Comparison of immunoglobulin free light chain (FLC), heavy chain/light chain (HLC) assays and immunofixation (IFE) in assessment of remission in multiple myeloma

Porównanie metod ilościowego oznaczania wolnych lekkich łańcuchów (FLC), Hevylite™ (HLC) i immunofiksacji (IFE) w ocenie remisji choroby w szpiczaku plazmocytowym

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Acta Haematologica Polonica; 43 (2a): 122–131

ABSTRACT

HLC separately measures in pairs light chain types of each intact immunoglobulin (Ig) class generating ratios of monoclonal Ig/non-involved polyclonal Ig concentrations; potentially simplifying assessment of monoclonal protein response. Normalization of FLC ratio is considered higher level of complete remission (CR) in multiple myeloma (MM). To compare IFE, HLC and FLC in assessment of remission we assayed sera from 44 MM patients who underwent autologous stem cell transplantation (ASCT). Of 44 patients in 26 (59%) after ASCT normalization of FLC ratio occurred. In 22 (84.6%) patients with normal FLC ratio also normalization of HLC ratio was noted but in 5 (19%) patients IFE was still positive. Concordance of three tests (IFE negative, HLC ratio normal, FLC normal) was found in 19 (73%) patients. In 21 of 44 (47%) patients after ASCT, normalization of FLC ratio documented sCR. In this group 5 (28%) patients relapsed. Median PFS was 24 months. In patients with CR, VGPR and PR 12 (60%) patients progressed. Median PFS was 12 months. One patient with IgAλ MM, sCR after ASCT and λ FLC clonal escape at relapse and at that time with negative IgAλ IFE and normal IgA HLC ratio illustrates the importance of serum FLC elevation for early detection of clinical relapse, in the absence of any other clinical and laboratory finding. Conclusion. IFE is more sensitive than HLC and FLC assays in detecting residual disease. CR with normalization of HLC and FLC ratios have impact on PFS.

Key words: Heavy/light chain assay, free light chains, multiple myeloma, minimal residual disease, remission

STRESZCZENIE

Test HLC pozwala na osobne oznaczanie ilościowe typów łańcuchów lekkich w parach każdej klasy immunoglobulinowej i oznaczanie stosunku stężenia monoklonalnej immunoglobuliny do stężenia poliklonalnej immunoglobuliny tej samej klasy, co potencjalnie upraszcza ocenę białkowej odpowiedzi monoklonalnej na leczenie. Normalizacja stosunku FLC w szpiczaku jest uważana za wyraz głębszej całkowitej remisji. Porównano skuteczność IFE, HLC i FLC w ocenie remisji u 44 chorych na szpiczaka poddanych autotransplantacji (ASCT). Spośród 44 chorych u 26 (59%) po ASCT wystąpiła normalizacja stosunku FLC. U 22 (84,6%) chorych z prawidłowym stosunkiem FLC wystąpiła także normalizacja stosunku HLC, ale u 5 (19%) chorych IFE była nadal dodatnia. Zgodność trzech testów (negatywna IFE, prawidłowy stosunek HLC, prawidłowy FLC) stwierdzono u 19 (73%) chorych. U 21 spośród 44 (47%) chorych normalizacja stosunku FLC po ASCT potwierdziła bezwzględną (*stringent*) CR. W tej grupie u 5 (28%) chorych wystąpiła progresja choroby. Mediana PFS wynosiła 24 miesiące. W grupie chorych z CR, VGPR i PR progresja wystąpiła u 12 (60%) chorych. Mediana PFS wynosiła 12 miesięcy. Jeden chory ze szpiczakiem IgAλ, sCR po ASCT i nawrotem wy-

Otrzymano: 14.03.2012
Zaakceptowano: 2.04.2012

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wołanym proliferacją klonu syntetyzującego λFLC i w tym czasie z IgAλ negatywną IFE i prawidłowym stosunkiem IgA HLC ilustruje wagę wzrostu FLC w surowicy we wczesnym wykrywaniu klinicznego nawrotu choroby, wzrostu FLC wyprzedzającego wystąpienie innych objawów klinicznych i laboratoryjnych nawrotu. Uzyskane wyniki wskazują, że IFE jest bardziej czuła niż testy HLC i FLC w wykrywaniu choroby resztkowej. CR z normalizacją stosunków HLC i FLC wpływa na PFS.

