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**6th International Symposium on
Clinical Applications of
Serum Free Light Chain Analysis (plus Hevylite)**

Bath, UK, 23-24 September 2010

Guest Editor: A.R. Bradwell

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6th International Symposium on Clinical Applications of Serum Free Light Chain Analysis (plus Hevylite)

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Guest Editors

A.R. Bradwell

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ORAL COMMUNICATIONS

REVIEW LECTURES

THE MONOCLONAL PROTEINS: A BRIEF HISTORY

R.A. Kyle

Mayo Clinic, Rochester, MN, USA

Less than a decade after the introduction of the term "protein," Thomas Alexander McBean, 44 years of age and a highly respectable grocer of temperate habits and exemplary conduct, developed easy fatigue. He took a vacation in the country in September, 1844 to regain his strength, but while vaulting out of an underground cavern, he developed sudden pain in his chest. He was treated with a strengthening plaster to the chest because movement produced pain. He was subsequently treated by the removal of a pound of blood and the application of leeches. Mr. McBean was seen in consultation on October 30, 1845, by Dr. William Macintyre, a 53-year-old Harley Street consultant, and, because of mild edema Macintyre personally examined the urine. The specimen was acidic and had a specific gravity of 1.035. When heated it became opaque and cleared with boiling, but the precipitate reappeared when it cooled. He sent a sample of the urine to Henry Bence Jones, a 31-year-old physician at St. George's Hospital, who had already established a reputation as a chemical pathologist. Jones calculated that the patient excreted more than 60 g/day and concluded that the protein was "hydrated deuterioxide of albumen." Although Macintyre described the heat properties of the urine, Jones emphasized its place in the diagnosis of myeloma, for he said "I need hardly remark on the importance of seeking for this oxide of albumen in other cases of mollities ossium (softening of the bone)." In 1846, Johann F. Heller, an Austrian chemist, described a protein in the urine that precipitated when warmed a little above 50° C and then disappeared on further heating, but did not recognize the precipitation of the protein when the urine cooled. The term Bence Jones protein was first used in 1880 by Fleischer. It was recognized in Dr. Loos, the famous patient of Otto Kahler. Bradshaw, in 1898, reported that meals had little or no effect on the amount of Bence Jones proteinuria and that there was no nocturnal variation. Bence Jones proteinuria was found in the blood in 1917 by Jacobson who postulated that the Bence Jones protein was "dammed back" in the bloodstream because of renal failure. Five years after the discovery of Bence Jones protein in the blood, Stanhope Bayne-Jones and D.W. Wilson immunized rabbits by intravenous injection of Bence Jones protein. Two distinct groups of antibodies were recognized and designated, group I and group II. Korngold and Lipari in 1956 rediscovered the two classes of Bence Jones protein and noted that they also reacted with myeloma proteins. As a tribute to Korngold and Lipari, the two classes of Bence Jones protein were designated as kappa and lambda. Putnam and Hardy found

that Bence Jones protein was rapidly excreted and they thought that it came from the nitrogen pool rather than from a plasma or tissue protein precursor. One hundred seventeen years after the description of the unique heat properties by Macintyre, Edelman and Gally showed that the light chains prepared from an IgG monoclonal protein and the Bence Jones protein from the same patient's urine were virtually identical. Hilschmann and Craig showed that each light chain was divided into a variable or V region and a constant or C region. Hyperproteinemia was not recognized until 1928 when Perlzweig et al. described a patient with multiple myeloma who had 9-11 g/dL of globulin in the serum. Arne Tiselius became Theodor Svedberg's research assistant in 1925 and began his electrophoretic studies of proteins. Svedberg had realized that colloids could be studied not only with ultracentrifugation but through migration in an electric field, i.e., electrophoresis. Svedberg had noted that light rays were bent when they passed through a concentration gradient. This refractive index was used to identify the protein boundaries in a U-tube. Tiselius received his doctorate degree by describing the moving boundary method for investigation of electrophoresis of proteins in 1930. His manuscript describing the apparatus for electrophoresis was rejected by the Biochemical Journal and was subsequently published in the Transactions of the Faraday Society in 1937. That same year, he described the separation of serum globulins into three components which he designated as alpha, beta, and gamma. Tiselius and Kabat then localized antibody activity to the gamma globulin fraction of plasma proteins. Longworth applied electrophoresis to the study of multiple myeloma and demonstrated the tall, narrow-based peak. However, the Tiselius U-tube apparatus was cumbersome and difficult to use. The original commercial models were 20 feet long and 5 feet high. A single electrophoretic run required a full day and the efforts of an experienced operator. Immunoelectrophoresis was reported in 1953 and immunofixation 11 years later. The concept of monoclonal and polyclonal gammopathies was presented in the Harvey Lecture series by Jan Waldenström in 1961. Monoclonal proteins were characterized by the presence of a tall, narrow based spike. Although many of these patients had multiple myeloma or macroglobulinemia, others had no evidence of malignancy and were considered as having 'essential hypergammaglobulinemia' or "benign monoclonal gammopathy". The term monoclonal gammopathy of undetermined significance (MGUS) was introduced in 1978 because in some of these patients multiple myeloma, macroglobulinemia, AL amyloidosis or a related disorder developed during long-term follow-up. Waldenström regarded the broad band in hypergammaglobulinemia as a polyclonal increase in proteins. This simple distinction is extremely important because patients with a monoclonal gammopathy either have a malignant process (multiple myeloma, etc.) or may develop a neoplastic process whereas patients with a polyclonal gammopathy have an inflam-

matory or reactive cause of their hypergammaglobulinemia. Free light chains (FLC) were separated from the serum in 1975 using column chromatography, but was impractical for routine use. In 2001, immunoassays, based on polyclonal antibodies, were developed that could measure kappa and lambda FLCs in normal serum. The FLC assay is most useful when following a patient with no measurable M-protein in serum and urine. It also plays a role in screening for an M-protein and for risk of progression of MGUS, smoldering multiple myeloma or solitary plasmacytoma of bone. It is of prognostic importance in multiple myeloma and AL amyloidosis.

SERUM FREE LIGHT CHAIN (SFLC) GUIDELINES FOR MONOCLONAL GAMMOPATHIES

B.G.M. Durie

MD Cedars-Sinai Cancer Center, Los Angeles, CA, USA

The widespread availability of sFLC testing has allowed assessment of sFLC results in a broad range of disease settings. The sFLC results are part of the risk stratification for both MGUS and smoldering myeloma. Identification of high risk smoldering myeloma has become especially important because of new intervention strategies with, for example, Lenalidomide therapy. It is particularly important to identify patients at risk of progression within the first 18-24 months. For patients with newly diagnosed active myeloma, sFLC can be used as a baseline stratification factor. For the subset of patients with non-secretory or hyposecretory disease, the availability of sFLC testing has allowed entry into frontline randomized trials as well as into new drug trials for relapse/refractory disease. Patients with light chain myeloma, systemic amyloidosis and light chain deposition diseases are particularly amenable to assessment using sFLC. Several studies now address the comparisons between sFLC measurements and urine M-component quantitation. The occurrence and significance of elevated sFLC at relapse is also being assessed in part because of the apparent more frequent development of "light-chain escape" in patients previously treated with the novel agents, Thalidomide, Bortezomib, and Lenalidomide. Potential cross correlations between light-chain escape, new bone events, PET/CT and MRI findings and other marker changes are under investigation. Evaluation of sFLC changes during the monitoring process is a particular focus of attention both in terms of early response and relapse. Several studies have already addressed the pros and cons of using the sFLC ratio as an indicator of strict complete response (sCR). Ongoing comparisons help validate the role of sFLC ratio as a correlate of PFS and OS outcomes. The additional availability of "Hevylite" testing will provide an extra tool for both monitoring and very sensitive determination of the completeness of response. Accurate, routine documentation of minimal residual disease (MRD) remains a challenge post-transplantation and consolidation as well as a part of maintenance monitoring. It has become important to know the relative benefit of 1-2 years of maintenance versus, for example, indefinite maintenance until progression. Careful MRD monitoring becomes especially relevant in

such comparisons which also incorporate immunophenotyping and PCR. It can be anticipated that many new uses for sFLC testing and **Hevylite** will emerge and contribute to more accurate disease assessment.

SERUM FLC ASSAY: TECHNICAL CONSIDERATIONS

H.D. Carr-Smith

The Binding Site Group Ltd, Birmingham, UK

The clinical utility of the serum free light chain (FLC) assays in the diagnosis, monitoring and prognosis of B-cell dyscrasias has been extensively researched and this in turn has lead to a wide adoption of the assay within clinical laboratories. It is therefore quite correct that the technical performance of such a widely used assay should be scrutinised and in this study I want to examine some of the technical considerations that have been discussed in the literature and describe some recent work explaining and addressing the issues highlighted. Antigen excess (AgXS) is always an issue when measuring a monoclonal protein with a greater than 10,000-fold range. There has been one study investigating the frequency of AgXS and a number of case reports detailing examples. AgXS is not a frequent occurrence, in the one study investigating it's frequency on the BNII FLC assays, of 7538 consecutive samples, 9 (0.12%) kappa and 0 lambda results were subject to AgXS. However, because it is impossible to predict which samples may exhibit AgXS it is advisable for laboratories to have strategies for dealing with it and the product inserts suggest a strategy. The most satisfactory solution is to use automated antigen excess detection (AAED). AAED is available on the The Binding Site (TBS) SPAPLUS and the Roche Integra and recently TBS have implemented AAED protocols for FLC on the BNII (see other abstracts). Sometimes the apparent FLC measurement is higher than the total protein measurement or seem unfeasibly high compared to the band visualised by serum or urine protein electrophoresis. This has lead to questions regarding the accuracy of the FLC measurement in these situations. Investigations at TBS laboratories have shown that this is due to polymerisation of FLC that leads to an over estimation of FLC levels by the assays, perhaps due to the availability of multiple epitopes on the large polymeric molecules (see other abstracts). This analytical issue cannot be rectified using direct homogeneous latex enhanced assays. Alternative assay formats have been investigated and patent protection has been applied for on two novel FLC assay formats. Firstly, we have developed a novel nephelometric/turbidimetric assay format for the FLC assays utilising the inhibition of immunocomplex formation. This assay format is unsusceptible to AgXS and does not over estimate polymerised FLC. Secondly, a monoclonal gammopathy screening assay has been developed utilising the enzyme immunoassay format and allows the simultaneous measurement of FLC and **Hevylite**. This assay is not susceptible to AgXS or over estimation of polymeric FLC.

ROLE OF SFCL ASSAYS IN THE LABORATORY

J.A. Katzmann

Mayo Clinic, Rochester, MN, USA

The use of quantitative immuno-nephelometric assays for serum free light chain (FLC) has changed the approaches to identifying and managing monoclonal gammopathies. The FLC assay has been incorporated into screening panels, prognostic testing, and disease monitoring. The impact on clinical practice has been significant because of the increased sensitivity of quantitative serum free light chains for significant plasma cell proliferative diseases, better discrimination between aggressive and more benign premalignant disorders, a better assessment of hematologic response in monoclonal light chain diseases, and simplified sample requirements for patient testing. The changes for the laboratory have been significant because the introduction of this test procedure adds a new assay that must be integrated into the electrophoretic and nephelometric processes used for evaluating plasma cell dyscrasias, reduces the need for highly concentrated urine, and increases the need for consistent laboratory test protocols for monitoring FLC.

Diagnosis. The sensitivity of serum FLC κ/λ ratio for detecting imbalances in kappa and lambda FLC has increased the diagnostic sensitivity of serum assays for detecting monoclonal free light chains. This has decreased the need for urine as part of the diagnostic screening panel for monoclonal gammopathies. The recommended screening panel for detection of monoclonal gammopathies is serum protein electrophoresis, serum immunofixation electrophoresis (IFE), and serum FLC with urine studies only suggested if primary amyloidosis is suspected. In order to further simplify the screening panel, we've investigated the effect of also removing serum IFE from the screening panel, and retrospective data shows that serum PEL and FLC provide a simplified screening panel with little loss of diagnostic sensitivity. A screening panel of serum PEL and FLC is sufficient for detecting multiple myeloma and Waldenström's macroglobulinemia with only a slight decrease (1%) in sensitivity for pri-

mary amyloidosis. The use of serum PEL and FLC with the selective use of serum and urine IFE is therefore an efficient approach to identifying monoclonal gammopathies, but requires that clinical judgment be integral to ordering screening panels for detection of monoclonal gammopathies. For the laboratory, this means that urine protein electrophoresis will be predominantly performed for assessment of renal function and quantitation of urine M-spikes but that the need for highly concentrated urine for sensitive diagnostic testing is greatly reduced.

Prognosis. The serum FLC κ/λ ratio is also a useful prognostic marker for progression of MGUS to MM. MGUS is a common premalignant disorder with a progression rate of 1%/yr. Patients with a normal FLC κ/λ ratio (0.25-1.65), an M-spike <15g/L, and a gamma heavy chain represent approximately half of all MGUS patients. This group of patients has a 0.1%/yr risk of progression (2% lifetime risk), and these low-risk patients don't have to be monitored with laboratory assessments unless there are clinical signs of progression. Identification of low-risk patients will decrease medical costs as well as patient anxiety, and conversely, identification of high-risk patients will focus approaches to interfere with malignant transformation. For the laboratory, the identification of low-risk MGUS patients means that we need to change our recommendations for long-term follow-up.

Monitoring. The concentration of monoclonal FLC (involved FLC) has a role in disease monitoring. To date, that role has been restricted to patients with no measurable serum or urine M-spike, and to confirm a stringent complete response in MM. The long-term variability of serum FLC and urine M-spike measurements, however, indicate that the urine M-spike and serum FLC may provide comparable assessments and allow monitoring without the need for a urine sample. This may require a change in the guidelines for disease monitoring and response criteria. For the laboratory, this means additional requirements to perform "delta" checks on serial samples for FLC quantitation, reduce inter-assay variability, and eliminate variability due to dilution artifacts and antigen excess.

