<0.4 g/L). Response to treatment was assessed by the IMWG criteria.[8] High-risk cytogenetics was defined according to the recommendations of the IMWG for the definition of high-risk disease.[9] Written informed consent for sample analysis was obtained from all patients. The study was conducted in accordance with the institutional guidelines with approval from the Institutional Review Board of Kameda Medical Center and in accordance with the principles of the Declaration of Helsinki.

Statistical analysis. The probability of overall survival after best response in patients diagnosed after 2009 and obtained best response after 2010 was calculated using the Kaplan–Meier method, and compared using the log-rank test. Categorical values were compared by Fisher's exact test. The associations of time to death with variables were assessed by multivariate analysis with Cox's proportional hazard regression model. Agreement in response between HLCR and FLCR was studied using the κ coefficient.[10]

Results

Clinical characteristics of the patients. Table 1 shows the clinical characteristics of the whole patient population. There were 41 patients with IgA (20 for IgAκ, 21 for IgAλ) and 79 patients with IgG (55 for IgGκ, 24 for IgGλ). Patients were followed up for a median of 28.2 months (range: 1.2 – 82.8 months). The median age of the entire patient population was 73 years (range: 44 – 89 years). The proportions of λ isotype and severe renal impairment (creatinine > 2 mg/dL) were significantly higher in patients with IgA subtype ($P = 0.004$ and $P = 0.021$, respectively). Other clinical characteristics included age, sex, international staging system (ISS) ≥ 2, hemoglobin, lactate dehydrogenase (LDH), high-risk cytogenetics, treatment, the number of patients receiving autologous stem cell transplantation, and proportion of patients with each response category were not significantly different between patients with IgA and those with IgG.

HLCR, FLCR, and uninvolved Ig suppression at various IMWG responses. Table 2 shows the percentage normal FLCR and HLCR at various responses among the 304 samples during treatment. Only one sample in each response category per patient was used for the analysis. Among them, FLCR data on 11 samples were not available. Percentages of samples with normal FLCR at presentation, partial response (PR), very good partial response (VGPR), ≥ complete response (CR), and stringent CR (sCR) were 11.1%, 29.2%, 75.9%, 72.0%, and 100% for IgA MM and 9.8%, 36.2%, 91.4%, 88.9%, and 100% for IgG MM, respectively. Whereas, percentage of normal HLCR at presentation, partial response (PR), very good partial response (VGPR), complete response (CR), and stringent CR (sCR) were 0%, 0%, 27.6%, 92.3%, and 91.3%, respectively for IgA MM and 0%, 12.5%, 64.3%, 86.8%, and 84.3% for IgG MM, respectively. Percentage of normal FLCR was significantly higher compared to percentage of normal HLCR at PR and VGPR samples in patients with both IgA and IgG MM

Table 1. Patients characteristics

Characteristics	Total n = 120	IgA n = 41	IgG n = 79	P
Age, years, median (range)	73.5 (44–89)	76 (50–87)	71 (44–89)	0.196
Male sex (%)	56	24 (58.5)	43 (54.4)	0.702
Light-chain isotype				
Kappa (%)	75 (62.5)	20 (48.8)	55 (69.6)	0.004
Lambda (%)	45 (37.5)	21 (51.2)	24 (30.4)	
ISS ≥2	94	31	63	0.644
Hb <10 g/dL	70	25	45	0.701
Creatinine >2 mg/dL	16	10	6	0.021
LDH>normal	26	6	20	0.244
High risk cytogenetics† (%)	31 (27.0)	10 (25.6)	21 (27.6)	1
Treatment				
Exposed to bortezomib	118	41	77	0.546
Exposed to IMiDs	103	33	70	0.273
Auto SCT	34	10	24	0.529
Best responses				
sCR	49	18	31	0.697
CR	10	4	6	0.734
VGPR	26	9	17	1
PR	30	10	20	1
SD	5	0	5	0.164

†High risk cytogenetics denote del17p, t(4,14) and t(14;16) by FISH, and del13q on conventional karyotype analysis. Number of positive patients per examined were 8/115 (7%), 18/119 (15.1%), 2/117 (1.7%), and 4/116 (3.4%) for del17p, t(4,14), t(14;16),and del13q on conventional karyotype, respectively.