Słowa kluczowe: Hevylite, wolne łańcuchy lekkie, szpiczak plazmocytowy, choroba resztkowa, remisja

Introduction

Serum protein electrophoresis (SPE) and immunofixation (IFE) are routinely used methods of identifying, characterizing and quantifying monoclonal proteins. In multiple myeloma (MM), concentrations of monoclonal immunoglobulin by quantification of protein bands in SPE is considered the best biomarker for monitoring and evaluation of response to treatment. However, SPE measurements can be inaccurate depending on the migration of the band. IFE is a more sensitive method but is not quantitative. Evaluation of immunoglobulin heavy/light chain (HLC) ratios is a quantitative addition to IFE [1] HLC is a novel antibody based assay that binds the unique junctional epitopes between the heavy chain and light chain constant regions of intact immunoglobulin molecules. It separately measures in pairs the light chain types of each immunoglobulin class generating ratios of monoclonal immunoglobulin/background polyclonal immunoglobulin concentrations (i.e IgGκ/IgGλ, IgAκ/IgAλ and IgMκ/IgMλ); potentially simplifying assessment of monoclonal protein response [1–3]. Free light chain (FLC) assay measures circulating unbound κ and λ light chains and is of diagnostic and prognostic value in plasma cell disorders [4–7]. Normalization of serum FLC ratio is considered a higher level of complete response (CR) in MM and has been incorporated into the definition of stringent complete response (sCR) in the International Myeloma Working Group Uniform Response Criteria [8]. Kumar et al. [10] reported that normalization of serum FLC ratio is associated with superior overall survival among myeloma patients achieving IFE negative state. Recently Hari et al. [3] found that normalization of FLC ratio among patients with > very good partial response (> VGPR)

Table I. Serum free light chain (FLC) and heavy/light chain (HLC) IgA concentrations in IgAκ (cases 1–3) and IgAλ (cases 4–10) multiple myeloma patients with negative immunofixation after autologous stem cell transplantation (ASCT)

Tabela I. Stężenie wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgA w surowicy chorych na szpiczaka plazmocytowego IgAκ (przypadki 1–3) i IgAλ (przypadki 4–10) z negatywną immunofiksacją po przeszczepie autologicznych komórek macierzystych (ASCT)

Case	Date of ASCT	Date of serum sample	HLC IgA g/l		HLC ratio IgAκ/IgAλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ
			IgAκ	IgAλ				
1.	III 2009	VII 2010	0.39↓	0.50 N	0.78 N	18.8	32.3	0.58
2.	IX 2009	XII 2010	0.39↓	0.31 N	1.29 N	15.4	15.1	1.02
3.	XII 2010	III 2011	0.43 N	0.19↓	2.24 N	6.65	6.02	1.10
4.	VII 2009	III 2010	0.59 N	0.54 N	1.08 N	1.23	2.38	0.51
5.	VII 2009	IV 2010	0.52 N	1.00 N	0.58 N	19.2	20.6	0.93
6.	VII 2011	X 2011	0.40 ↓	0.19↓	2.12 N	6.90	8.63	0.80
7.	VII 2010	VIII 2011	0.16↓	0.07↓	2.36 N	1.45	1.87	0.78
8.	XI 2010	XI 2011	0.51 N	0.63 N	0.81 N	7.84	7.00	1.12
9.	III 2009	VIII 2009	0.93 N	0.94 N	0.98N	12.0	18.1	0.66
10.	V 2009	V 2010	1.53 N	0.96 N	1.59 N	7.99	16.6	0.48
Blood donor sera acc. to Bradwell et al. [2] min. and Katzmann et al. [12] max.			0.43 0.36	0.4 1.73	0.58 2.52	3.3 19.4	5.7 26.3	0.26 1.65

Table II. Serum free light chain (FLC) and heavy/light chain (HLC) IgG concentrations in IgGκ (cases 1-7) and IgGλ (cases 8–10) multiple myeloma patients

Tabela II. Stężenie wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgG w surowicy chorych na szpiczaka plazmocytowego IgGκ (przypadki 1–7) i IgGλ (przypadki 8–10)

Case	Date of ASCT	Date of serum sample	IFE	HLC IgG g/l		HLC ratio IgGκ/IgGλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ
				IgGκ	IgGλ				
1.	V 2010	VI 2010	–	5.56 N	3.10N	1.54N	8.55	9.52	0.90
2.	I 2011	V 2011	del	3.86↓	1.55↓	2.50 N	6.30	8.11	0.78
3.	II 2009	XII 2010	–	7.84 N	6.15 ↑	1.27 N	20.4	18.6	1.10
4.	IV 2009	I 2010	–	5.21N	2.52N	2.07 N	7.96	5.65	1.40
5.	III 2010	III 2011	del	6.19 N	2.94N	2.11 N	12.0	10.1	1.19
6.		VIII 2008	–	11.80 N	3.62 N	3.27 N	13.6	9.38	1.45
7.		XII 2011	–	3.86 ↓	2.64 N	1.46 N	11.60	21.2	0.55
8.	IV 2007	VII 2007	+	0.80 ↓	27.2 ↑	0.03 ↓	8.82	10.6	0.83
9.	XI 2010	VIII 2010 XI 2011	del del	4.76 N 5.77 N	4.55 N 5.64 N	1.07 ↓ 1.02 ↓	8.57 9.91	12.6 20.6	0.68 0.48
10.	IX 2009	V 2011	–	7.64N	6.24N	1.22N	17.9	13.8	1.30
Blood donor sera acc. to Bradwell et al. [2] min. and Katzmann et al. [12] max.				4.23 12.18	2.37 5.91	1.26 3.2	3.3 19.4	5.7 26.3	0.26 1.65