FREE LIGHT CHAINS AND THE KIDNEY

USE OF HIGH CUT-OFF HAEMODIALYSIS FOR TREATMENT OF MYELOMA KIDNEY: WORLDWIDE EXPERIENCE TO DATE

C.A. Hutchison

Clinical lecturer, Renal Institute of Birmingham, University of Birmingham, Birmingham, UK

Irreversible severe renal failure greatly increases the morbidity and mortality of patients with multiple myeloma.^{1,2} Once confounding factors, such as hypercalcaemia and non-steroidal anti-inflammatory drugs, have been excluded the principal renal pathology is myeloma kidney (cast nephropathy). This tubulointerstitial lesion occurs as a direct consequence of the high concentrations of circulating monoclonal immunoglobulin free light chains (FLCs). Recent work has demonstrated that an early reduction in serum FLC concentrations predicts the clinical outcomes of patients with myeloma kidney.^{3,4} Achieving this early reduction has therefore become a key focus in the management of these patients. Growing evidence suggests that novel chemotherapeutic agents, such as thalidomide and bortezomib, have a role for rapidly reducing FLC production in this setting and in uncontrolled studies have been associated with favorable clinical outcomes.^{5,6} The impact of effective chemotherapy alone on the serum concentrations of FLCs can however be limited by the prolonged serum half-lives of FLCs in the context of established renal failure.⁷ Therefore, strategies to directly remove FLCs from the serum have been developed to be used in combination with chemotherapy. Principally plasma exchange has been used, however, results have been disappointing.⁸ A mathematical model of FLC kinetics suggests this failure of plasma exchange relates to the inefficiency of the procedure to remove large quantities of FLCs.⁹ More recently haemodialysis using a dialysis membrane with a uniquely high molecular weight cut-off has been described as an alternative strategy.¹⁰ The capacity of high cut-off haemodialysis (HCO-HD), for FLC removal, is approximately 5 fold greater than plasma exchange. This new treatment is currently being evaluated by the European Trial of Free Light Chain Removal by Extended Haemodialysis in Cast Nephropathy (EuLITE),¹¹ a multi-centre randomised controlled trial based in the United Kingdom and Germany.

Study purpose. The current study was undertaken to describe how FLC removal by HCO-HD has been adopted into clinical practice internationally and report the clinical outcomes associated with its use.

Web-based electronic case report forms were used for data collection. Clinicians from centers across 13 countries were approached in an unselected manner. Data collection included basic demographic details, the patients' haematological and renal presentations, treatments received and clinical outcomes.

Results. In total 16 centres, across 9 countries enrolled 67 patients, between November 10, 2008 and September 24, 2009. The population was predominately male (62.7%)

and had a median age of 65.1 years (SD=10.01). The majority had new presentation of multiple myeloma (n=50, 74.6%). Nearly a third of the population had a light chain only myeloma (lambda 13.4%, kappa 16.4%), but the most frequent myeloma type was an intact IgG, with associated kappa or lambda FLCs (total 55.3%). The FLC type was closely distributed with 46% of patients having a kappa FLC and 54% a lambda FLC. Sixty percent of the patients had immunoparesis at presentation. Only 11 patients (16%) were known to have pre-existing chronic kidney disease. The majority of patients were assessed with a renal biopsy (56.7%), of these myeloma kidney was the predominant diagnosis (86.7%). The majority of the population received a chemotherapy regime including 'novel' chemotherapeutic agents, with 57.6% receiving a bortezomib based regime. On average patients received 12.5 (SD=8) HCO-HD sessions. Ninety-seven percent of the population received an extended dialysis schedule (greater than 4 hours) per session, predominately this was on one HCO dialyser not two. Dialysis related side-effects were reported in 6% of the patients.

Sustained reductions in serum FLCs. Forty one patients (61.2%) were reported to have a sustained reduction in serum FLC concentrations by day 12, and 42 patients (62.7%) by day 21. Of these 71% and 69% subsequently became independent of dialysis, respectively. Among patients who achieved a sustained reduction in serum FLC concentrations, 68.3% had a decline of greater than 50% on day 12 and 83.3% had a decline of greater than 50% on day 21.

Figure 1. Percentage of the population who became independent of dialysis and the reduction in serum FLCs at day 12.

Predictors of dialysis independence

The achievement of a sustained reduction in serum FLC concentrations by days 12 and 21 significantly increased the probability of becoming dialysis independent OR=29 ($p=0.002$) and OR=22.3 ($p=0.005$), respectively. Moreover, the probability of achieving dialysis independence increased linearly with increasing levels of reduction in FLC concentrations on day 12 (Figure 1). Using an unadjusted logistic regression model only the delay in initiating HCO-HD and the degree of FLC reduction at days 12 and 21 significantly predicted dialysis independence. Bortezomib chemotherapy, number of dialysis sessions,

duration of dialysis, number of dialysers, time from renal failure to chemotherapy, time from chemotherapy to HCO initiation, new or relapsing multiple myeloma, and known chronic kidney disease were not statistically significantly predictors.

In summary, FLC removal by HCO-HD was well tolerated and resulted in sustained reductions in serum FLC concentrations in the majority of patients. The degree of early reduction in serum FLC concentrations achieved and the time to initiating HCO-HD were the only factors which independently predicted dialysis independence.

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EuLITE TRIAL: RESULTS TO DATE

M. Cook

*University Hospital Birmingham NHS Foundation Trust
Birmingham, UK*

Myeloma is an incurable malignancy of plasma cells. Renal complications are common, either as a direct consequence of the disease (cast nephropathy) or due to other features such as infection, hypercalcaemia and dehydration. Indeed, it is estimated that up to 50% of myeloma patients will have some degree of renal impairment at least once in their disease pathway. Up to 10% of patients will present with acute renal failure requiring dialysis at diagnosis. The commonest renal lesion in this circumstance is cast nephropathy, which occurs when clonal free light chains, circulating in excess, are filtered and retained within the renal tubules. The end product is the deposition of an insoluble waxy cast resulting in renal failure. Acute renal failure has significant negative prognostic implications in this patient group. Survival for patients presenting needing dialysis is significantly reduced compared to a non-dialysis cohort with death due to infection a significant problem. For those who survive initial treatment and are young enough to undergo autologous transplantation, the morbidity and mortality of the procedure is significantly increased. Continued requirement of haemodialysis also has a marked negative impact on quality of life. There is therefore a compelling argument to try and reverse the renal failure of myeloma. If successful, it is hoped that subsequent treatment with conventional anti-myeloma therapy can be delivered in a safer fashion with a positive impact on response and survival. The ambition therefore is to return the patient to a 'standard' pathway of care with expectations of survival similar to patients who did not present needing dialysis and a consequent positive impact on quality of life.

In a previous pilot study, we examined the role of a high cutoff dialysis membrane (HCO1100, Gambro) as an aid to prompt reduction in circulating light chain levels (Hutchison et al. *Clin J Am Soc Nephrol* 2009 4(4) 745-754). In combination with anti-myeloma chemotherapy, a significant reduction (median 85%) in light chain levels was seen and as a clinical consequence, reversal of dialysis dependency in over 70% of patients. Renal response was linked to the ability to receive anti-myeloma treatment in a sustained fashion- there was only one renal response seen in the cohort of patients unable to receive regular anti-myeloma therapy. Of note was that most of these patients had relapsed myeloma and had had at least one prior line of therapy. More recently, there has been evidence that in patients with various degrees of renal impairment, renal response is linked to the response of myeloma to anti-myeloma therapy (Ludwig et al *Blood* 2009 114 Abs#3862).

The European trial of free Light chain removal by exTENDED haemodialysis in cast nephropathy (EuLITE) study is a randomised phase II study designed to evaluate the impact of extended high cut off dialysis in newly diagnosed myeloma patients presenting with acute renal failure requiring dialysis. Entry criteria include biopsy proven symptomatic myeloma and confirmation of cast

nephropathy on renal biopsy. All patients receive modified PAD chemotherapy (bortezomib, doxorubicin, dexamethasone given in a 3 week cycle) as anti-myeloma therapy. Patients can receive up to 8 cycles of chemotherapy. Randomisation is between extended dialysis with the HCO1100 and conventional dialysis. Patients also receive appropriate anti-infective prophylaxis. The primary endpoint is independence of dialysis at 3 months after trial entry. Secondary endpoints are duration of dialysis, reduction in serum free light chains, myeloma response and survival.

After slow initial recruitment (including an initial safety cohort of 6 patients), at the time of writing, 19 patients have been recruited. There are now over 10 participating centres in the UK and recently, German partner centres completed the regulatory work required for the study to open in Germany. We now expect recruitment to increase significantly. The target number of patients for treatment within the study is 90. Interim analyses are planned after 25 and 50 patients.

Treatment has been well-tolerated in the majority of patients. An update on patient recruitment, adverse events and projected timeline for key landmarks in the study will be given at the meeting.

Note. The EuLITE study is registered ISRCTN45967602. The study is sponsored by UHB NHS Foundation Trust. Gambro (Hechingen, Germany) have kindly provided equipment and financial support and Ortho-Biotech (High Wycombe, UK) have kindly provided bortezomib.

POLYCLONAL FLC AND CHRONIC KIDNEY DISEASE

S. Stringer

Renal Institute of Birmingham, University of Birmingham, Birmingham, UK

Chronic kidney disease (CKD) is a significant cause of morbidity and mortality in the UK with around 10% of the population having some form of CKD. There are many causes of CKD but for most there is no disease specific treatment and the mainstay of management is to reduce the risk of further decline in renal function and to reduce cardiovascular (CV) risk. Patients with CKD are at enhanced CV risk by virtue of several factors; the risk factors for CV disease are the same as those for CKD (hypertension, diabetes and smoking to name but three) and the likely presence of a low grade inflammatory state mediated by CKD resulting in endothelial dysfunction and atherogenic plaque formation; the role of free light chains (FLC) in this process is considered below.

As kidney function declines (the glomerular filtration rate (GFR) is reduced) levels of serum FLC rise. Declining kidney function is associated with a pro-inflammatory state; a consequence of this is increased polyclonal FLC production. While the clearance of polyclonal FLC is normally via the kidneys, when there is impaired kidney function the mode of clearance becomes predominantly reticulo-endothelial, unlike the renal route this method of clearance is not related to molecular size so the serum half lives of kappa (κ) and lambda (λ) converge as renal function worsens. FLC are filtered at the glomerulus and absorbed at the proximal tubule, when the capacity for

reabsorption at the proximal tubule is overwhelmed the FLC appear in the urine. The renal toxicity of monoclonal FLC is well understood (the formation of casts of FLC and Tamm-Horsfall protein in myeloma kidney being an example of this). However a role for direct renal pathogenicity of polyclonal FLC has not been shown to date.

To address this we have (i) analysed a large cohort of patients with CKD to assess the relationship between serum polyclonal FLC and clinical outcomes (ii) studied the pathogenic potential of polyclonal FLC in human kidney disease.

A large cohort of patients ($n=1328$) with varying stages of CKD were recruited in Birmingham; the cohort comprised patients recruited from secondary care clinics and included all stages of kidney disease. The cohort was followed prospectively for a median of 43 months. The outcomes examined were death, cardiovascular events and progression of renal disease where appropriate. Eighteen patients had an abnormal κ/λ ratio and were excluded from analysis ($n=18$). The total free light chains were significantly higher in dialysis patients (median 237 versus 57 for CKD and 50 in the transplant cohort, $p<0.001$). The survival analysis showed that the factors that were significantly associated with patient survival were the total FLC ($p<0.001$), the serum κ ($p<0.001$) and serum λ ($p<0.001$), for all three of these variables higher levels were associated with an increased chance of death at any time and thus worse survival, on a log scale a one-unit increase in total FLC caused the hazard of death to increase three-fold. The multi-variable analysis demonstrated that total FLCs, age, pre-existing CV disease, highly sensitive CRP and classification (CKD, dialysis or transplant) were all significantly associated with patient survival, after adjustment for these none of the other variables were significant, in particular GFR was not a predictor of death.

Renal progression was analysed in those patients who were not dialysis dependent at recruitment. The measure of renal progression was the delta eGFR (the change of eGFR divided by the length of follow up). In the multivariable analysis both age and total FLC had a significant effect upon delta GFR ($p<0.001$ and $p=0.02$ respectively). Other factors including ACR were not significant (though this tended towards significance).

To address the possible mechanisms of rate of deterioration of kidney function, ex vivo and in vitro studies were performed. These showed that polyclonal FLC, unlike monoclonal FLC, do not activate proximal tubular epithelial cells. However there is a relationship between distal tubular casts containing FLC and in situ surrogates for progressive CKD. Furthermore polyclonal FLC co-precipitates with Tamm-Horsfall Protein, the major protein of the renal tract, confirming the potential for polyclonal FLC to act as a co-factor for cast formation in CKD.

These data indicate that total FLC may be a risk marker for (i) death and (ii) progression of renal disease. Whilst the biological basis for these relationships requires further investigation, the association with mortality may reflect both systemic inflammation and reticulo-endothelial dysfunction. For renal function, further studies are required to assess the interplay between polyclonal FLC and known co-factors for the progression of kidney disease.

se including proteinuria and ischaemia

The clinical outcome results are the first from a series of large cohorts of patients with CKD, which should accurately define the utility of polyclonal FLC as an aid to risk factor assessment and directed clinical management in patients with CKD.

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DISEASES ASSOCIATED WITH MONOCLONAL FREE LIGHT CHAINS

AL AMYLOIDOSIS - ROLE OF FLC ASSAY IN RISK ESTIMATION

S. Kumar

Mayo Clinic, Rochester, MN, USA

Light chain amyloidosis (AL amyloidosis), where monoclonal immunoglobulin free light chains form the substrate for amyloid fibril formation, represents the most common form of systemic amyloidosis. The ability to directly measure the amount of free kappa and lambda light chains in the serum has had a profound impact on the management of AL amyloidosis. Studies have shown that levels of serum free light chains at diagnosis directly correlate with the degree of organ involvement and have been shown to predict the outcome in patients with AL amyloidosis. Serum free light chain measurements can be integrated with the current staging parameters such as cardiac biomarkers such as cardiac troponin T (cTnT) and N terminal pro Brain natriuretic peptide (NT-ProBNP) in order to develop improved risk stratification systems. Among patients undergoing stem cell transplantation for AL amyloidosis, the baseline FLC levels as well as the magnitude of FLC change following therapy can be used to predict outcome. In addition, the type of light chain (kappa or lambda) also predicts the spectrum of organ involvement in the disease. Presence of free light chain unaccompanied by a detectable heavy chain also appears to predict poorer outcome among these patients. In addition to its role in risk stratifying patients with AL amyloidosis, FLC estimates offer a better marker for assessment of disease response compared to the intact monoclonal protein. Studies have shown that response assessment using FLC reduction allows better estimate of outcome based on response and moreover the majority of patients do not have measurable levels of intact monoclonal protein in order to apply conventional definitions of hematological response.