patients. Four of 36 (11.1%) and 6 of 61 (9.8%) of patients with IgA and IgG patients had normal FLCR even at presentation, respectively. None of the patients had normal HLCR at presentation either IgA or IgM. Although the percentages of normal HLCR at various responses were different between the samples of IgA and IgG, normalization of HLCR at PR and VGPR was more frequently seen in IgG MM compared to IgA MM (PR; 12.5% vs 0%, respectively, $P = 0.169$, VGPR; 64.3% vs 27.6%, respectively, $P = 0.004$) indicating lower sensitivity of HLC assay for detecting of clonality in samples from patients with IgG MM than from those with IgA MM. Table 3 shows the association between uninvolved Ig suppression and normalization of HLCR and FLCR. Uninvolved Ig suppression was seen in 223 samples (73.4%) at various IMWG responses. Samples with uninvolved Ig suppression had significantly higher percentages of abnormal HLCR and FLCR compared to those without uninvolved Ig suppression (73.9% vs 44.2%, respectively, $P = 0.002$ and 58.7% vs 27.5%, respectively, $P = 0.001$). We also looked at the agreement between uninvolved Ig suppression and the presence or absence of normal HLCR and FLCR after treatment. Number of samples with both HLCR and FLCR normal and HLCR and FLCR abnormal was 75 and 139, respectively. The agreement between HLCR and FLCR, therefore, was 73.0%. However, it should be noted that the percentage of the agreement depends on the selection of samples. If it contains many samples at

Table 2. Percentages of normal free light chain ratio (FLCR) and heavy/light chain ratio (HLCR) at various responses status in samples from IgA and IgG myeloma patients

Response	IgA			IgG		
	FLCR	HLCR	*P*	FLCR	HLCR	*P*
At presentation	11.1 (4/36)	0 (0/36)	0.115	9.8 (6/61)	0 (0/61)	0.028
PR or less	29.2 (7/24)	0 (0/24)	0.009	36.2 (17/47)†	12.5 (6/48)	0.009
VGPR	75.9 (22/29)	27.6 (8/29)	0.001	91.4 (32/35)†	64.3 (27/42)	0.006
≥CR	72.0 (18/25)†	92.3 (24/26)	0.075	88.9 (32/36)†	86.8 (33/38)	1.000
sCR	100 (18/18)	91.3 (16/18)	0.486	100 (32/32)	84.3 (27/32)	0.053

†FLCR was not available on 11 samples. CR, complete response; IgA, immunoglobulin A; IgG, immunoglobulin G; PR, partial response; sCR, stringent CR; VGPR, very good partial response.

Table 3. Associations between presence or absence of uninvolved immunoglobulin suppression and normal or abnormal heavy/light chain ratio (HLCR) and free light chain ratio (FLCR)

	Uninvolved Ig suppression	
	Yes (*n* = 230)	No (*n* = 104)
HLCR		
Normal (%)	60 (26.1)	58 (55.8)
Abnormal (%)	170 (73.9)	46 (44.2)
FLCR		
Normal (%)	95 (41.3)	65 (62.5)
Abnormal (%)	135 (58.7)	39 (37.5)

A total of 18 samples were excluded from the analysis due to the lack of data. Ig, immunoglobulin.

diagnosis or CR, an agreement will also increase and vice versa.

Relevance of abnormal HLCR on survival. As a majority of the patients that achieved less than PR response did not obtain normal HLCR after treatment (Table 2) and were expected to have shorter survival periods, we excluded patients with PR or less response and analyzed survival limited to the patients with VGPR or better. Therefore, the survival analysis was limited on the 85 patients diagnosed after 2009 and obtained ≥VGPR due to the availability of serial serum samples. Survival analysis was limited on OS because of the diverse treatments used. Maintenance therapy with different novel agents did not allow performing the progression-free survival (PFS) properly. Figure 1 shows the Kaplan–Meier curve of OS in 85 patients with ≥VGPR according to the presence or absence of uninvolved polyclonal Ig suppression. No significant difference in OS was observed between the patients with or without uninvolved Ig suppression of either IgG, IgA, or IgM and OS if they obtained ≥VGPR (median survival: not reached *vs* not reached, *P* = 0.859) (Fig. 1a). However, patients with HLCR remaining abnormal that achieved VGPR or better at their best response had significantly shorter survival compared to those achieving normal HLCR (median survival not reached *vs* 55.5 months, respectively, *P* = 0.032) (Fig. 1b). As the sensitivity of HLC assay for detecting clonality may differ between IgG and IgA MM [5], OS was analyzed according to the presence or absence of abnormal HLCR in patients with IgG and IgA myeloma separately. Survival of IgG MM patients with ≥ VGPR did not differ regardless of achievement of normal HLCR (median OS; not reached *vs* not reached, *P* = 0.453), while OS was significantly shorter in IgA MM patients without normal

HLCR compared to those with normal HLCR (median OS; 30.1 months *vs* not reached, *P* = 0.014) (Fig. 2a,b). These data clearly showed the different impact of abnormal HLCR on survival among the patients with IgG and IgA MM.