"–" means negative; del – delicate; N – normal

disease state did not impact progression free survival (PFS) suggesting that stringent CR criteria may need further validation.

The aim of the present study was comparison of IFE, HLC and FLC methods in assessment of MM remission and to answer the question whether CR with using the FLC ratio and HLC ratio criteria is prognostic for PFS or overall survival (OS).

Material and methods

We assayed fresh and stored sera from 44 MM (25 F, 19 M, median age 55 years, 29 IgG, 13 IgA, 1 IgD, 1 IgE, 2 Bence Jones, 1 nonsecretory; DS stage I 8, stage II 10, stage III 26) patients who underwent autologous stem cell transplantation (ASCT). All patients were diagnosed [11], treated and follow-up at the Institute of Hematology and Transfusion Medicine in Warsaw.

Serum protein electrophoresis and immunofixation were performed on agarose media with densitometric scanning using Hydrasys™ 2 apparatus (Sebia, France) and antisera from the same company. Nephelometric immunoglobulin assays, performed using a Siemens BN™ II nephelometer, were used to measure IgGκ/IgGλ, IgAκ/IgAλ (HLC) and also to quantify FLC in the fresh and archived frozen sera of assessed patients. In this method there were applied antibodies (Hevylite TM Human IgG Kappa Kit; IgG lambda Kit, Hevylite™ Human IgA Kappa Kit; IgA Lambda Kit; The Binding Site, Ltd, Birmingham, UK) specific for IgGκ, IgGλ, IgAκ, IgAλ, and antibodies (Freelite®; The Binding Site, Ltd Birmingham, UK) specific for κ and λ light chains in free form, not bound to the heavy chain.

Results

Of the 44 patients who underwent ASCT in 26 (59%) normalization of serum FLC ratio occurred. Comparisons of analyzed tests were made in this group of patients with normalization of FLC ratio. In 22 of these 26 (84.6%) patients with normal FLC ratio also normalization of HLC ratio was observed but in 5 (19%) patients IFE was still positive. Among 22 patients with normalization of HLC ratio in 5 (19%) IFE was slightly positive. Concordance of three tests (IFE negative, HLC ratio normal, FLC ratio normal) was found in 19 (73%) patients.

Table I illustrates concordance of serum IFE, HLC ratio and FLC ratio in individual 10 IgA MM patients. Table II presents serum FLC and HLC concentrations and ratios in individual 10 IgG MM patients. Table III and Table IV present results of serial sample analysis of HLC and FLC in 5 IgG MM patients and 1 light chain MM patient.

Serial samples analysis of the IgGκ MM patient (Tab. III case 1; Fig 1) showed persistent normalization of IgG HLC ratio during 41 months of follow-up, whereas IFE was constantly positive. Concordance between IgG HLC ratio and FLC ratio illustrates case 2 Table III; Figure 2. Partial discrepancy

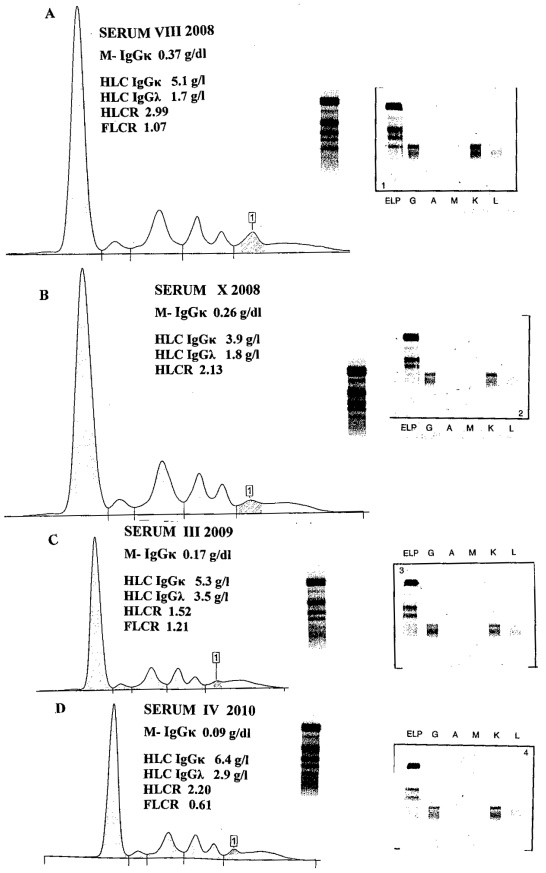

Fig. 1 Serum protein picture during 3-years follow-up after tandem ASCT in a patient with IgGκ MM. IgG HLC ratio and FLC ratio are normal while SPE and IFE constantly show M-protein IgGκ