FLCs IN THE AMYLOIDOSIS CLINIC

P. Hawkins

National Amyloidosis Centre, UCL Medical School, London, UK

AL amyloidosis is the cause of death of about 1 in 1000 individuals in the UK, and it is associated with a wide spectrum of clonal B-cell dyscrasias. Although all AL amyloid fibrils are derived from monoclonal immunoglobulin light chains and the deposits can be very extensive, the underlying B-cell dyscrasias are often extremely subtle, and conventional serum and urine immunofixation are each negative in about 20% of cases. A whole serum paraprotein greater than 10 g/L, i.e. a serially quantifiable level, is present in only about 10% of patients. Quantitative measurements of serum free light chains (FLC) using the robust, sensitive, **Freelite** immunoassay has had a major impact on the clinical management of AL

amyloidosis during the past 10 years. The combination of serum and urine immunofixation and serum FLC measurements are recommended in UK and US guidelines for evaluation of patients with possible AL amyloidosis, with diagnostic performance approaching 100%. Although demonstration of a monoclonal immunoglobulin product plainly supports the diagnosis of AL in patient with proven or suspected amyloidosis, it remains vital to confirm the AL nature of the deposits through immunohistochemical staining, or proteomic approaches when the latter does not yield a definitive result. Subtle monoclonal gammopathies are not infrequent in the general population, illustrated by their misleading incidental presence in many older patients with hereditary and senile cardiac amyloidosis.

Serial FLC measurements are usually the most effective means for evaluating the early effects of chemotherapy and the need for ongoing treatment, supporting the approach of cyclic combination chemotherapy which is now pursued in the majority of AL patients. Evidence of response to cyclophosphamide, thalidomide and dexamethasone can be determined within 2-3 cycles of treatment, enabling an early change of therapy if unsuccessful, whilst response to bortezomib usually becomes evident during the first cycle.

The degree of reduction of the involved FLC following chemotherapy is the single most important modifier of outcome in AL amyloidosis, and correlates strongly with the likelihood of gradual regression of existing amyloid deposits. However, SAP scintigraphy has revealed that the rate of turnover of amyloid varies substantially between patients, and between different organs within individual patients, governing the potential within a given patient for regression of amyloid to occur in a clinically beneficial timescale. A favorable outcome in cardiac amyloidosis is unlikely to occur unless the involved FLC has been suppressed by more than 90%, and the greatest likelihood of a renal response also occurs among patients who achieve >90% FLC response.

Various challenges in the utility of FLC assays remain, including difficulties in their interpretation in renal failure due to retention of healthy polyclonal light chains, and in up to 20% of patients in whom FLC results are only slightly abnormal or not abnormal at all. Explanations for the latter include anti-light chain antibodies not binding certain monoclonal FLCs or very efficient sequestration of highly amyloidogenic FLCs into amyloid deposits. Use of the dFLC method, a simple formula in which the concentration of the uninvolved class of light chain is subtracted from that of the aberrant amyloidogenic light chain, is often more informative in renal failure, and in many of the one-third of AL patients without renal failure in whom the involved FLC values is <100mg/L at presentation.

SERUM FREE LIGHT CHAINS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

G. Pratt

University of Birmingham, Birmingham, UK

Background. Serum free light chain (sFLC) assays have improved screening and monitoring and provided prognostic information, for a range of plasma cell disorders. Data has also emerged indicating a prognostic significance for an abnormal serum FLC ratio in some non-plasma cell lymphoid malignancies. Whereas an abnormal ratio, reflecting clonal production, has prognostic relevance in plasma cell disorders it may be that elevated polyclonal sFLC may be more relevant as a prognostic marker in non-plasma cell disorders, reflecting generalised B cell activation.

Aims. The aim of the current study was to determine whether both monoclonal and polyclonal sFLC elevations have prognostic significance in patients with chronic lymphocytic leukaemia (CLL).

Methods. We retrospectively analysed sFLC in 181 untreated/pre-treatment and 78 treated CLL patients from three UK centres.

Results. An abnormal serum FLC ratio was found in 39% (100/259) of the CLL cohort. Using multivariate analysis (n=194), four independent prognostic variables for overall survival were identified namely Zap-70 ($p=0.0001$), β_2M ($p=0.01$), IgV_H gene mutation status ($p=0.017$) and an abnormal sFLC ratio ($p=0.024$). For patients with mutated Vh genes, reduced κ/λ ratios were adversely prognostic and associated with the poor prognostic Vh 3.21, Vh 3.48 and Vh 3.53 subgroups, suggesting an abnormal sFLC ratio may reflect biological subgroups within CLL. Patients with abnormal sFLC ratios were significantly more likely to have unmutated IgV_H status ($p=0.001$), Zap-70 positivity ($p=0.034$), lymphocyte doubling time (LDT) less than 12 months ($p=0.004$) and have a high β_2M ($p=0.001$) than CLL patients with a normal FLC ratio. Where available surface expression of light chain in the majority of CLL cases correlated with an abnormal FLC ratio of the same type except for 8 patients. Analysis of a subpopulation of 167 untreated stage A patients, with normal kappa / lambda sFLC ratios identified summated polyclonal sFLC >50mg/L as being significantly associated with progressive disease and poorer outcome. 35/120 patients had serum FLC >50mg/L and had the following characteristics Median age 79 (range 62-98), Mutated v Unmutated 22 v 7; 6 unknown), Zap 70 pos v neg (9 vs 23, 3 unknown), CD38 pos v neg (11 vs 23, 1 unknown), 18/35 patients have progressed to treatment and 10/35 patients died. Median time to first treatment was 88 months compared to 170 months for patients with a normal FLC, ($p=0.023$). Median survival points were not reached, 75%ile survival was significantly shorted in patients with sFLC >50mg/L compared to those with a normal sFLC concentration (98 vs 201 months $p=0.006$).

Summary/Conclusion. This study shows that in an unselected population of CLL patients an abnormal serum FLC ratio has independent prognostic significance and that a serum FLC >50mg/L can independently identify a group of CLL patients with progressive disease and a poorer outcome.

ok. Here we present our data and review other relevant studies of sFLC in CLL.

SERUM FREE LIGHT CHAINS IN NON-HODGKIN LYMPHOMA

M.J. Maurer, J.A. Katzmman, B.K. Link, J.R. Cerhan, T.E. Witzig

Mayo Clinic, Rochester, MN, USA

Serum FLC abnormalities in NHL. The first report of serum free light chain (FLC) abnormalities assessed via the **Freelite** assay in NHL were by Martin and colleagues at the Mayo Clinic (Martin et al). Using frozen sera from the Mayo Clinic/ Lymphoma SPORE serum bank, they evaluated 208 NHL samples. Twenty seven samples (13%) had an abnormal FLC ratio. Prevalence of abnormal FLC ratio was varied across NHL subtype, ranging from 36% of mantle cell samples (9/25) to 4% of follicular lymphoma samples (3/75). To further characterize the prevalence of FLC abnormalities in NHL, we recently assessed serum FLC in a cohort of over 1400 lymphoma patients from the Mayo Clinic and University of Iowa SPORE Molecular Epidemiology Resources (MER). The MER offers enrollment to any newly diagnosed lymphoma patient seen at Mayo Clinic and the University of Iowa. Similar to the Martin analysis, we saw a range of abnormal FLC ratio prevalence across subtypes, with the highest prevalence in high grade B-cell lymphoma (50%), CLL/SLL (33.7%), and mantle cell lymphoma (26%) (see table). We also assessed the percentage of patients with elevated FLC, as defined by a kappa or lambda concentration above the established reference ranges (Katzmann et al). Elevated FLC was most common in mantle cell lymphoma (63%) and was generally present in 25-35% of patient across subtypes. Notably, elevated FLC was not restricted to B-cell NHL, as 25% of Hodgkin lymphomas and 37% of T-cell lymphomas had an elevated kappa or lambda.

FLC and outcome in DLBCL. We recently reported at ASH on serum FLC in untreated DLBCL patients from NCCTG N0489, a phase II study of epratuzumab + R-CHOP (Maurer et al). Serum was collected both prior to treatment as well as at restaging after 2 and 6 cycles of treatment. We found a similar prevalence of FLC abnormalities to the Martin publication with 12% of patients have abnormal FLC ratio and 34% of patients having elevated FLC. Elevated FLC was highly associated with poor outcome on the clinical trial. Patients with elevated FLC had a significantly worse event-free survival (EFS HR=3.06, $p=0.01$) and overall survival (OS HR=3.16, $p=0.02$) than patients with kappa and lambda in the normal range. Abnormal FLC ratio was positively associated with survival, though not statistically significant (EFS, HR=1.87, $p=0.26$, OS HR=2.58, $p=0.10$). Serum FLC was reduced significantly during treatment in all patients, with a median reduction in total FLC of 42% and 57% after 2 and 6 cycles of treatment, respectively. In summary, FLC abnormalities are quite prevalent in lymphoma. Elevated FLC concentrations are associated with poor outcome in patients with DLBCL. Additional studies of FLC in lymphoma are warranted to better understand the mechanisms of elevated FLC and reasons for poor outcome in these patients.

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Table 1. Prevalence of FLC Abnormalities in Lymphoma in Mayo Clinic/University of Iowa SPORE Molecular Epidemiology Registry.

Lymphoma Subtype	N	Elevated FLC	Abnormal FLC Ratio	Both Elevated FLC and Abnormal FLC Ratio
DLBCL	318	27.4%	17.0%	8.5%
Mediastinal Large B-cell	16	6.3%	6.7%	0%
PCNSL	25	8.0%	29.2%	4.0%
PTLD	11	27.3%	9.1%	0%
Follicular	283	13.1%	11.8%	3.9%
FL I	150	13.3%	10.7%	4.0%
FL II	85	11.8%	10.8%	3.5%
FL III	47	12.8%	17.0%	4.3%
Mantle Cell	72	62.5%	26.4%	25.0%
T-Cell	71	36.6%	8.8%	2.8%
Post Follicular	106	40.6%	28.9%	19.8%
Splenic / Nodal	19	42.1%	38.9%	21.1%
Marginal Zone	70	30.0%	17.4%	10.0%
MALT	17	82.4%	64.7%	58.8%
LPL				
Hodgkin Lymphoma	144	24.8%	6.3%	0.7%
Burkitt / High Grade	15	26.7%	50.0%	20.0%
CLL/SLL	380	32.6%	33.7%	18.4%

PROGRESSING SERUM IMMUNE ABNORMALITIES IN MYELOMAGENESIS

O. Landgren

National Cancer Institute, National Institutes of Health, Center for Cancer Research, Medical Oncology Branch, Bethesda, MD, USA

Monoclonal gammopathy of undetermined significance (MGUS) is a pre-malignant plasma-cell proliferative disorder associated with a life-long risk of progression to multiple myeloma (MM). In fact, long-term follow-up studies of MGUS cases have shown that the excess average annual MM risk does not diminish even after 25 to 35 years. Consequently, most individuals diagnosed with MGUS are typically followed for life by their responsible primary-care provider. Until recently, it has been impossible to determine if a protracted premalignant phase (MGUS) precedes MM in all patients. Taking advantage of the large nationwide U.S. PLCO (Prostate, Lung, Colorectal, and Ovarian) Cancer Screening Trial, we employed a unique study design to conduct the first prospective study to address this question (Blood 2009;113:5412-7). Among 77,469 persons in the screened arm who were cancer-free at baseline, we identified 71 individuals who developed MM during the course of the study in whom serially-collected pre-diagnostic serum samples obtained at least 2 years (up to 9.8 years) prior to MM diagnosis were available. Using multiple pre-diagnostic blood samples (up to 6 samples) obtained annually in the same individual; we applied serum protein electrophoresis, immunofixation, and kappa-lambda free light chain (FLC) assays to define the prevalence of MGUS prior to the diagnosis of MM, and characterized longitudinally patterns of M-protein abnormalities prior to MM diagnosis. MGUS was present in 100.0% (87.2-100.0%), 98.3% (90.8-100.0%), 97.9% (88.9-100.0%), 94.6% (81.8-99.3%), 100.0% (86.3-100.0%), 93.3% (68.1-99.8%), and 82.4% (56.6-96.2%) at 2, 3, 4, 5, 6, 7, and 8+ years prior to MM diagnosis, respectively. The median for M-protein concentration exhibited a year-by-year ($p_{\text{trend}}=0.025$) increase; at 8+ and 2 years prior to MM diagnosis, the median (range) concentration was 0.9 (0.5-1.8) and 1.6 (0.4-3.7) grams per deciliter, respectively. Simultaneously,

we found that the proportion of study subjects with an abnormal kappa-lambda FLC-ratio at 8+ to 2 years prior to MM varied between 47% and 85%. This pattern adds substantially to our previous investigation where we found that about 30% of all MGUS cases had an abnormal kappa-lambda FLC-ratio, and if the ratio was abnormal, there was a 2.6-fold increase for progression. In the present study, when we assessed FLC variations on a group level, year-by-year in a 10-year interval prior to MM diagnosis, there was no statistical association over time. Interestingly, in accord with recent observations, when we evaluated M-protein and involved-FLC ratios over time in individual patients, about half the study population showed a year-by-year increase prior to MM diagnosis, while the other half maintained largely stable abnormal serum protein level up to the diagnosis of MM. Although it remains to be confirmed, others have speculated that “evolving MGUS” potentially could be a marker for an early myeloma with a slow rate of progression. In summary, we found MM to be consistently preceded by MGUS. The median M-protein concentration exhibited a year-by-year increase over time. Two years prior to MM diagnosis, the proportion of MGUS cases with an abnormal FLC-ratio was 85%, supporting a role for FLC assays in routine MGUS follow-up. In about half the study population, the M-protein concentration and involved FLC-ratio levels showed a year-by-year increase prior to MM diagnosis, while the other half maintained largely stable abnormal serum protein level up to the diagnosis of MM. Thus, stable M-protein or FLC levels do not exclude the development of MM development. Until better molecular markers for progression to MM are available, clinicians have to use clinical measures in combination with routine blood test (including renal function, hemoglobin, and serum calcium) and serum and urine M-protein markers in their monitoring of MGUS cases. Future studies are needed to provide insights to the pathogenesis of MGUS and predictors for MM progression. A better understanding of mechanisms mediating monoclonal plasma-cell proliferation, survival, and migration in the bone marrow microenvironment will improve our insights on myelomagenesis and help us to identify novel molecular targets. Larger prospective samples will be required to further refine these patterns.

DISEASES ASSOCIATED WITH ELEVATED POLYCLONAL FREE LIGHT CHAINS – I

DISEASES ASSOCIATED WITH POLYCLONAL FREE LIGHT CHAINS - CONCEPTS

G. Mead

University of Birmingham, Birmingham, UK and The Binding Site Group Ltd, Birmingham, UK

To date, the main interest in the use of serum free light chain FLC measurement has been as an aid in the diagnosis and monitoring of patients with monoclonal plasma cell disorders, particularly multiple myeloma and AL amyloidosis. Diagnostically, the guidance has always been that it is the presence of an abnormal FLC ratio which is indicative of monoclonal FLC production. However, any laboratory that has been using FLC as part of a screening protocol for lymphoproliferative disorders will see a number of results with a normal FLC ratio but FLC concentrations above the reference range. Such results indicate a polyclonal FLC increase and the potential causes of such elevations are manifold. As with any serum protein, an increased concentration may result from increased production or from reduced clearance.