Univariate and multivariate analysis of HLCR and other clinical variables for survival. We next performed univariate and multivariate analyses of HLCR and other clinical variables for survival in IgA MM patients that obtained ≥ VGPR (Table 4). Only abnormal HLCR at best response significantly affected OS on univariate analysis and remained significant on multivariate analysis. Similar analyses were performed in IgG MM patients with ≥ VGPR, but none of the factors affected on survival on univariate and multivariate analysis (data not shown).

Discussion

Accurate quantification of monoclonal protein (M-protein) is essential for response assessment, management, and prognostic prediction in patients with multiple myeloma (MM). The International Myeloma Working Group (IMWG) defined very good partial response (VGPR) as ≥ 90% reduction of M-protein or disappearance of M-peak on serum protein electrophoresis (SPEP).[11] However, SPEP has limitations when M-protein comigrates into the β-fraction or M-protein fails to show a distinct sharp spike at low monoclonal Ig levels on densitometry.[12,13] In addition, judgment of the disappearance of the M-peak on SPEP is often arbitrary, and nephelometric measurement of Ig contains both involved and uninvolved Ig.

The present study was performed to assess the clinical utility of immunoglobulin heavy/light chain assay for patients with IgA and IgG myeloma at diagnosis and during follow-up after treatment. Although both HLC assay and FLC assay identify the monoclonal proteins through abnormal κ/λ ratio, FLC assay does not directly measure the intact monoclonal immunoglobulin whereas HLC assay directly measures the involved intact immunoglobulin of kappa and lambda subtype separately. The amount of intact monoclonal Ig is not necessarily proportional to that of involved monoclonal FLC in the considerable portion of patients with intact Ig myeloma (IIMM). Some patients show a high intact monoclonal