Ryc. 1 Obraz białek surowicy w czasie 3-letniej obserwacji po tandemowej ASCT chorego na szpiczaka IgGκ. Stosunki IgG HLC i FLC prawidłowe, a IgGκ nadal wykrywalna w SPE i IFE.

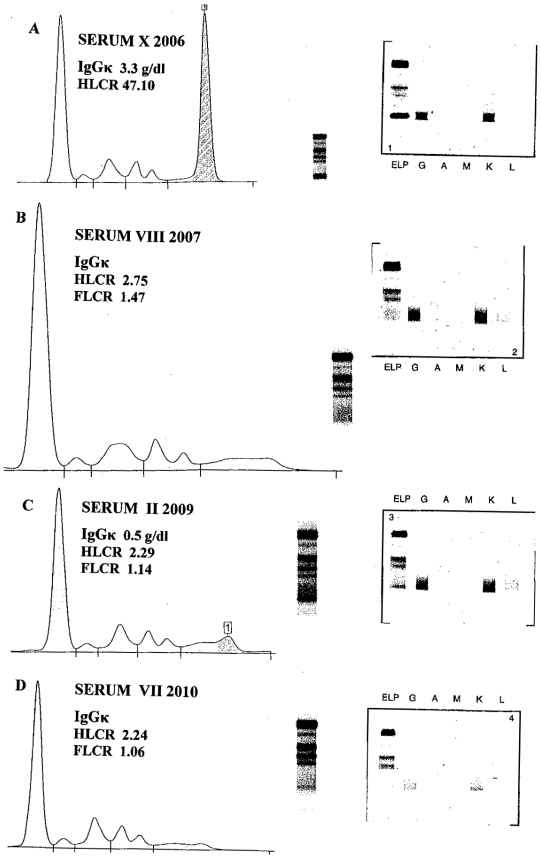

Fig. 2 Serum protein picture at IgGκ MM diagnosis (A) and at remission: after first ASCT (B), 100 days after second ASCT (C) and 20 months after second ASCT (D); A-IgGκ seen in SPE, IFE and HLC; BCD – IgGκ present in SPE and IFE while HLC ratio and FLC ratio are normal

Ryc. 2 Obraz białek surowicy w chwili rozpoznania szpiczaka IgGκ (A) i w czasie remisji po pierwszej ASCT (B), 100 dni po drugiej ASCT (C) i 20 miesięcy po drugiej ASCT (D). A – SEP, IFE, HLC obecna IgGκ; BCD – w SPE i IFE obecna IgGκ, a stosunki IgG HLC i FLC prawidłowe.

between results of IgG HLC ratio and FLC ratio illustrates case 3 Table III. In IgGλ MM patient (Tab. IV, case 2; Fig 3) 11 months after ASCT serum IFE revealed oligoclonal IgGκ; at that time values of IgG HLC ratio and FLC ratio remained normal. In one IgGλ MM patient (Tab. IV, case 3) during 5 years of observation after ASCT IgG HLC ratio was constantly abnormal while FLC ratio remained normal even at the time of disease progression which was revealed in bone marrow increase of plasma cells up to 30%. In one patient (Tab. IV case 1) at the time of disease diagnosis serum monoclonal protein was IFE mistakenly identified as IgGκ. Stored serum sample HLC and FLC analysis allowed to establish correct diagnosis of κ light chain MM.

In 21 of 44 (47%) MM patients after ASCT normalization of FLC ratio documented sCR. In this group 5 (28%) patients relapsed. Median PFS was 24 months (range 7–35). In the group of patients with CR, VGPR and PR (n=20), 12 (60%) patients progressed. Median PFS was 12 months (13–74). Three patients not responded. Median follow-up period after ASCT of patients with sCR is 22 months (7-64), of patients with CR, VGPR and PR 25 months (5–108) and of non-responders 9 months (3–25). So far one patient died (Tab. V).

Table VI and Figure 4 summarize results of investigation of a patient with IgAλ MM, sCR after ASCT and λ FLC clonal escape at relapse and at that time at relapse with serum negative IgAλ IFE, normal IgA HLC ratio and normal total IgA concentration 1.38 g/dl. Abnormal FLC ratio in this case was 9 months ahead of the disease progression that was revealed in IFE, bone marrow histology and appearance of osteolysis.