The predominant clearance mechanism for FLC is via the kidneys and this determines the serum half-life of only 2-6 hours.¹ There is also a second mechanism for clearance of FLC and that is via pinocytosis by metabolically active cells, a route that operates for all other serum proteins as well. In the absence of any renal clearance, this mechanism would remove FLC with a half-life of 2-3 days.⁸

The main source of FLC production is thought to be plasma cells and studies following the clearance of radio-labelled FLC and IgG indicated that, in addition to the light chains that were paired with heavy chains to form intact immunoglobulin, there was an excess synthesis of approximately 40%, which was released as FLC.¹ Even if FLC production is solely a “by-product” of immunoglobulin synthesis, the measurement of serum FLC concentrations will provide different information than overall immunoglobulin levels. Immunoglobulins have serum half-lives of several days (IgA ~5 days, IgG ~20 days).¹³ So serum FLC concentrations, with a serum half-life of a few hours, will reflect current production rates more accurately. Furthermore, the range of IgG concentrations is limited because of its concentration-dependent catabolism: at 5g/L the serum half-life of IgG is close to 30 days whereas it is approximately 10 days when the concentration is 30g/L. This is because the Fc receptor which recycles IgG, (avoiding catabolism in endosomal vesicles) saturates at higher IgG levels.^{13,14,15} However, plasma cells are not the only B-lymphocytes capable of FLC production. Cells earlier in the process of B-cell maturation are also able to express FLC and this is reflected in the number of non-Hodgkin lymphoma patients who have abnormal FLC ratios.^{11,12} FLC production in lymphomas is, however, considerably less than that seen with most plasma cell disorders. Therefore, in consideration of the above, a polyclonal FLC elevation may be caused by any disorder that adversely affects either of the FLC clearance mechanisms or that increases FLC production by plasma cells or B-lymphocytes.

The most common disease associated with reduced FLC clearance is chronic kidney disease, and this, in turn, is associated with other co-morbidities, notably cardiovascular disorders.⁶ At ~24kDa (monomeric kappa) and ~50kDa (dimeric lambda) FLCs are more sensitive markers of glomerular filtration rate (GFR) than serum creatinine.⁵ It is generally recognised that serum creatinine is insensitive to minor changes in GFR.⁷ Disorders associated with increased FLC production include, autoimmune disease, infections and inflammatory disorders. It is worth noting that FLC is not an acute phase protein and it correlates poorly with C-reactive protein (CRP). An observational study of serum markers in 14 patients during treatment in an intensive care unit² revealed that CRP, FLC and immunoglobulins frequently showed very different concentrations and changes in concentration. Correcting FLC values using cystatin C, to account for any changes in renal function, did not significantly improve the correlations. Furthermore, significantly raised concentrations of FLC are seen with viral infections^{9,10} whereas CRP is known to be poorly responsive and an unreliable indicator of viral infections. In addition to the diseases already discussed, preliminary studies, measuring serum FLC concentrations in patients with liver disease, showed levels that were significantly elevated in some patients. Surprisingly, the elevations seen with alcoholic liver disease were higher than those with other disorders such as autoimmune hepatitis.³ These differences persisted, after correction for renal function and it was speculated that reduced pinocytotic clearance of FLC, due to liver fibrosis, might have contributed to the raised concentrations observed. Although pinocytotic clearance of FLC is much slower than renal clearance, a mathematical model of the processes indicated that reductions in pinocytosis could have a significant effect on FLC concentrations, particularly if there was some degree of concurrent renal impairment.⁴ In conclusion, a polyclonal serum FLC increase may be the result of a number of diverse disorders; it is not a specific marker of any particular disease process. FLC measurement could, however, form part of an initial examination and, if indicating that something is amiss, prompt further, more specific, investigations. There are a number of studies now exploring these possibilities in more detail.

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NON-CLONAL SERUM IMMUNOGLOBULIN FREE LIGHT CHAINS (FLC) AS MARKERS OF OVERALL SURVIVAL

A. Dispenzieri, J.A. Katzmann, R.A. Kyle, D. Larson, S.V. Rajkumar

Mayo Clinic, Rochester, MN, USA

Background. When screening for clonal disease, the kappa (κ) to lambda (λ) FLC ratio (FLC-R) is employed to correct for both impaired catabolism (renal function) and non-specific production (generalized immune overstimulation). This adjustment makes it possible to identify patients with relative imbalances between κ and λ levels, which typically qualifies for clonal excess and has been of value in screening for and prognosticating in patients with most clonal plasma cell disorders. We previously performed a population-based study among Olmsted County residents to ascertain the prevalence of monoclonal gammopathy of undetermined significance (MGUS) and light chain MGUS. We hypothesized that by using this cohort, we could identify whether the FLC assay provides information that would be prognostic in a general population of people 50-years and older.

Methods. The Olmsted MGUS prevalence cohort was comprised of 21,463 of the 28,038 enumerated individuals over the age of 50 who were living Olmsted between 1/1/95 and 11/21/2003. The final sample size of individuals for the present study was 15839 due to patient los-

ses secondary to blinding, inadequate sample to perform the FLC assay, and known MGUS or LC-MGUS. For the multivariate analyses, another 772 were excluded due lack of coincident creatinine measurement. The FLC measurements were performed using the FLC assay (Binding Site, Birmingham, UK). The respective reference ranges for κ FLC and λ FLC are 0.33-1.94 mg/dL and 0.57-2.63; therefore, the reference range for the sum of κ and λ FLC (Σ FLC) is 0.90-4.57 mg/dL. Survival time was calculated from the time of sample ascertainment.

Results. Nearly 45% of the cohort was male. The median age was 63 years (range 50, 109). On univariate analysis, the hazard ratio for death for those patients with the highest decile of Σ FLC relative to the other 90% of patients in the cohort was 4.3 (95%CI 4.01, 4.62), Figure 1. Because serum immunoglobulin FLC rise with age and with renal insufficiency, a multivariate analysis was performed to exclude the possibility that Σ FLC was merely a surrogate for these other parameters. With these other variables included, the hazard ratio for death with highest Σ FLC dropped 2.0 (95%1.88, 2.2).

Conclusions. The finding that Σ FLC above the normal range (i.e. highest decile) among individuals above the age of 50, excluding patients with known MGUS or LC-MGUS, is intriguing. Although there is an association between Σ FLC, renal function, gender, and age, excess risk of death still exists. The mechanism by which Σ FLC predicts for outcomes is uncertain, but one could postulate that conditions resulting in overactivation of the immune system contributes to excess risk of death. Whether the Σ FLC adds above and beyond other conventional inflammatory markers like ESR or CRP cannot be determined by the present study, but is certainly worthy of further investigation.

Figure 1.

POLYCLONAL FREE LIGHT CHAIN ELEVATION AND MORTALITY IN THE GERMAN HEINZ NIXDORF RECALL STUDY

J. Dürig,¹ L. Eisele,¹ A. Hüttmann,¹ U. Dührsen,¹ A. Führer,¹ S. Kieruzel,¹ B. Bokhof,³ R. Erbel,³ K. Mann,³ K.-H. Jöckel,³ S. Moebus³ on behalf of the Heinz Nixdorf Recall study Investigative Group

¹Department of Hematology, University Hospital Essen, Germany; ²Department of Endocrinology and Division of Laboratory Research, University Hospital Essen, Germany; ³Heinz Nixdorf Recall study Investigative Group

Abnormalities of the λ free light chain (FLC) ratio can detect monoclonal FLC elevations and are a valuable tool in the diagnosis and follow-up of plasma cell dyscrasias. However, due to their generation in active cells of the immune system and their renal metabolism, polyclonal FLC elevations might also provide valuable hints to other pathologic conditions. Recent reports suggest e.g. a role in predicting outcome in chronic viral infectious diseases. In a previous study, we screened the cohort of the German Heinz Nixdorf Recall study for monoclonal gammopathies by combined serum protein electrophoresis and screening immunofixation¹ and also measured FLC concentration by nephelometric immunoassays (The Binding Site Group Ltd, UK) in all available samples. We here report our first preliminary results of the analysis of polyclonal FLC elevation with regard to all-cause mortality in the Heinz Nixdorf Recall cohort. After exclusion of samples with monoclonal FLC elevation, laboratory results together with clinical information from 4350 study subjects (2180 male, 2170 female) were available for analysis. We used summated FLC (total FLC, tFLC) as a measure for polyclonal elevation. tFLC ranged from 2.7 to 275 mg/l with a median of 30.2 mg/l. High levels of tFLC were associated with high-sensitive CRP (hsCRP) and chronic kidney disease (CKD). Both quintiles of tFLC and CKD stage were associated with shorter survival in univariate analysis. Using the median as cutoff, tFLC still separated groups with different survival within CKD stages 0 and 1. tFLC remained an independent predictor of survival in multivariable cox regression analysis adjusted for sex, age, hsCRP and CKD stage (HR 1.13 (95%CI 1.03 – 1.24 per quintile, $p=0.0068$). For the 274 deaths that occurred during a median observational time of 5 years we had information available from death certificates. Causes of death were categorized into cardiopulmonary, infectious, cancer, and other. The number of deaths increased from the lowest to the highest tFLC quintile (34 vs. 98), however we found no associations of tFLC with categorized causes of death. Polyclonal FLC measurements are affected by a variety of health conditions and may thus be subject to fluctuations over time. We are currently measuring FLC in the 5-year follow-up samples of the Heinz Nixdorf Recall study. This will provide us with a more precise estimate of polyclonal FLC elevation and will help us to further define their role in predicting mortality. These results will be reported at the conference.

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CIRCULATING SERUM FREE LIGHT CHAINS AS PREDICTORS OF AIDS LYMPHOMA AND BEYOND

O. Landgren

National Cancer Institute, National Institutes of Health, Center for Cancer Research, Medical Oncology Branch, Bethesda, MD, USA

Chronic HIV infection leads to progressive immunosuppression (i.e., AIDS). As a result, HIV-infected individuals have a markedly increased risk for certain cancers, including non-Hodgkin's lymphoma (NHL); this risk remains increased in the era of effective HIV therapy. Although the hallmark of HIV infection is progressive loss of CD4 lymphocytes, HIV-infected individuals at all stages of progression also manifest abnormalities in B-cell function. B-cell dysfunction is characterized by abnormally low levels of antibodies to specific pathogens and poor immune responses to vaccines. Paradoxically, total serum levels of immunoglobulin (Ig; mostly of Ig isotype G) are elevated, reflecting nonspecific polyclonal B-cell activation. In some, but not other, studies, high levels of serum globulins (mostly Ig) have been reported to be predictive of the development of NHL. Also, based on small numbers, it has been suggested that monoclonal gammopathy of undetermined significance (MGUS) may be elevated among HIV-infected people and it is thus possible that MGUS could predict AIDS NHL among HIV-infected people. Recently (J Clin Oncol 2010;28:773-9), we took advantage of three established cohorts of HIV-infected people, and evaluated the role of B-cell dysfunction in the etiology of AIDS-related NHL by directly measuring serum-based markers of B-cell stimulation. Among approximately 5,000 HIV-infected people, we identified 66 individuals who developed AIDS NHL and for whom serially collected blood samples collected before NHL diagnosis were available. We applied protein electrophoresis, immunofixation, and assays for free light chains (FLCs) to define patterns of serum protein abnormalities before NHL diagnosis. For comparison, we also evaluated 225 HIV-infected matched controls who were free from NHL. Our study tested the hypothesis that the risk of lymphomagenesis is related to an altered immunoregulatory state, reflected in circulating polyclonal or monoclonal Ig levels. Serum/plasma samples obtained 0 to 2 years and 2 to 5 years before diagnosis/selection were assayed for IgG, IgM, and IgA levels; monoclonal (M) Igs; and kappa and lambda FLC levels. Patients and matched controls were compared by using conditional logistic regression. The kappa and lambda FLCs were both significantly higher in patients (e.g., in 2- to 5-year window: median kappa, 4.24 v 3.43 mg/dL; median lambda, 4.04 v 3.09 mg/dL) and strongly predicted NHL in a

dose-response manner up to 2 to 5 years before diagnosis/selection (e.g., NHL risk 3.76-fold higher with kappa concentration >2 times the upper limit of normal (ULN), and 8.13-fold higher with lambda concentration >2 times ULN compared with normal levels). In contrast, IgG, IgM, and IgA levels were similar in patients and controls. M proteins were detected in only two patients with NHL (3%) and in nine controls (4%), and they were not significantly associated with NHL risk. In summary, we found that the presence of elevated FLC levels, a marker of polyclonal B-cell activation, is a strong risk factor for AIDS-related NHL among HIV-infected individuals. On a clinical note, we speculate that FLC measurement may have utility in assessing risk for NHL and perhaps other AIDS-related outcomes. At present, decisions regarding initiation of HAART are guided mainly by the CD4 count and, to a secondary degree, the HIV viral load. Commonly, a threshold CD4 count of 350 cells/mm³ is used, but recent evidence suggests that earlier initiation of HAART may be beneficial to prevent HIV-related complications. One possibility is that clinicians could utilize FLC levels to help determine which patients might benefit most in starting HAART. Although this is a preliminary suggestion, this approach would find support from our observations that FLCs were especially predictive of NHL risk among patients not on HAART who had a relatively high CD4 count (>100 cells/mm³) and that this predictive relationship was obtained over a prolonged period of 2 to 5 years after FLC measurement. Although NHL risk has declined substantially in the HAART era, lymphoma still remains a considerably important morbidity in the setting of HIV infection. On the basis of our findings, additional elucidation of the pathogenetic mechanisms of AIDS-related NHL should continue to be a priority in epidemiologic studies and clinical trials, and this elucidation may help in the development of improved prevention strategies and the identification of novel therapeutic targets.

DISEASES ASSOCIATED WITH ELEVATED POLYCLONAL FREE LIGHT CHAINS – II

HEPATITIS C VIRUS AND LYMPHOMA DEVELOPMENT

B. Terrier, P. Cacoub

Department of Internal Medicine, Groupe Hospitalier Pitié-Salpêtrière, Université Paris 6, Paris, France

Besides hepatic manifestations, chronic hepatitis C virus (HCV) infection is associated with B-cell disorders, mainly mixed cryoglobulinemia (MC) which can evolve in some cases into overt B-cell non Hodgkin lymphoma (B-NHL). The estimated risk for B-NHL in patients with HCV-related MC is 35-fold higher compared with the general population. Epidemiological studies have demonstrated the association between HCV infection and B-NHL, mainly low-grade marginal zone lymphomas, and more rarely lymphoplasmacytic lymphomas and large B cell lymphomas. The finding that HCV-associated splenic lymphoma with villous lymphocytes responds to antiviral therapy with a strong correlation between viral load and tumor burden supports the existence of a causal relationship between HCV chronic antigenic stimulation and the marginal zone lymphomatous process. However, a direct transformation of B-cells may be at play in some cases. In patients infected with HCV, the diagnosis of B-NHL is often difficult, and early biomarkers are lacking. Free light chain (FLC) assessment could be used as a surrogate marker of B-cell lymphoproliferative disorders. An abnormal serum FLC ratio is consistently associated with the presence of MC vasculitis and/or B-NHL. Moreover, after antiviral therapy, the serum FLC ratio could be used as marker of the control of the HCV-related lymphoproliferation.