Ig with extremely low involved FLC and vice versa. Our data comparing normal FLCR and HLCR showed the higher sensitivity of HLCR compare to FLCR for detecting intact monoclonal Ig (Table 2). HLC assay can better assess the clonality of involved Ig isotype than simple measurement of the total involved Ig. Therefore, it is expected to provide a more precise measure of the amount of monoclonal Ig after treatment, which is important for response assessment in patients with MM.
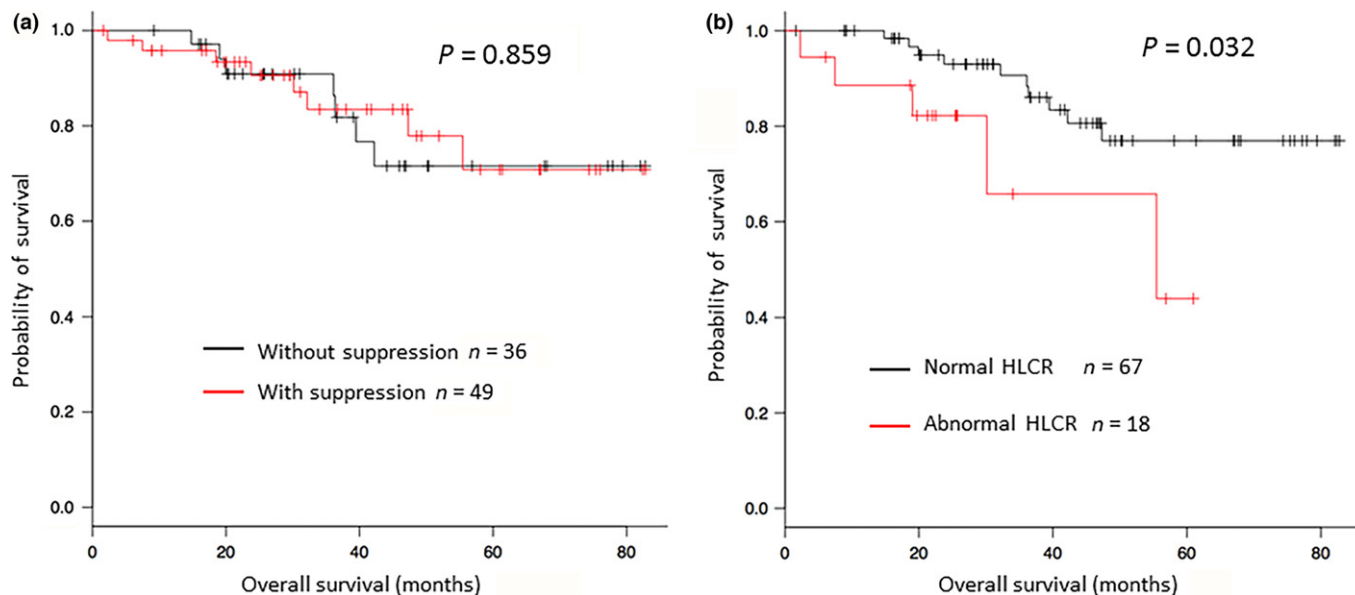
Fig. 1. Overall survival of patients with very good partial response (VGPR) or better according to the presence or absence of uninvolved polyclonal Ig suppression (a); and abnormal heavy/light chain ratio (HLCR) (b).
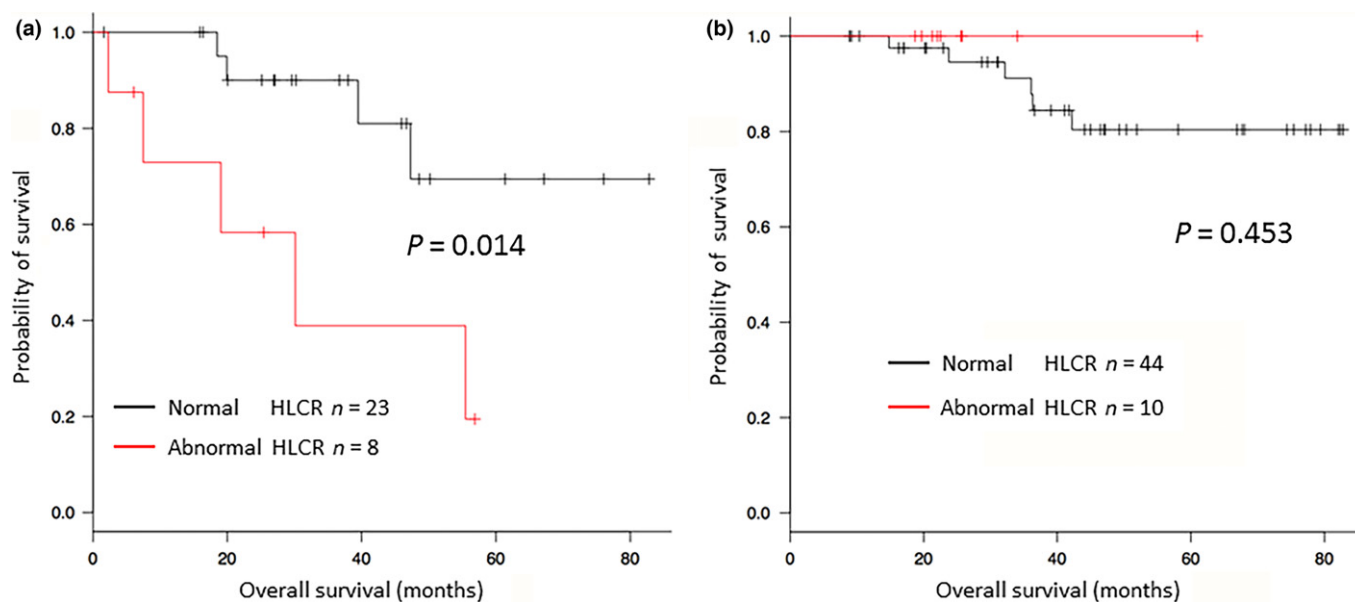

Fig. 2. Overall survival of IgG and IgA myeloma patients with very good partial response (VGPR) or better according to heavy/light chain ratio (HLCR) normalization (a) Patients with IgG myeloma; (b) Patients with IgA myeloma.

Markedly altered baseline HLCR has been shown to be of prognostic value, predicting progression in MGUS and smoldering MM, and survival in MM. Ludwig et al.[14] recently reported the prognostic impact of HLC-matched pair suppression in 203 patients with MM. Severe (> 50%) HLC-matched pair suppression at baseline, identified in 54.5% of 156 newly diagnosed patients, was associated with significantly shorter survival. This correlation was seen in IgG myeloma patients, but not in IgA myeloma patients. Harutyunyan et al.[15] also reported that abnormal HLC-paired suppression or abnormally elevated involved HLC levels were correlated the clinical responses after treatment. However, these studies did not examine the role of HLC assay focused on patients with good myeloma responses (i.e., ≥VGPR) after treatments. Residual

monoclonal components after therapy, especially in patients with a good response, would be more relevant to prognosis.