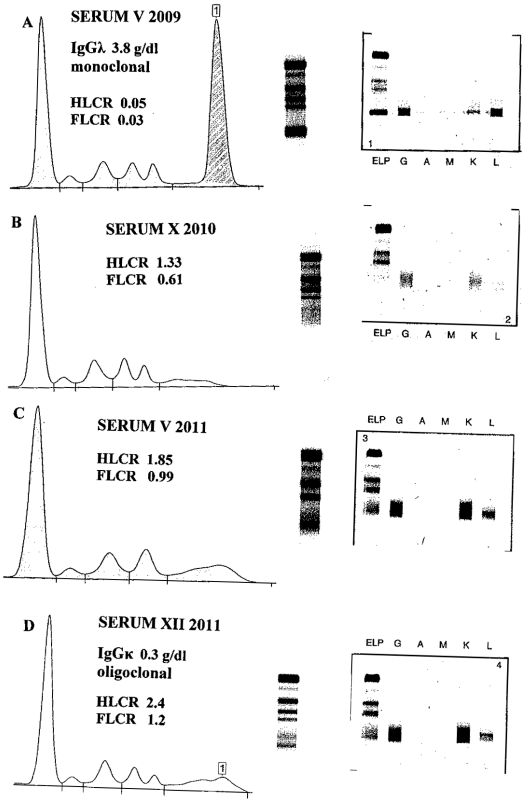

Fig. 3 Serum protein picture at IgGλ MM diagnosis (A) and at remission (BCD); A – SPE, IFE, HLC, FLC indicate presence of monoclonal IgGλ; BC – concordance of tests SPE, IFE, HLC and FLC; D- SPE, IFE show presence of oligoclonal IgGκ while HLC ratio and FLC ratio are normal

Ryc. 3 Obraz białek surowicy w chwili rozpoznania szpiczaka IgGλ (A) i w czasie remisji (BCD). A – SEP, IFE, HLC, FLC świadczą o obecności monoklonalnej IgGλ; BC – zgodność testów SPE, IFE, HLC i FLC; D – w SPE i IFE obecna oligoklonalna IgGκ podczas gdy stosunki HLC i FLC są prawidłowe.

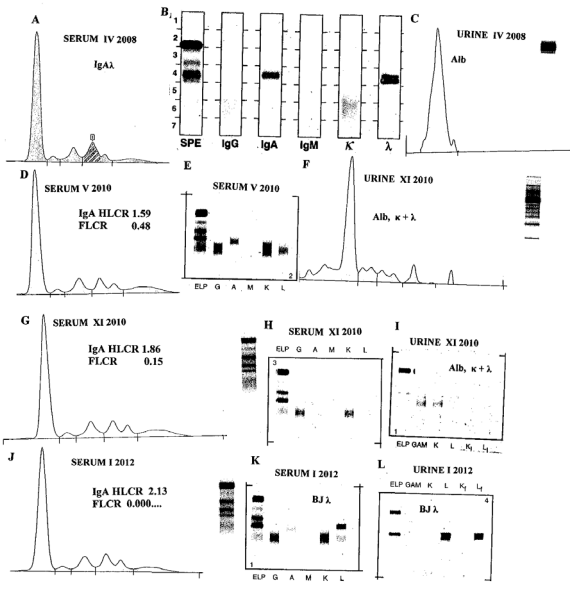

Fig. 4 Serum and urine protein pictures at IgAλ MM diagnosis (ABC), remission (DEFGHI) and relapse (JKL). ABC – in serum presence of IgAλ (AB), in urine albumins (C); DEF – monoclonal protein undetectable in SPE (DF) and IFE (E). GH – monoclonal protein undetectable in SPE (G), IFE (HI) and IgA HLC while FLC ratio is abnormal (first symptom of relapse); JKL – serum and urine IFE (KL) and FLC ratio indicate presence of lambda Bence Jones protein

Ryc. 4 Obraz białek surowicy i moczu w czasie rozpoznania szpiczaka IgAλ (ABC), remisji (DEFGHI) i nawrotu (JKL). ABC – w surowicy obecna IgA (AB), a w moczu albuminy (C); DEF – białko monoklonalne niewykrywalne w SPE (DF) i IFE (E); GH – białko monoklonalne niewykrywalne w SPE (G), IFE (HI) i IgA HLC, ale stosunek FLC jest nieprawidłowy (pierwszy zwiastun nawrotu); JKL – IFE surowicy i moczu [KL] oraz stosunek FLC świadczą o obecności białka monoklonalnego Bence Jonesa typu lambda.