FREE LIGHT CHAINS OF IMMUNOGLOBULINS AS DISEASE ACTIVITY MARKERS IN AUTOIMMUNE DISEASES

J. Gottenberg

National Reference Center for Autoimmune Diseases, Strasbourg University Hospital, France

Objectives. B lymphocytes play a pathogenic role in autoimmune diseases. Serum levels of free light chains of immunoglobulins might represent markers of B-cell activation and disease activity markers in rheumatoid arthritis (RA) and primary Sjögren's syndrome (pSS). **Methods.** Serum FLCs were assessed using nephelometry, as well as other markers of B-cell activation, such as total levels of Igs, beta2-microglobulin, B-cell-activating factor of the TNF family (BAFF), in prospective cohorts of patients with RA and patients with pSS.

Results. Increased levels of FLCs and other markers of B-cell activation were observed in patients with RA and pSS compared with controls. Kappa to lambda ratio was not altered. Serum FLC levels were associated with higher disease activity in both RA and pSS. In early RA, increased kappa levels were associated with a higher fre-

quency of radiographic lesions.

Conclusions. Serum markers of B-cell activation, including FLCs, are increased in autoimmune diseases and correlate with disease activity. An extended follow-up is necessary to determine whether the increase of FLCs might help identify patients at risk to develop lymphomas, which are more frequent in autoimmune diseases.

CLINICAL AND BIOLOGICAL RELEVANCE OF SERUM FREE LIGHT CHAINS (sFLC) ASSESSMENT IN PATIENTS WITH HODGKIN'S LYMPHOMA

A. Pinto,¹ G. Iaccarino,¹ F. Russo,¹ B. Amoroso,² E. Morelli,¹ C. Riemma,¹ R. De Filippi^{1,3}

¹Hematology-Oncology and Stem Cell Transplantation Unit, Istituto Nazionale Tumori, Fondazione 'G.Pascale', IRCCS, Naples, Italy; ²The Binding Site Group Ltd, Rome, Italy; ³Faculty of Biotechnological Sciences, Federico II University, Naples, Italy

The role of cellular microenvironment in the evolution and progression of tumours has recently emerged also as an important determinant of prognosis and response to treatments. A suitable and well studied model of tumour-microenvironment interactions is represented by classical Hodgkin's lymphoma (cHL). The tumor cells of HL, the Hodgkin and Reed-Sternberg (H-RS) cells, derive from germinal center B-cells with a deranged B-cell transcription program due to epigenetic silencing and acquired genetic lesions. Tissue H-RS cells are surrounded by a preponderant infiltrate of mixed non-malignant reactive cells which are recruited and/or induced to proliferate by tumour cells and in turn produce soluble or membrane-bound molecules essential for tumour cell growth and survival. Microenvironmental cell types of cHL include non-neoplastic B and T small lymphocytes, plasma cells, eosinophils, mast cells and histiocytes/macrophages in addition to a great number of fibroblast-like cells and interdigitating reticulum cells which often associate with HRS cells within the collagen bands of nodular sclerosis cHL. While tumor H-RS mostly lost the typical B cell surface and functional phenotype and display an abnormal repertoire of membrane markers and transcriptional regulator of multiple lympho-haemopoietic lineages, normal functionally mature B-lymphocytes may account for 20% to 50% of infiltrating reactive cells in HL lesions. These non-malignant B cells are able to deliver survival and proliferation signals to H-RS cells through the secretion of multiple cytokines and the presentation of TNF-superfamily ligands such as CD30L, CD40L and OX40L. More recently, it has been shown that small percentages (0.2%; range 0–2.4% of CD19+) of clonotypic B cells, i.e. clonally related to HRS cells, circulate in the peripheral blood of HL patients, probably representing HL-initiating cells. The role of reactive B-cells in HL is still poorly understood. While some data indicate that high intratumoral B-cell counts may predict for a better outcome, the activity of rituximab in cHL, regardless of CD20 expression on H-RS cells, was suggested to result by depletion from the HL microenvironment of normal B lymphocytes required for tumor cell growth. Since the serum free light chain (sFLC) assay detects and quantifies monoclonal and polyclonal B-

cell populations expanding in lymphohemopoietic tissues, we exploited the sFLC testing to further explore the biologic significance of B-lymphocytes in HL. Since tumor cells of HL do not produce immunoglobulins, we assumed that results would directly reflect changes in the reactive B-cells pool. To this end frozen (-80°C) serum samples from 119 newly diagnosed cHL patients and two separate groups of 26 relapses and 30 patients in complete remission from at least two years, were assayed by immunonephelometry (Freelite, The Binding Site Group Ltd., UK). After quantization of free κ and λ concentrations (normal ranges, κ : 3.3-19.4 mg/L; λ : 5.71-26.3 mg/L), the sFLC κ/λ ratio was calculated (reference range 0.26-1.65) and results were correlated to clinico-pathologic findings at diagnosis and clinical outcome. Patients were required to have a normal renal function and normal serum immunonephelometry at study entry. This because serum levels of polyclonal and monoclonal sFLC are known to increase as a function of the progressive renal impairment. The median age was 31 years, with 22% of patients aged ≥ 45 years. Histology was nodular sclerosis in 85 pts (71.4%) and 72 (60.5%) were in stage I–II. According to GHSG criteria, 16% had early favorable, 29% early unfavorable and 55% advanced disease. The International Prognostic Score (IPS) was of 0-2 and ≥ 3 in 66.4% and 33.6% of pts, respectively. The EBV status was available for 111 patients as assessed by LMP-1 immunostaining. Results showed that 48% and 29% of newly diagnosed patients displayed abnormally elevated serum levels of κ and λ sFLC respectively, consistent with the presence of an expanded polyclonal B-cell population. Interestingly, about one fourth of patients displayed the simultaneous elevation of both κ and λ levels. In contrast, sFLC testing was consistent with the presence of monoclonal B-cells in only 7% of patients, who appeared to bear a B-cell population clonally restricted for κ chains in about 90% of the cases. Similar results were obtained when comparing the median absolute levels of κ and λ sFLC among the different patients subsets. A statistically significant difference was evident between newly diagnosed or relapsed patients as compared to those in complete remission for the absolute levels of both κ and λ sFLC, suggesting that an abnormal Freelite test may correlate with the presence of an active disease. The presence of concurrently elevated levels of both κ and λ sFLC statistically correlated with some clinical features at presentation including older age, leukocytosis, abnormal erythrocyte sedimentation rate and a low albumin. The presence of elevated levels of sFLC was also statistically correlated to an unfavourable IPS score, ≥ 3 . This was not, however, unexpected given that IPS includes most of the above features. Similar results were obtained by analyzing separately patients with elevated kappa or lambda levels. Conversely, no correlations with other prognostic parameters, including the EBV status were found. To assess the effects of the abnormal κ and λ sFLC values on the clinical outcome we performed an event free survival (EFS) analysis. Patients were divided into tertiles for κ and λ values and no statistically significant differences emerged when the whole patient population was analyzed, according sFLC levels. In contrast, while we found no significant association with response rate, baseline elevation in sFLC predicted for event free

survival in 51 evaluable pts with early stage disease treated with 4 to 6 ABVD cycles with or without radiotherapy. The best break point value for predicting EFS coincided with the upper limit of the highest tertile for both κ (>24.43 mg/L) or λ ($>$ than 23.33 mg/L) sFLC. Patients in the top tertile had the worst outcome compared with the 2 lower tertiles. Outcomes for the 2 lower tertiles were comparable. The worst outcome, in terms of EFS, was recorded in a small group of 13 early stage patients who displayed, at diagnosis, the concurrent elevation of both κ and λ sFLC with values in the the respective 3rd tertiles. The predictive effect of κ and λ tertiles was maintained within the low and high risk IPS groups IPS. In contrast, baseline sFLC levels, kappa or lambda, were not predictive for EFS in pts with advanced disease. The presence of an expanded polyclonal B-cell population in about 50% of patients, as witnessed by our **Freelite** testing data may be relevant to the pathophysiology of HL under several aspects. First, polyclonal B-cells may directly deliver survival and stimulatory signals to H-RS cells and/or their progenitors promoting tumor cell growth. Second, an excess of FLCs in HL tissues may promote and sustain inflammatory processes typical for this disease by activating several reactive cells which in turn stimulate H-RS cells and maintain the tumour microenvironment. Under this light different groups have shown that FLCs stimulate tissue sclerosis through fibroblast activation, and promote survival and activation of both neutrophils and mast cells. These latter cell types are able in turn to stimulate tumor cells through a wide array of soluble and membrane-bound molecules. Our study support that elevated FLC levels may reflect an increased polyclonal B cell activity in HL microenvironment which appears to negatively influence the outcome of pts in early stage disease. This effect is lost in advanced disease, suggesting that rituximab might result more active for pts in early than advanced stages. Finally, whether such polyclonal B-cell expansion, present from the early phases of disease development, and at relapse, may facilitate the emergence and/or maintainment of the recently described putative HL-initiating small B-cells, remains an intriguing possibility to be further assessed.

HEVYLITE

HEVYLITE: CONCEPTS

A.R. Bradwell

Department of Immunity and Infection, University of Birmingham and The Binding Site Group Ltd, Birmingham, UK

Introduction. Intact immunoglobulin - heavy chain/light chain (HLC) ratios (**Hevylite**)^{1,2} The important clinical applications of serum FLC κ/λ ratios have stimulated the analysis of κ/λ ratios for the M-spike based upon specific immunoassays for IgG κ , IgG λ , IgA κ and IgA λ . Of particular interest is the ability of the HLC assays, as Ig κ /Ig λ ratios, to compensate for changes in FcRn turnover, blood volume and haematocrit variations. Furthermore they allow measurement of the suppression of the un-involved, polyclonal Igs of the same isotype as that produced by the tumor. This has not previously been possible. Intact immunoglobulin molecules contain unique junctional epitopes between the heavy chain (CH1) and light chain (CL) constant regions. These are the target of **Hevylite** antibodies. Hence, they can separately identify the different light chain types of each immunoglobulin class, i.e., IgG κ , IgG λ , IgA κ , IgA λ and IgM κ , IgM λ .

FcRn Receptors.^{3,4,5} Under normal circumstances, serum proteins that are too large for renal filtration (>65 kDa) have a half-life of 2-3 days. The removal mechanism is by pinocytosis which occurs in nucleated cells as they obtain their essential nutrients from plasma. However, for IgG the half-life is prolonged to 20-25 days by FcRn receptors. These heterodimeric FcRn molecules protect internalised IgG and albumin from acid digestion and recycle them back to the cell surfaces to be released in the slightly alkaline environment of the blood. The process occurs many times under normal circumstances so that the half-lives of both IgG and albumin extend from 3 to 21 days. Interestingly, IgG and albumin molecules do not compete for the same receptor sites, but the exact mechanism and sites of binding are unknown. FcRn proteins have a structure similar to Class I MHC molecules with a heavy chain of 3 domains and a single domain light chain comprising β_2 -microglobulin. They are the same receptors that transport IgG from the mother to the developing foetus in the last trimester of pregnancy. When there are no functioning FcRn receptors such as in familial hypercatabolic hypoproteinaemia (a disease associated with a genetic deficiency of β_2 -microglobulin) the half-lives of IgG and albumin are only 2-3 days. Such patients have hypogammaglobulinaemia, not from failure of production, but simply from excess catabolism. An additional feature of the FcRn recycling system is that it saturates, presumably because of limited supplies of FcRn molecules. This results in a continuous relationship between IgG concentrations and its serum half-life. IgG half-life is 21 days at normal concentrations but at high IgG concentrations this falls towards 3 days as there are insufficient FcRn receptors to protect all IgG molecules. Hence, a patient presenting with, for example, a monoclonal IgG of 90 g/L is producing far more than 3 times the amount of IgG than a patient presenting with 30 g/L of IgG. In

contrast, at low IgG concentrations, when FcRn receptor protection is maximal, IgG half-life extends for many weeks. For IgA and IgM, clearance rates are not concentration dependent, although there is presumably some mechanism that prolongs their half-lives beyond 2-3 days (5 days for IgM and 6 days for IgA compared with only 3 days for IgD). This variation in catabolic rate has several consequences for serum measurements of IgG in patients with intact immunoglobulin multiple myeloma:

- 1) Concentrations do not accurately relate to tumour production rates (in addition to any variations in the efficiency of Ig production by myeloma cells).
- 2) Incremental increases in concentrations under-estimate tumour growth.
- 3) Incremental falls in concentrations under-estimate reductions in tumour size.
- 4) At high concentrations, the rate of IgG fall after successful tumour killing is much faster than 21 days, while for low concentrations it is slower.
- 5) Background IgG is low when monoclonal IgG is high because of FcRn saturation. This is manifest as suppression of polyclonal IgG.⁶ In addition, there is bone marrow suppression of IgG producing plasma cells in many patients.