In the present study, we showed that normalization of HLCR occurred more frequently in patients with good response and was correlated with clinical response. Notably, 12.5% of PR and 64.3% of VGPR samples of IgG myeloma showed normal HLCR, while none of the PR and only 27.6% of VGPR samples of IgA myeloma showed normal HLCR. These observations indicated the difference in sensitivity of HLC assay between IgA and IgG myeloma. Bradwell et al.[1] suggested the lower sensitivity of IgG HLC assay for detecting monoclonal immunoglobulin compared to IgM and IgA HLC assay in their previous study. Recently, Katzmann et al.[5] showed that the sensitivity of HLCR was almost the same as that of

Table 4. Univariate and multivariate analysis of variables on overall survival among IgA-MM patients with ≥ very good partial response (VGPR)

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P*-value	Hazard Ratio	95% CI	*P*-value
Age > 75	3.14	0.631, 15.65	0.162	2.775	0.497, 15.480	0.245
Durie-Salmon stage III	1.84	0.226, 15	0.568			
ISS ≥ 2	2.59	0.318, 21.1	0.374	0.743	0.065, 8.486	0.811
Hb <10 g/dL	1.79	0.360, 8.868	0.477			
LDH >Normal	0.89	0.109, 7.251	0.913			
Creatinine >2 mg/dL	1.85	0.366, 9.339	0.457	3.214	0.484, 21.350	0.227
Uninvolved Ig suppression at best response	1.18	0.279, 4.939	0.826			
Abnormal HLCR at best response	8.79	2.043, 37.81	0.004	8.768	1.624, 47.340	0.012

iFLC, involved free light chain; ISS, International staging system.

SPEP for detecting the presence of clonal M-protein in IgG myeloma, while HLC assay was more sensitive than SPEP and could be substituted for SPEP and IFx in IgA myeloma. In line with their observations, our findings indicated the lower sensitivity of HLCR in IgG myeloma compared to IgA myeloma for detecting clonal M-protein after treatment.

However, patients with ≥VGPR and abnormal HLCR at their best response had significantly shorter survival compared to those with normal HLCR. This correlation was only seen in IgA myeloma patients and was not seen in IgG myeloma patients. This indicates that IgA MM patients with normalized HLCR had deeper response compared to those with abnormal HLCR. These observations were consistent with a previous study in patients with MGUS where abnormal HLCR was more frequent in patients with IgA MGUS compared to IgG MGUS (97% *vs* 56%, respectively).[2] The low sensitivity of HLC assay in IgG myeloma could be explained by the relatively large pool of polyclonal IgG compared to those of IgA and IgM. It is likely that a low concentration of monoclonal IgG would be less likely to results in abnormal HLCR, as suggested by Bradwell *et al.*[1] previously. We also speculated that patients with IgG myeloma could retain relatively larger numbers of normal polyclonal plasma cells even after treatment compared to those with IgA myeloma. These normal plasma cells may be less sensitive to anti-myeloma treatment.

In contrast to the abnormal HLCR, polyclonal Ig suppression in patients with ≥ VGPR at their best response did not have a prognostic impact in our study. Although the precise mechanism of polyclonal Ig suppression in myeloma patients remains speculative, our observations suggest that different mechanisms are responsible for suppression of isotype specific polyclonal Ig and delay of uninvolved HLC-pair Ig after treatment that could result in abnormal HLCR.

Univariate analysis of factors that may affect OS in patients with IgA myeloma that achieved VGPR or better indicated that age > 75 years and abnormal HLCR at best response were the significant predictor for shorter OS, but only abnormal HLCR at best response remained on multivariate analysis.

Our study was limited by the small sample size, its retrospective nature, and the lack of predefined schedules for response assessments. The variety of anti-myeloma therapies including the introduction of novel agents and use of maintenance therapies also limited the survival analysis.

In conclusion, HLC assay is useful for accurate monitoring of monoclonal protein in patients with myeloma. The results suggested that obtaining normal HLCR indicated a more favorable prognosis in patients with IgA myeloma, but not IgG myeloma, who achieved VGPR or better response. Although our data provided important insights for disease monitoring and prognostic prediction by the use of heavy/light chain assay in myeloma patients after treatment, further studies with more homogenous patient populations and treatment regimens will clarify the appropriate use of this assay.

Acknowledgments

Disclosure Statement

KE is an employee of The Binding Site. The other authors have no conflicts of interest.

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