Discussion

In our study in 22 (84.6%) patients with normalization of FLC ratio after ASCT, also normalization of HLC ratio was observed but in 5 (19%) of these patients IFE was still positive. In all IgA MM patients concordance of 3 tests (IFE negative, HLC ratio normal, FLC ratio normal) was found (Tab. I). Discrepancy between IFE and HLC and FLC results (IFE positive, HLC ratio normal, FLC ratio normal) was found in patients with IgG MM (Tab. II, III).

Avet-Loiseau et al. [13] compared the use of HLC assays to SPE and IFE for monitoring MM patients. Sequential archived sera from 156 patients enrolled onto the 2007–01 IFM trial were respectively analysed. Comparisons were made at complete response. At presentation HLC ratio was abnormal in 43/43 IgA and 112/112 IgG MM patients. Post-ASCT 92% of IgA patients were negative by SPE with 63% of patients achieving a CR; 55% of patients had a normal IgA HLC ratio. For IgG patients post-ASCT, 45% were negative by SPE with 26% of patients achieving a CR. 39% of patients had a normal IgG HLC ratio. They concluded "HLC ratio have a greater sensitivity than IFE for detection of minimal residual disease in IgA MM, but are slightly less sensitive in IgG MM" [13]. Decaux et al. [14] found that in 5 MM patients who achieved VGPR serial serum samples analysis showed persistent normalization of HLC ratio after 6–9 months of treatment, whereas IFE was still positive.

Olivero et al. [15] analysed sensitivity of the HLC in minimal residual disease (MRD) assessment in comparison with IFE, serum FLC κ/λ ratio and the six-color flow cytometry (FC) from bone marrow aspirations. Both serum and bone marrow samples (1–3 per patient) from 27 patients enrolled in the IFM 2008 trial (15 IgG, 8 IgA and 4 light chain MM), were analysed at 3 times: respectively, pre-stem cell transplantation (n=11, MRD 1 stage), post-ASCT (n=25, MRD 2 stages) and post-consolidation (n=23, MRD3). For 50 intact immunoglobulin MM samples,

Table III. Serum free light chain (FLC) and heavy /light chain (HLC) IgG concentrations in IgGκ multiple myeloma patients with positive immunofixation after autologous stem cell transplantation (ASCT)

Tabela III. Stężenia wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgG w surowicy chorych na szpiczaka plazmocytowego IgGκ z pozytywną immunofiksacją po przeszczepie autologicznych komórek macierzystych

Case	Date of ASCT	Date of serum sample	HLC IgG g/l		HLC ratio IgGκ/IgGλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ
			IgGκ	IgGλ				
1.		X 2006 at diagnosis	24.50↑	0.48↓	51.1↑			
	/1/ II 2008	VIII 2008	5.13 N	1.72 ↓	2.99N	9.65	8.99	1.07
	/2/ XII 2008	III 2009	5.33 N	3.52 N	1.52N	8.44	6.95	1.21
		IV 2010	6.48 N	2.94N	2.20N	9.77	15.90	0.61
		I 2012	3.92N	1.83	2.13N	11.4	22.30	0.51
2.		X 2006 at diagnosis	40.4↑	0.86↓	47.10↑			
	/1/ V 2007	VIII 2007	6.06 N	2.2↓	2.75 N	8.52	5.81	1.47
		II 2008	7.03 N	1.8↓	3.79↑			
	/2/ XI 2008	II 2009	8.59 N	3.7 N	2.29N	9.35	8.20	1.14
		X 2009	11.80N	2.0↓	5.88↑			
		VII 2010	3.41↓	1.5↓	2.24 N	9.77	9.23	1.06
3.		VI 2007 at diagnosis						
		I 2008	5.27N	2.46N	2.14 N	8.22	7.37	1.12
	VII 2008	VII 2010				10.90	6.20	1.76
		III 2011				13.30	7.11	1.88
		7 X 2011				53.70	7.56	7.11
		31 X 2011	10.60N	1.29↓	8.22↑	16.90	12.80	1.32
		XI 2011				6.97	8.71	0.80
		I 2012	7.51 N	1.36↓	5.53↑	1.81	1.55	1.16
		II 2012				16.30	10.00	1.63

FC and IFE showed almost the same sensitivity: 60 and 58% respectively. HLC and FLC were abnormal in 46% and 30% respectively. In details FC MRD1 analysis showed better sensitivity than IFE and HLC: 100% *vs* 80% and 70% respectively. They have not observed significative different sensitivities at MRD2 and MRD3 points between FC, IFE and HLC. FLC data showed the same low sensitivity at all points (30%). The HLC ratios were normal in 9 light chain MM samples while FC remained positive in 2.