Hence, the apparent speed of tumour response is much greater when monoclonal IgG concentrations are very high compared with when they are low. Since high concentrations are associated with larger tumour masses, and normally a worse outcome, these patients may have a fast initial fall in IgG concentrations but then may relapse quickly. Therefore, landmark studies of patients at 6 or 12 months, where patients have responded fast initially may identify patients with worse prognosis. These processes mean that measurements of, and changes in, monoclonal IgG concentrations bear less relationship to tumour production rates than might be hoped. Early guidelines for MM included measurements of monoclonal immunoglobulin concentrations for tumour staging but it was later realised that such measurements were unhelpful. This was probably because of these mechanisms. FcRn receptors are functional in many different cells including the podocytes of the glomerular filtration apparatus. Proteins diffusing through the glomerular basement membrane pass through the slit membrane of the podocytes into the urinary space of the Bowman's capsule. Proteins of <60kDa are readily filtered but larger proteins cannot pass so they remain in the basement membrane where they could block fluid flow. FcRn molecules actively transport albumin (65kDa) and immunoglobulins (150kDa) from the basement membrane, through the podocyte cells into the urinary space, thus, by-passing podocyte slit fenestrations. This provides a mechanism whereby monoclonal IgG molecules could enter the urine in the absence of glomerular damage. IgG molecules can subsequently be taken up by cells of the proximal tubules, partially degraded into monoclonal FLCs and re-enter the urine. This may explain why some patients with monoclonal serum IgG but no monoclonal serum FLCs may have monoclonal IgG and monoclonal FLCs in urine.^{7,8} Blood volume changes in monoclonal gammopathies.⁹ Changes in red cell volume (haematocrit) affect measurements of serum immunoglobulins in a direct manner. If,

for example, haematocrit rises from 20 to 40% during treatment there is less blood volume available for immunoglobulin molecules so their concentrations increase (assuming no changes in immunoglobulin mass). Moreover, changes in haematocrit do not affect the serum concentrations of IgG, A and M to the same extent. Because of their differing molecular sizes, 90% of the IgM, 50% of IgG and only 20% of free light chains (FLCs) are located in the vascular compartment. Hence, serum FLCs are least affected because of their large extra-vascular distribution. Furthermore, using serum FLC ratios compensates for any errors in the individual FLCs or immunoglobulin concentrations, making them inherently more reliable for judging changes in tumour mass. Blood volume changes also occur in monoclonal gammopathies. Immunoglobulin molecules are osmotically active so that high serum concentrations lead to increases in plasma volume. This relates to the relative amounts in serum compared with the extra-vascular compartment, so again, molecular size is relevant. As the mass of monoclonal immunoglobulins fall during treatment, there is a reduction in the plasma volume and vice-versa. Thus, changes in serum measurements under-represent changes in tumour production. Again, serum FLC ratios are little affected by changes in plasma volume. All these various factors: long half-life of serum IgG, saturable receptors and changes in haematocrit and blood volume, may explain why intact Ig measurements at diagnosis are not helpful prognostically while serum FLCs and serum FLC ratios are prognostic.

Antibody specificity. Inevitably, one of the most demanding aspects of HLC assay production is ensuring good specificity. As for FLC immunoassays, the reagents are polyclonal antibodies produced in sheep. Immunisation and adsorption techniques are designed to ensure no cross-reactivity. For example, IgGκ reagents do not react with free κ or IgGλ, and any other immunoglobulin. There are 4 HLC epitope regions per immunoglobulin molecule - one on each side of the heavy chain/light chain contact regions and the same on the other arm of the molecule. Because there are 4 per molecule, immune complexes readily form to produce good homogeneous immunoassays that are suitable for nephelometry and turbidimetry.

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DIAGNOSTIC ROLE OF HEAVY/LIGHT CHAIN ANALYSIS

S. Harding

The Binding Site Group Ltd, Birmingham, UK

Background

International guidelines recommend serum protein electrophoresis (SPE) and immunofixation (IFE) for the detection, quantification and typing of monoclonal immunoglobulins. This approach is adequate for the majority of intact immunoglobulins but inadequate in identifying and monitoring monoclonal serum free light chains (FLC). Nephelometric tests are currently used to measure FLC and their use has been recommended in screening algorithms. However, FLC testing is not routinely used in screening and many laboratories utilise SPE alone as the primary screen, reflexing to IFE if an abnormality is seen. SPE is relatively insensitive and quantification of monoclonal proteins below 1g/L may not be accurate due to the presence of a normal polyclonal immunoglobulin background. Furthermore, identification and quantification of IgA, and to a lesser extent IgM, monoclonal proteins by SPE can be difficult due to their co-migration with other serum proteins such as transferrin and beta-2 microglobulin. IFE can detect such proteins but it is qualitative and subjective; guidelines recommend the use of total immunoglobulin measurements for monitoring these patients. Total immunoglobulin measurements are analytically sensitive but inherently include both monoclonal and polyclonal proteins, which make them clinically insensitive when immunoglobulin concentrations are close to normal.

Monospecific antisera have now been produced which recognise junctional, conformational epitopes between the bound kappa or lambda light chain and their heavy chain partners. These antisera have been used to generate nephelometric tests for the measurement of serum IgGκ / IgGλ, IgAκ / IgAλ and IgMκ / IgMλ which allow the determination of heavy/light chain ratios (HLC). Normal reference ranges were established for IgG, IgA and IgM HLC ratios from healthy blood donors.

Sensitivity limits for the detection of monoclonal immunoglobulins were determined experimentally by diluting a known concentration of monoclonal protein into normal sera. For 3 IgG monoclonal proteins SPE had a greater sensitivity than the IgGκ / IgGλ ratio. For IgA and IgM monoclonal proteins sensitivities for SPE and HLC ratios were similar. Arithmetically it is easy to understand why the IgG HLC ratio may not be as sensitive as IgA or IgM in this system. 1g/L of monoclonal protein in a normal (16g/L) IgG polyclonal background represents less than

10% of the total IgG, by contrast 1g/L monoclonal protein in normal (2g/L) IgA or IgM represents 33% of the total Ig. This concentration of monoclonal protein would be within the detection limits of SPE, although perhaps at the limit of accurate quantification. Furthermore, greater sensitivity for HLC ratios would be expected in clinical samples because of the suppression of the non-tumour immunoglobulin production.

Retrospectively, 530 IgG (374 IgGκ and 156 IgGλ) presentation sera from patients with multiple myeloma (MM) have been analysed. Abnormal ratios were reported in 526/530 patients. All 4 patients with normal IgGκ / IgGλ ratios had monoclonal protein levels below the detection limit of SPE and had total IgG concentrations of less than 10g/L but within the normal range. Of these patients 2/4 had high levels of κFLC and abnormal FLC ratios. A third patient was reclassified as a nonsecretory multiple myeloma patient and was negative by IFE and FLC. The final patient's sample had degraded and accurate evaluation was not possible.

In addition, 210 IgA (145 IgAκ and 65 IgAλ) MM presentation sera were analysed; abnormal HLC ratios were found in all 210 sera. 83/210 sera had monoclonal proteins migrating anodally and accurate quantification was not possible by SPE. Finally, of 92 IgM (71 IgMκ and 21 IgMλ) Waldenström's Macroglobulinemia (WM) patients tested, 91/92 sera had an abnormal IgMκ / IgMλ ratio. For the 1 WM patient with a normal ratio, there was no obvious paraprotein by SPE densitometry and the total IgM concentration was less than 2g/L.

Similar diagnostic sensitivities have been seen in monoclonal gammopathy of undetermined significance (MGUS) and smouldering MM (SMM). In presentation sera from 23 IgA MGUS (13 IgAκ and 10 IgAλ) and 4 SMM (2IgAκ and 2 IgAλ), 22/23 and 4/4 patients respectively had abnormal IgA HLC ratios. The 1 IgA MGUS patient with a normal ratio had no obvious paraprotein by SPE and a barely detectable band by IFE; total IgA was less than 2g/L. In presentation sera from 7 IgG MGUS (6 IgGκ and 1IgGλ) and 30 SMM (20 IgGκ and 10 IgGλ), 5/7 MGUS and 28/30 SMM patients had abnormal ratios. All those patients with normal IgG HLC ratios had monoclonal proteins below 1g/L and had normal levels of polyclonal IgG. In the majority of patients immunosuppression of polyclonal Ig means that HLC ratios are as sensitive, or more sensitive than SPE. A few IgG patients with low level monoclonal production and normal polyclonal IgG serum concentrations have normal IgG HLC ratios.

Preliminary data is now available from a study comparing IFE to HLC ratio sensitivities. Approximately 1500 sera were screened with SPE and FLC, 41 patients were identified as having abnormal SPE (24 of which were also abnormal by FLC) and an additional 42 patients had FLC abnormalities alone. IgA HLC ratio matched IFE in the detection of monoclonal proteins. IgG HLC ratios detected the clinically diagnosed MM and plasmacytoma patients. There were discordant IgG HLC and IFE results in 7 patients, 2 of whom had MGUS with monoclonal protein production less than 1g/L. The remaining 5 patients had diseases not normally associated with monoclonal protein production. Similar results were returned with the IgM HLC assay. In the patients with a normal

SPE and who were only identified by abnormal FLC ratio, HLC ratios detected 1 small lymphocytic lymphoma patient, 3 chronic lymphocytic leukaemia patients, 1 WM patient and 1 patient with progressing MGUS. There were other malignancies identified not normally associated with monoclonal protein production and final clinical outcome for all the patients has yet to be established.

Analysis of HLC ratios is a sensitive tool for the diagnosis of monoclonal gammopathies. Low concentrations of IgG monoclonal proteins may be missed by the assay, but these are unlikely to be symptomatic. In addition the assay is more sensitive than SPE at detecting B-cell non-Hodgkin lymphoma and may aid in early detection.

MONITORING MULTIPLE MYELOMA PATIENTS WITH HEAVY/LIGHT CHAIN ANTIBODIES FOR IgA AND IgG

H. Ludwig, L. Mirbahai, N. Zojer, A.R. Bradwell, S. Harding
Center for Oncology and Haematology, Wilhelminenspital, Vienna, Austria

Background. Serum protein electrophoresis (SPE) densitometry is the most commonly used method for the quantification of monoclonal proteins (M-proteins). In multiple myeloma (MM) changes in M-protein concentrations are routinely used to monitor the course of the disease. However, quantification of the M-protein by SPE can be inaccurate. In approximately 30% of IgA MM patients the M-protein co-migrates with other serum proteins and small IgG monoclonal proteins can be difficult to quantify. Nephelometric measurements of total immunoglobulins would avoid such problems. However, inherently the measurements will include monoclonal and polyclonal immunoglobulins making response evaluation and monitoring difficult particularly at normal serum concentrations. Nephelometric assays have been produced which can quantify the different light chain types of each of the IgA and IgG immunoglobulin classes. Here we report results obtained using these assays with 102 MM patients (53 IgA and 49 IgG) followed through the course of their disease. In addition, we compared results obtained with the novel assays with those obtained using SPE, IFE and free light chain (FLC) measurements.

Materials and Methods. SPE and immunofixation (IFE) were performed using a SEBIA Hydrasys system, according to the manufacturer's instructions. FLC and heavy/light chain (HLC) analysis was performed on a Siemens Dade Behring BNII analyzer. Longitudinal samples from 53 IgA (29 IgAk / 24IgA λ) and 49 IgG (31 IgGk / 18 IgG λ) MM patients were analysed (median number of samples = 4, range 1-12). 68 (29 IgA and 39 IgG) patients had baseline samples available.

Results. IgA HLC ratios correctly identified monoclonal protein in 29/29 baseline samples and matched IFE. In 14 of the 53 IgA patients the monoclonal protein was obscured by other proteins and accurate quantification with SPE densitometry was not possible. In these cases the HLC ratio could be used to monitor the patients through the course of their disease. IgG HLC ratios correctly identified monoclonal protein in 39/39 baseline samples and

matched IFE. In 41 IgG MM patients, measurement of HLC ratios allowed response evaluation and monitoring of the disease. In 6/41 patient sera at presentation total IgG immunoglobulin levels were within the normal range (6-16 g/L) with monoclonal protein measurements between 2-10g/L. In 3/6 instances patients monoclonal protein levels fell below 2 g/L and accurate monitoring was not possible with conventional SPE. HLC measurements were able to accurately monitor the patients in all cases. In an additional 3/41 cases HLC ratios remained abnormal when there was no M-protein identifiable by SPE. At relapse in 5/49 IgG patients HLC ratios became abnormal before any other test, in 3/5 cases the abnormal ratio correctly identified clinical disease progression (75-282 days) before other tests. In 2/5 patients disease progression occurred >1000 days after the ratio had become abnormal.

Conclusions. The HLC IgG and IgA assays correctly identified monoclonal proteins at presentation. Furthermore, HLC measurements allowed monitoring of IgA monoclonal proteins, even when they were obscured by other proteins on SPE and were able to monitor IgG patients where accurate SPE densitometry was not possible. HLC assays improve the detection and measurement of paraproteins enabling the accurate response evaluation and monitoring of patients with IgG or IgA paraproteins.

PROGNOSTIC ROLE OF HEVYLITE IN MYELOMA

H. Avet-Loiseau

Laboratoire d'Hématologie, Institut de Biologie, Nantes, France

Serum concentrations of the immunoglobulin (Ig) heavy chain/light chain (HLC) subsets; IgGk, IgG λ , IgAk and IgA λ , were evaluated in relation to outcome in patients with multiple myeloma, alongside IgG, IgA, IgM and other measurements. 339 patients were studied from the French IFM 2005-01 trial that compared Velcade (V) and dexamethasone (D) with vincristine-adriamycin plus D. Results showed that the HLC ratio of the tumor Ig isotype correlated with progression free survival (PFS) (Cox regression analysis hazard ratio [HR] 1.9; $p=0.00018$) that was largely due to suppression of the polyclonal component (HR 1.8; $p=0.002$). Furthermore, greater HLC ratios were associated with shorter PFS ($p<0.001$). There was no association between PFS and concentrations of tumor produced IgG or IgA, serum free light chain / ratios or suppression of non-tumor Igs, implying the presence of Ig specific, myeloma niches in the bone marrow. In multivariate Cox regression analysis, only β_2 -microglobulin (β_2 -M) ($p=0.045$) and HLC ratios ($p=0.001$) were of prognostic significance, even including cytogenetic abnormalities. Using the International Staging System, comprising β_2 -M plus albumin, correlation with PFS was less significant (HR 1.3; $p=0.017$) than β_2 -M plus HLC ratios (HR 1.7; $p=0.00002$). Thus, HLC measurements were prognostic and may have a role in staging patients with multiple myeloma.

PROGNOSTIC ROLE OF HEVYLITE IN MGUS

J.A. Katzmann,¹ R. Clark,¹ A. Dispenzieri,¹ R.A. Kyle,¹ O. Landgren,² A.R. Bradwell,³ S.V. Rajkumar¹

¹Mayo Clinic, Rochester, MN, USA; ²National Cancer Institute, Bethesda, MD, USA; ³The Binding Site Group Ltd, Birmingham, UK

Background. Prognostic factors for progression of MGUS include the size of the M-spike, heavy chain isotype, and free light chain (FLC) κ/λ ratio. The combined use of these variables provides approximately a 10-fold difference in risk for progression. Suppression of uninvolved immunoglobulins has been an inconsistent predictor of progression. The **Hevylite** assay measures concentrations of each isotype-specific heavy and light chain (HLC) pair enabling us to determine if isotype-specific suppression of the uninvolved HLC pair is occurring [e.g. GL concentration in a patient with a monoclonal GK] and if it is predictive of progression of MGUS to MM.

Methods. Total immunoglobulins were quantitated by immunonephelometry, and suppression was defined as concentrations less than the lower limit of the reference range. HLC concentrations were quantitated with **Hevylite** reagents. Reference ranges were derived from 276 serum donors aged 22-90 years. We compared the proportion of patients who had isotype specific suppression of the opposite HLC pair in 40 MGUS patients (30 IgG, 10 IgA) who eventually progressed to MM versus 40 long-term stable (36 IgG, 4 IgA). To confirm the relationship of isotype specific HLC pair suppression compared to suppression of uninvolved immunoglobulins of other heavy chain classes, we tested a second cohort of 39 IgG and 14 IgA MGUS patient samples that were obtained just prior to the development of MM.