Hari et al. [3] in order to assess the prognostic impact of HLC and FLC assays and to correlate them with SPE and IFE assessment, analysed 497 stored serum samples from patients enrolled in the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0102 clinical trial, sponsored by the NHLBI and NCI, of tandem autologous vs tandem autologous–allogeneic hematopoietic cell transplantation. Samples were collected prior to the first ASCT. HLC remission was defined as normalization of composite heavy and light chain ratios across all 3 measured heavy/light chain pairs or the normalization of clonal isotype with normal ratios of uninvolved pairs. Of the 211 patients with baseline SPE response better than or equal to a very good partial response (> VGPR), 188 also had an HLC remission (sensitivity = 89%). Comparison of the HLC remission with the > VGPR disease state also identified a specificity of 52%, positive predictive value (PPV) of 58% and a negative predictive value (NPV) of 87%. Similarly, all 56 patients in CR by SPE/IFE were in HLC remission. Compared with conventional CR assessment, sensitivity of HLC remission was 100%, specificity 39%, PPV and NPV values of 17% and 100% respectively. FLC remission correlated with > VGPR disease state had sensitivity of 47%, specificity of 81% and PPV and NPV of 64% and 67% respectively. It was found that there was a lower risk of treatment failure and superior PFS for patients who achieved an HLC remission. Normalization of FLC ratio among patients with > VGPR disease state did not impact PFS. These results indicate that abnormal HLC after induction therapy has a high

Table IV. Serum free light chain (FLC) and heavy/light chain (HLC) IgG concentrations in κ light chain (case 1) and IgGλ (cases 2, 3) multiple myeloma patients

Tabela IV. Stężenie wolnych łańcuchów lekkich (FLC) i związanych z łańcuchem ciężkim (HLC) IgG w surowicy chorych na szpiczaka plazmocytowego Bence Jonesa κ (przypadek 1) i IgGλ (przypadki 2, 3).

Case	Date of ASCT	Date of serum sample	HLC IgG g/l		HLC ratio IgGκ/IgGλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ
			IgGκ	IgGλ				
1.		V 2008 diagnosis	2.66↓	1.34↓	1.98N	11400	1.50	7620
		X 2008	3.40↓	1.55 ↓	2.19 N			
	XII 2008	III 2009	6.33 N	3.81 N	1.66 N	1.82	10.6	1.25
	VIII 2009 URD	XI 2009	3.71↓	1.67↓	2.23 N	1.82	1.84	0.99
		II 2010	5.99 N	2.94 N	2.04 N	8.34	11.50	0.73
		IX 2011	8.90 N	3.96 N	2.25 N	15.90	16.30	0.98
2.		V 2009	1.76↓	34.30↑	0.05↓	10.00	348	0.03
		X 2010	3.21↓	2.42 N	1.33 N	1.39	2.28	0.61
	I 2011	V 2011	8.28 N	4.47 N	1.85 N	14.8	15.0	0.99
		VIII 2011	9.79 N	4.14 N	2.37 N	25.5	19.0	1.34
		XII 2011	9.55 N	3.88 N	2.40 N	27.4	21.2	1.29
3.	III 2004	I 2006	5.86 N	5.38 N	1.09 ↓	12.7	13.1	0.96
		IV 2008	5.94 N	5.48 N	1.08 ↓	9.64	15.8	0.61
		IX 2009	5.59 N	6.20 ↑	0.90 ↓			
		VIII 2010	5.57 N	6.92 ↑	0.81↓	12.7	48.0	0.27

negative predictive value for identifying patients not achieving CR by uniform response criteria and is also associated with shorter PFS after transplant.

Koulieris et al. [16] reported that the depth of response correlated to PFS and MM patients in sCR, CR and nCR had a longer PFS than the others. HLC ratios normalization only was a strong parameter of increased PFS (p=0.016) after treatment at any line. Also Avet Loiseau et al. [17] found that increasingly abnormal HLC ratios correlated with shorter PFS in patients with intact immunoglobulin MM.

Preliminary results of our study and congress publications indicate that it is possible to monitor response to treatment using HLC ratio. HLC ratio may serve as a parameter for myeloma induced immunoparesis and serve as a new marker for validating remission deph and relapse probabilities [18–23] and as illustrates case reported by Willenbacher et al. [23] may serve as new diagnostic tool for rational treatment allocation, especially with respect to maintenance and consolidation strategies. However further studies are necessary to define more precisely the interest of this new assays among traditional methods [14].

In 21 of 44 our MM patients who underwent ASCT, normalization of serum FLC ratio confirmed sCR. PFS in this group of patients was 24 months vs. 12 months (p<0.07) in patients with CR, VGPR and PR (Tab. V). Our results suggest that CR with using the FLC ratio criteria is prognostic for PFS, as was reported by Kumar et al. [10]. Our study is too immature for overall survival analysis. However, so far 1 of 44 patients died. This suggests that FLC ratio have no impact on overall survival.