Results. The adult 95% reference ranges were $G\kappa = 407-1130$ mg/dL, $G\lambda = 188-583$ mg/dL, $A\kappa = 55-292$ mg/dL, and $A\lambda = 39-249$ mg/dL. Using quantile regression, no age or gender effects existed for any of the assays. The $G\kappa/G\lambda$ ratio (median = 1.9) was different than the $A\kappa/A\lambda$ ratio (median = 1.4). In the diagnostic samples from 36 IgG MGUS patients who had stable MGUS, 64% had an abnormal $G\kappa/G\lambda$ ratio and 22% had isotype specific suppression of the opposite HLC pair. Only 6% had suppression of uninvolved intact immunoglobulins of IgA and/or IgM. In baseline samples from 30 IgG MGUS patients who eventually progressed to myeloma or related malignancy, 83% had an abnormal $G\kappa/G\lambda$ ratio. Compared to the stable MGUS patients the same percentage (7%) had suppression of IgA and/or IgM, but 53% had isotype specific suppression of the opposite IgG HLC pair. In these 2 groups of MGUS patients, HLC pair suppression was 4-8-fold more frequent than isotype suppression of immunoglobulin heavy chain classes. In addition, MGUS patients who eventually progressed had a 2 to 3-fold higher rate of isotype specific HLC pair suppression than stable MGUS patients. These same studies were performed using the diagnostic samples from IgA MGUS patients who remained stable or who eventually progressed. These results were not the same as for IgG MGUS patients. The IgA MGUS patients all had abnormal $A\kappa/A\lambda$ ratios. In addition, whether the IgA patients were stable or eventually progressed, heavy chain isotype sup-

pression (IgG or IgM) was approximately equal to the opposite HLC pair suppression. Stable IgA MGUS patients had 25% heavy chain isotype suppression and 25% HLC pair suppression. Non-stable IgA MGUS patients had 50% and 40% suppression. Thus the HLC pair suppression was similar to the IgG MGUS patients, but the heavy chain isotype suppression was greater than the IgG patients. We tested these findings on a second cohort of MGUS patients who progressed to myeloma. In the initial samples from 39 IgG MGUS patients, 44% had suppression of other heavy chain classes (IgA or IgM), while 87% had suppression of the isotype specific HLC by the **Hevylite** assay. In the last samples prior to progression, the isotype suppression of heavy chain classes increased to 54%, while isotype specific HLC suppression rose to 95%. Interestingly, the 14 IgA patients had equivalent suppression of gamma or mu heavy chain classes as compared to the HLC pair suppression, with 43% and 50% of the patients having heavy chain isotype and HLC pair suppression in the initial sample and 79% for both in the last sample prior to MM.

Discussion. We have studied the prognostic value of isotype specific HLC suppression of uninvolved immunoglobulins as a predictor of progression in MGUS. Previous attempts to study isotype suppression had inconsistent conclusions. The availability of reagents to separately quantitate the kappa and lambda containing proteins of each immunoglobulin isotype provides a different tool to study polyclonal immunoglobulin suppression in monoclonal gammopathy patients. The HLC data in the IgG MGUS patients indicate: 1) IgA and/or IgM isotype suppression is rare early in the disease process but increases prior to diagnosis of MM, 2) HLC pair suppression is more common than IgA or IgM suppression in each group of MGUS patients (3-4-fold early in stable MGUS, 7-8-fold early in eventual progressive MGUS, 2-fold just prior to progression), and 3) HLC pair isotype suppression is more frequent in MGUS patients that eventually progress to MM. The HLC data in the IgA MGUS patients indicate: 1) IgG and/or IgM isotype suppression in early disease is more common than in IgG MGUS and increases prior to diagnosis of MM, 2) HLC pair suppression is equal to IgG or IgM suppression in each group of MGUS patients, and 3) HLC pair isotype suppression is more frequent in MGUS patients that eventually progress to MM. Our findings suggest that clonal IgG plasma cells are able to suppress other IgG producing non-clonal plasma cells far more effectively than they can suppress IgA or IgM secreting cells, and this occurs more frequently with cells that will eventually undergo malignant transformation.

Conclusions. 1. Suppression of the opposite IgG HLC pair is a predictor of MGUS patients destined for progression. 2. Isotype specific HLC pair suppression is more frequent in IgG MGUS patients than isotype suppression of other heavy chain classes. 3. We were not able to demonstrate a similar differential effect of heavy chain isotype suppression compared to HLC pair suppression in IgA MGUS. Larger studies are underway to determine if HLC pair suppression is independent of M-spike quantitation, heavy chain isotype, and FLC ratio for prediction of risk for MGUS progression.

CASE STUDIES

G. Mead, E. Koulieres, M-C Kyrtsolis, M. Drayson, H. Lachmann, D. Riebe, D. Bruegel, D. Schauseil, S. Harding

University of Birmingham, Birmingham, UK and The Binding Site Group Ltd, Birmingham, UK

Introduction. Heavy/light chain (HLC) assays appear to have a utility in the diagnosis of monoclonal gammopathies; as a reflex test after abnormal serum protein electrophoresis results. They may also facilitate monitoring in some patients, particularly those where the monoclonal immunoglobulins are hidden on the serum protein electrophoresis (SPE) gel. Here we present some case studies, which illustrate the use of HLC assays in diagnostic and monitoring settings.

Diagnostic Case studies. While measuring HLC ratios is frequently more sensitive than SPE at identifying monoclonal immunoglobulin production, the relative sensitivity of immunofixation electrophoresis (IFE) and HLC ratios is more closely aligned. There are, however, instances where HLC measurements can help resolve equivocal IFE results.

Case study 1. A serum sample from a patient under investigation for a possible plasma cell disorder. At $> 16\text{g/L}$, the IgA was abnormally high. Immunofixation did not show any monoclonal bands but there was concern about the result because the density of polyclonal staining could have hidden a monoclonal band. HLC analysis indicated approximately 9g/L of both IgA κ and IgA λ , confirming that there was a polyclonal elevation.

Case study 2. A presentation serum from a patient with suspected heavy chain disease because of immunofixation results showing a clear monoclonal band in the anti- α chain track but no corresponding band in either of the light chain tracks. The IgA κ /IgA λ ratio was 0.39 (normal range: 0.8-2.0) indicating that there was monoclonal IgA λ production but the λ light chain was not recognised by the immunofixation antiserum. A FLC ratio of 0.12 supported this interpretation.

Case study 3. A patient with AL amyloidosis and a small, IgG κ monoclonal protein which disappeared after treat-

ment. During a subsequent relapse a truncated, isolated heavy chain was visible (ie. Heavy chain disease), but no IgG κ by IFE. Retrospective HLC analysis of stored sera revealed an abnormal IgG κ /IgG λ ratio, indicating the return of monoclonal IgG κ production below the sensitivity of IFE detection.

Monitoring Case studies. In addition to those patients who can never be monitored quantitatively because their monoclonal immunoglobulin (usually IgA) co-migrates with other proteins, there are instances where HLC ratios appear to be indicating different clinical interpretations than SPE measurements of the monoclonal immunoglobulins.

Case study 4. An IgA κ multiple myeloma (MM) patient who achieved a stringent complete response (sCR) after induction chemotherapy, which was also reflected by normalisation of their IgA κ /IgA λ HLC ratio. After 7 months, there was a clinical relapse which was corroborated by SPE and a worsening HLC ratio. Salvage chemotherapy with velcade resulted in a fall in the SPE densitometry measurement but the HLC ratio continued to deteriorate. This was associated with continued clinical decline and extensive bone marrow plasma cell infiltration.

Case study 5. An IgG λ MM patient, enrolled on a MRC myeloma trial, whose frozen serum samples were retrospectively analysed with HLC assays. Response to the initial therapy resulted in a CR and temporary normalisation of the HLC ratio. There was a relapse after ~400 days accompanied by the return of the monoclonal band on SPE and a worsening HLC ratio. Salvage chemotherapy produced a temporary fall in the SPE results but no change in the HLC ratio. This was then followed by steady decline in both parameters.

Case study 6. An IgA λ MM patient given VAD induction therapy. SPE measurements indicated a partial response at 111 days but the HLC ratio was progressively becoming more abnormal. There was continued clinical deterioration and the patient re-presented with spontaneous fractures 6 months later and was found to have 100% bone marrow plasma cell infiltration with severe anaemia. Treatment with velcade and high-dose melphalan (with stem cell rescue) resulted in a sCR and normalisation of the HLC ratio.

POSTERS

FREE LIGHT CHAIN EVALUATIONS AND ANALYTICAL ISSUES

A1 - COMPARISON OF THROUGHPUT OF FREE LIGHT CHAIN ASSAYS WITH AUTOMATED ANTIGEN EXCESS DETECTION ON THE SIEMENS BNII ANALYSER™ & THE BINDING SITE SPAPLUS

M. Solanki, M. Dominic, E. Hardy

The Binding Site Group Ltd, Birmingham, UK

Background. In response to customer demand, automated antigen excess detection (AAED) parameters for free light chain (FLC) assays on the Siemens BNII have been developed. Binding Site (TBS) SPAPLUS FLC assays already feature AAED. **Aims.** To evaluate the assay throughput on the Siemens BNII analyser (with and without AAED) & the Binding Site SPAPLUS, when the FLC assays were run in isolation and with 3 other assays running concomitantly. **Methods.** Calibration curves, QC, 10 normal donor serum samples & 10 myeloma patient serum samples were tested on both platforms and both sets of BNII parameters for kappa & lambda FLC alone & with concomitant IgG1, IgG2 & IgG3 assays. The samples were assayed according to the kit inserts & the processing time was monitored for calibration curves, QC, normal samples & myeloma samples. The inserts for the current kappa and lambda FLC BNII assays (without AAED) state that samples suspected of antigen excess should be re-assayed at a 1/2000 dilution. The requests for the additional 1/2000 testing against the FLC assays without AAED for the myeloma patient samples were carried out after reporting of the initial results. These additional re-dilutions were not required for the FLC AAED assays on the BNII or SPAPLUS. **Results.** Table 1: Total time required to carry out assessment of calibration curves, QC & serum samples.

Table 1.

Testing Details	Total Time For Completion	Total Number of Tests
FLC with Automated Antigen Excess Assay (Siemens BNII) + IgG1, IgG2, IgG3 Assays	4 Hours 50 Minutes	195
Current FLC Assay (Siemens BNII) + IgG1, IgG2, IgG3 Assays	4 Hours 28 Minutes	190
FLC Assay (TBS SPAPLUS) + IgG1, IgG2, IgG3 Assays	3 Hours 02 Minutes	214
FLC with Automated Antigen Excess Assay (Siemens BNII) – Only	3 Hours 37 Minutes	77
Current FLC Assay (Siemens BNII) – Only	3 Hours 53 Minutes	77
FLC Assay (TBS SPAPLUS) – Only	2 Hours 17 Minutes	72

Summary/Conclusion. The Siemens BNII AAED FLC parameters had higher throughput than the current BNII FLC assay when run alone, but not with addition of other assays. The TBS SPAPLUS assays had faster throughput of samples compared to the Siemens BNII assays (with and without AAED) both with other assays alongside and for FLC assays alone.

A2 - INCIDENCE AND HANDLING OF ANTIGEN EXCESS WHEN MEASURING FREE LIGHT CHAINS

B. Pepperkok,¹ A. Zeuch,¹ R. Jehlicka-Rose,² J. Bartel¹

¹Labor Dr. Limbach und Kollegen, Heidelberg (Germany)

²The Binding Site GmbH, Schwetzingen (Germany)

Background. The measurement of serum free light chains (FLC) is recommended for diagnosis, monitoring and prognosis of monoclonal gammopathies. For monoclonal proteins like FLCs cases of non-linearity and antigen excess have been reported. These phenomena can sometimes affect the interpretation of the results as at different analyser dilutions different FLC concentrations can be obtained. **Aims.** The aim of this study was to determine the frequency of antigen excess and to evaluate the manufacturer's recommendations on how to distinguish between both phenomena which ensure a consistent reporting of results. **Methods.** 342 serum samples requested for FLC testing were analysed for both FLCs by nephelometry using the standard and a higher sample dilution. A factor between the results of both dilutions was calculated by dividing the result of the higher dilution by the result of the standard dilution allowing discrimination between antigen excess and non-linearity. **Results.** For 42 samples results at both dilutions were obtained. For 33 samples results showed a factor of ≤ 4 between both dilutions and for 9 samples the factor was >4 . This indicates that there may be different underlying causes leading to these phenomena. All samples showing results with a factor ≤ 4 between both dilutions were grouped as non-linear samples and the result of the standard dilution was reported. All samples showing results with a factor >4 were categorised to be in antigen excess and the results of the higher dilution were reported. **Summary/Conclusion.** The incidence of antigen excess was 1.3% for both free kappa and free lambda. The use of factor 4 as a "cut-off" to distinguish between non-linearity and antigen excess allows a consistent and reliable reporting of the FLC concentrations.