One patient with IgAλ MM, sCR after ASCT and λ FLC clonal escape at relapse and at that time with negative IgAλ IFE and normal IgA HLC ratio (Tab. VI, Fig. 4) illustrates the importance of serum FLC elevation for early detection of clinical relapse, in the absence of any other clinical and laboratory finding.

Relapse of disease might be associated with differential expansion of plasma cell clones producing intact immunoglobulin and/or FLCs. The term "light chain escape" is applied when patients with multiple myeloma whose tumor produced intact immunoglobulin at presentation, relapse with stable or falling intact immunoglobulin concentrations but rising FLCs. Case reports have suggested that "light

Table 5. Progression free survival (PFS) and duration of follow-up in multiple myeloma patients with various depth of response after autologous stem cell transplantation (ASCT)

Tabela 5. Czas wolny od progresji i czas obserwacji chorych po autotransplantacji z uwzględnieniem głębokości uzyskanej remisji

	Total number of patients	Number of patients with progression	PFS since ASCT median range (months)	Follow-up after ASCT median range (months)
Patients with sCR	21	6 (28%)	24 7–35	22 7–64
Patients with CR+VG-PR+PR	20	12 (60%)	12 3–74	25 1–108
Patients with no response	3		0	9 3–25

Table VI. Serum free light chain (FLC) and heavy/light chain (HLC) concentrations in a patient with IgAλ multiple myeloma at the time of remission after ASCT and with "clonal light-chain escape" at relapse.

Tabela VI. Stężenia wolnych łańcuchów lekkich (FLC) i HLC IgA w surowicy chorego na szpiczaka plazmocytowego IgAλ, w czasie remisji po ASCT i w czasie nawrotu choroby z „wymykającymi się łańcuchami lekkimi"

Date of MM diagnosis// ASCT	Date of serum sample	Bone marrow plasmocytes (%)	Osteolysis	M-protein acc. to IFE		HLC IgA g/l		HLC ratioIgAκ/IgAλ	κ FLC (mg/l)	λ FLC (mg/l)	FLC ratio κ/λ
				Serum	Urine	IgAκ	IgAλ				
IV 2008/ IV 2009	V 2010	3	-	-	-	1.530	0.960	1.59	7.99	16.60	0.48
	XI 2010			-	Alb; κ + λ	1.640	0.884	1.86	8.45	55.20	0.15
	III 2011			-	Alb; κ + λ 0.6 g/24h	1.500	0.706	2.13	10.80	179.00	0.06
	IV 2011			-	BJλ + alb; 1.5 g/24h				6.69	207.00	0.03
	XII 2011	15	+	BJλ	BJλ+alb				1.95	3170.00	0.00
	I 2012			BJλ	BJλ+alb				1.49	3990.00	0.000

chain escape" might be more prevalent with use of modern therapeutic agents and detected earlier by serum FLC analyses [22, 24– 26]. In a study of Koulieris et al. [27] light chain escape was observed during diseases course in 8 of 51 (15%) patients with intact immunoglobulin MM. Median time from onset of light chain clonal escape to clinical relapse was 6 months (2–11 months).

Dawson et al. [26] reported three patients with florid extramedullary relapse with a marked increase in serum FLC in the absence of a parallel rise in intact immunoglobulin – light chain escape from plateau phase. The most plausible explanation for the lack of heavy chain secretion seen in light chain escape from plateau phase is the accumulation of genetic mutations during clonal evolution that compromise the production of intact heavy chains. Mechanisms including lack of IgH mRNA transcription, instability or degradation of IgH mRNA or translation errors could underlie this loss of capacity. The case presented in table VI emphasizes the need to monitor intact immunoglobulin multiple myeloma patients with both serum protein electrophoresis/ immunofixation and serum FLC or urine protein analysis for objective assessment of treatment efficacy and to detect all forms of disease relapse.

In a study of Hassoun et al. [28] patients were treated with doxorubicin and dexamethasone for 2 or 3 months followed by thalidomide and dexamethasone for 2 months. The authors found that normalization of the serum FLC ratio after one or two cycles of treatment, which occurred in 8 of 37 patients, was significantly associated with the achievement of complete response or near complete response. Hajek et al. [29] found the utility of the serum FLC assay for early detection of resistance to bortezomib-based regimens. Serum FLC assay should be done in all patients who have achieved a complete remission to determine whether they have attained a stringent complete remission or residual disease [9, 30].

Acknowledgement

The authors thank The Binding Site Company Ltd. Birmingham, UK, Dr. B. Olszewska and Dr. D. Rutkowski from Biokom company for providing the reagents for Hevylite tests.

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