A3 - MONOCLONAL IgM PROTEINS CAN CAUSE ABERRANT NEPHELOMETRIC FREE LIGHT CHAIN RESULTS MAKING DATA INTERPRETATION DIFFICULT

D. White,¹ N. Gardner,¹ S. Harding,¹ H. Stang,² H. Gisslinger,³ T. Perkmann,³ S.J. Naides⁴

¹The Binding Site Group Ltd, Birmingham, UK

²Evergreen Haematology Oncology, Spokane, WA

³AKH Wien - Universitätsklinken

⁴Quest Diagnostics Nichols Institute, USA

Background. Serum free light chain (FLC) testing is routinely performed on nephelometers and turbidimeters worldwide. The assay is useful in AL Amyloidosis, Multiple myeloma (MM), Waldenström's macroglobulinemia and monoclonal gammopathy of undetermined significance (MGUS). Increasingly, clinicians are requesting the test for diseases including chronic lymphocytic leukaemia, non-Hodgkin's lymphoma and chronic kidney disease. Occasionally samples behave unexpectedly on the assay and require further testing, most commonly from antigen excess. Cross reaction with intact immunoglobulins or other serum proteins has not been reported. Monoclonal IgM is an inherently unstable and difficult protein to quantify in the laboratory and has been identified as potentially interfering in a number of nephelometric assays. In a few cases these proteins have been identified as possibly causing aberrant results in the nephelometric quantification of FLC. **Aims.** To investigate the role of IgM monoclonal proteins in two samples with atypical serum FLC results. **Methods.** The Siemens Dade Behring BNII nephelometer was used to measure serum FLC and to evaluate 'blank' latex reagents. Patient sera were fractionated by size exclusion chromatography under native conditions. Fractions were analysed by FLC assay, SDS-PAGE gels and characterised by Western blotting using isotype specific antisera. Where appropriate, additional gel techniques including Ouchterlony plates, and immuno-fixation electrophoresis (IFE) were employed. **Results.** Sample A had an IgM kappa monoclonal protein with FLC results initially being reported in antigen excess. The final results reported were 7,490mg/L kappa and 2,710mg/L lambda. Neither IFE nor Ouchterlony identified free kappa or lambda light chains in the sample. Size exclusion chromatography demonstrated protein peaks of 60kDa, 150kDa and >600kDa. Analysis of the fractions using the FLC assay showed the majority of the activity was present in the peak which corresponded to >600kDa. Replacing the FLC latex with blank reagents showed that the sample precipitated under reaction conditions in the absence of specific antisera. Ouchterlony analysis showed there was no specific reaction between the anti-FLC antibodies, BSA (which is used to coat latex) or sheep IgG in these fractions. A second sample from a patient with an IgM kappa monoclonal protein and trace IgG lambda monoclonal protein, with FLC values of 5,290mg/L kappa and 1,500mg/L lambda was similarly separated and analysed. After size exclusion chromatography kappa and lambda activity was only found in the largest (>600kDa) peak. Non-specific precipitation was shown using a blank latex assay and Ouchterlony plates as before. **Summary/Conclusion.** High elevations of free kappa and lambda light chain in the

same sample have been reported but are rare. Two samples with both κ and λ FLC levels >1000mg/L and no other indication of monoclonal protein apart from IgM kappa protein by IFE aroused suspicion. On investigation, the apparent elevations of FLC were due to non-specific reactions of the IgM protein with the latex particles. No similar response has been identified in other monoclonal IgM samples.

A4 - EVALUATION OF PARAMETERS FOR MEASURING FREE IMMUNOGLOBULIN LIGHT-CHAINS WITH AUTOMATED ANTIGEN EXCESS DETECTION ON THE SIEMENS BNII ANALYSER

M. Solanki, D. Matters

The Binding Site Group Ltd, Birmingham, UK

Background. Assays specific for serum immunoglobulin free light chains (FLC) on automated analysers are available. Although a rare occurrence, one aspect of these assays that has been criticised is their susceptibility to antigen excess. Automated antigen excess detection (AAED) is available on The Binding Site Group Ltd (TBS) SPAPLUS and Roche Integra 400 & 800 analysers. **Aims.** To evaluate newly developed serum FLC assays with AAED for use on the Siemens BNII analyser. **Methods.** Total precision was assessed at three antigen levels for both assays. The assay linearity was calculated over an antigen concentration range of 2.6 -124.2mg/L for kappa FLC and 2.7 -146.0mg/L for lambda FLC. Results from serially diluted polyclonal serum samples were compared with calculated expected results for these samples. Possible interference from co-existing substances was tested by adding haemoglobin, bilirubin, and triglyceride to serum samples and comparing to an equivalent sample blank at the minimum sample dilution (1/5). Comparison was made with the current TBS FLC assays for the Siemens BNII analyser by measuring samples from normal subjects and subjects with a prior diagnosis of multiple myeloma and carrying out Passing-Bablok regression analysis. Efficacy of the AAED parameters was challenged using samples which had previously exhibited antigen excess on the Siemens BNII FLC assays (Table).

Table.

Test		Kappa FLC	Lambda FLC
NCCLS total precision	Low precision	8.2% (9.43mg/L)	6.5% (14.49mg/L)
	Mid precision	8.8% (36.05mg/L)	6.6% (56.14mg/L)
	High precision	11.0% (106.58mg/L)	12.1% (134.36mg/L)
Haemoglobin interference (5g/L)	1/5 analyser dilution	-9%	-6%
Bilirubin interference (100mg/L for kappa, 200mg/L for lambda)	1/5 analyser dilution	6%	-5%
Lipid interference (Chyle @ 250FTU for kappa, 100FTU for lambda)	1/5 analyser dilution	-6%	-10%
Linearity (total across 1/20 & 1/100 analyser dilutions)		1.00x + 0.11	1.03x - 0.21
Comparison of AAED FLC BNII assay vs. current FLC BNII assay		1.06x + 0.02	1.02x - 2.01

Results. One lambda and 64 kappa FLC samples which displayed antigen excess on the current BNII FLC assays, auto-repeated correctly on the AAED FLC parameters. **Summary/Conclusion.** The FLC assays with AAED for the Siemens BNII provide a precise method of measuring FLC in serum and show good agreement with existing assays. Both assays correctly detected antigen excess in all samples tested, although, due to rarity of antigen excess in lambda samples only one was tested.

A5 - A STUDY OF THE USE OF FREE LIGHT CHAINS TO IMPROVE THE INTERPRETATION OF CAPILLARY ELECTROPHORESIS

A. Boyle

Department of Biochemistry, Royal Lancaster Infirmary, Ashton Road Lancaster, UK

Background. Since April 2007 the laboratory has offered a diagnostic and monitoring service for patients with Myeloma and related diseases. The tests available when the service began were serum Capillary Zone Electrophoresis (CZE), Immunotyping (IFE) and urine gel electrophoresis (UPE). In response to clinical demand serum free light chain (FLC) assays were added in 2008. **Aims.** To identify situations where further tests are required to interpret Capillary Zone Electrophoresis. **Methods.** All requests received from 1st April 2009 to 31st May 2010 were assessed and results obtained for each of the three tests were compared. **Results.** In total, 6,648 CZE, 1,347 UPE and 951 FLCs were assayed. Of the CZE, 18% had a paraprotein, with 28% of these (334 patients) having no previous history of Myeloma. The FLCs were analysed and the results tabulated against the CZE results (Table 1). 6% of all CZE requests were reported as abnormal but with no band identified. This group included samples with Beta/Gamma bridging, small peaks in the gamma and irregular patterns in the Beta region which cannot be conclusively identified by a combination of CZE and IFE. Of the UPEs, 50 abnormal bands were detected (4%). Three patients had an abnormal band in the urine and no abnormalities detected by CZE or FLCs. One patient had an 8.5 g/L paraprotein nine months later but neither remaining patient had a subsequent sample analysed.

Table.

SERUM ELP REPORT	FREE LIGHT CHAINS ABNORMAL	FREE LIGHT CHAINS NORMAL
PARAPROTEIN IDENTIFIED	288 (62%)	175 (38%)
PARAPROTEIN IN BETA	82 (75%)	28 (25%)
NO ABNORMAL BANDS	56 (27%)	150 (73%)
HYPOGAMMA GAMMA <5g/L	36 (64%)	20 (36%)
IRREGULAR	6 (27%)	16 (73%)

Summary/Conclusion. CZE combined with IFE allows identification of paraprotein bands down to 2 g/L when they migrate in the gamma region. FLCs are essential for the identification of patients with paraproteins which run in the beta region and for patients with hypogammaglobulinaemia.

A6 - SERUM FREE LIGHT CHAIN ANALYSIS CORRELATION WITH SERUM PROTEIN ELECTROPHORESIS IN MULTIPLE MYELOMA - AN INDIAN STUDY

M. Noorgahan, M. Goyal, S. Murthy, D. Palanki, S. Nirni

Indo-American Cancer Institute & Research Centre, Hyderabad, India

Background. Quantitative measurement of serum free light chains (serum FLC) has now been adopted into screening algorithms for multiple myeloma. The assays indicate monoclonal FLC production by the presence of abnormal kappa or lambda free light chain ratios (reference range 0.26-1.65). **Aim.** We report our experience with serum FLC assays in the diagnosis of multiple myeloma in an Indian population in comparison with serum protein electrophoresis (SPE). **Methods.** Review of 42 cases of multiple myeloma was undertaken. Multiple myeloma was diagnosed in these cases according to the CRAB criteria. Serum FLCs were measured by a turbidimetric method using **Freelite** (Binding Site). SPE was done on cellulose acetate strip according to procedure provided by GenioLilac medicare. **Result.** All 42 cases of multiple myeloma patients showed abnormal ratio of serum FLCs while SPE could detect monoclonal protein in 36 (85.7%) out of 42 cases. By SPE, the detection limit for monoclonal protein is 1-2 g/L where as for the serum FLCs it is 1mg/L. **Conclusion.** These findings indicate that serum FLC assay enables us to detect multiple myeloma patients with very low monoclonal proteins. The incorporation of quantitative measurement of serum FLCs with serum kappa/lambda FLC ratio is useful in the detection of monoclonal proteins particularly when it is not detected by SPE but there is strong clinical suspicion.

A7 - A SINGLE, LATEX-ENHANCED TURBIDIMETRIC ASSAY FOR TOTAL KAPPA AND LAMBDA FREE LIGHT CHAINS IN SERUM

P.J. Showell, C.J. Buckley, G.P. Mead

The Binding Site Ltd, Warstock Road, Kings Heath, Birmingham, UK

Background. A number of recent studies have indicated that the measurement of polyclonal free light chains (FLC) may be useful for monitoring a variety of inflammatory diseases. This utility appears to derive from the dual role of FLCs as markers of B-Cell activation and of renal function. As both kappa and lambda FLC change in parallel in these instances, and there is a normal FLC ratio, there would be a practical advantage in having a single assay which measures the combined concentration of free kappa and free lambda (tFLC). **Aims.** Here we describe a single, latex-enhanced, turbidimetric assay for combined measurement of both kappa and lambda free light chains. **Methods.**

Monospecific, polyclonal antibodies directed to both kappa and lambda FLC were bound to latex microparticles. Assay parameters were generated for the resultant reagent on the Binding Site SPAPLUS turbidimetric analyser. The analyser was programmed to construct a calibration curve from a 6 point calibration set. Curves were validated by assay of control fluids. Samples were initially measured at a 1/10 dilution and, if out of range, the instrument automatically re-measured the samples at a 1/20 dilution. All dilutions were made with the instrument's on-board pipetting system. The measuring range was from 6.25-200 mg/L at a 1/10 sample dilution, with a reflex range of 12.5-400 mg/L at 1/20, and sensitivity of 0.625 mg/L at neat. The assay took 10 minutes and was read at end-point. To assess analytical sensitivity, ten replicates of two samples with concentrations equivalent to 140% and 200% of the curve bottom point were run and assessed by paired t-test. Intra-assay precision was assessed at three antigen levels (n=20). Inter-assay precision was assessed in duplicate at three levels over ten days. Linearity was assessed by mixing a high polyclonal sample pool with a normal sample pool according to NCCLS EP6-A over a range of 30-160 mg/L. The assay was tested for possible interference from co-existing substances by adding haemoglobin (5g/L), bilirubin (200 mg/L) and Chyle (1500 FTU's) to serum samples and comparing to an equivalent sample blank. Comparison was made with summated values from the Binding Site kappa & lambda FLC kits for the SPAPLUS by measuring samples from normal (n=52), myeloma (n=30) and chronic kidney disease patients (n=21), and performing Passing and Babcock regression analysis. *Result.* The analytical sensitivity of the assay was confirmed through measurement of low level samples – 8.3 mg/L (CV 5.5%), 12.6 mg/L (CV 4.7%), $p < 0.0001$. Intra-assay precision generated CVs of 2.0% (188 mg/L), 3.1% (56 mg/L), and 5.5% (23 mg/L). Inter-assay precision generated CVs of 2.9% (188 mg/L), 3.8% (56 mg/L), and 5.6% (23 mg/L). Assay linearity was confirmed by linear regression – $y = 1.009x - 3.6$, $R^2 = 0.999$. Interference was within $\pm 6\%$ when haemoglobin, bilirubin or chyle were added to serum samples of known total free light chain concentrations. Good agreement was observed when the assay was compared with summated FLC values: $y = 1.03x - 1.24$. *Summary/Conclusion.* We conclude that this combined FLC assay provides a more rapid, cost-effective means of measuring tFLCs than separate FLC tests and shows good agreement with summated values from existing assays.

A8 - MONOCLONAL GAMMOPATHY DIAGNOSIS USING AN ANTIBODY COATED BEAD ARRAY IMMUNOASSAY, FOR FREE KAPPA AND LAMBDA LIGHT CHAIN AND IgG, IgA AND IgM KAPPA AND LAMBDA

R. Budd,¹ D. Taylor,¹ A. Bunce,² S. Harding,¹ A.R. Bradwell,³ G. Mead¹

¹The Binding Site Group Ltd, Birmingham, UK; ²Dynex Technologies Inc, Chantilly, USA; ³Division of Immunity and Infection, The Medical School, University of Birmingham, Birmingham, UK

Background. Laboratory screening guidelines written by

the International Myeloma Working Group, recommend serum protein electrophoresis (SPE) plus immunofixation electrophoresis (IFE), for identifying monoclonal intact immunoglobulins, and determining serum free light chain (FLC) ratios for identifying monoclonal free light chain production. This protocol thus entails a combination of both nephelometry/turbidimetry and gel electrophoresis, which can require a degree of expert interpretation. Recent studies utilising assays specific for heavy chain/light chain (HLC) pairs have indicated that HLC ratios (IgG κ /IgG λ , IgA κ /IgA λ , IgM κ /IgM λ) provide a sensitive indication of monoclonal intact immunoglobulin production. This raises the possibility of developing monoclonal gammopathy screening protocols based upon the use of both HLC and FLC ratios. Combining these in one multiplex assay would have obvious time and cost benefits. *Aims.* To develop a single assay to screen for monoclonal intact immunoglobulin and monoclonal FLC production based on a prototype bead array assay concept developed by Dynex Technologies using the Binding Site polyclonal antibodies. *Methods.* Ninety six sera from patients previously identified as having a monoclonal gammopathy (16 IgG κ , 13 IgA κ , 17 IgM κ , 1 IgD κ , one free κ ; 18 IgG λ , 6 IgA λ , 15 IgM λ , 9 free λ) and 48 normal blood donor sera (NBDS) were screened. The assay was automated using a modified Dynex DS2 instrument. Diluted samples were added to wells containing specific, sheep antibody-coated beads to the following targets: κ FLC, λ FLC, IgG κ , IgG λ , IgA κ , IgA λ , IgM κ and IgM λ and incubating for 30 minutes, followed by a wash step. Due to individual dilution constraints, three wells were required per sample. Bound FLC or HLC was detected using horseradish peroxidase conjugated antibodies to total κ and λ LC, and incubated for 30 minutes. Following a final wash, luminol substrate was added for two minutes. Luminescence was captured using a Photometrics camera; images were interpreted using Array-Pro software. *Result.* 144 samples were tested; this generated 8 results for each sample, 1,152 in total. The assay correctly picked out abnormal FLC κ / λ ratios in 10/10 (100%) of the sera from patients with FLC myeloma and normal ratios in all NBDS samples. All 13 IgA κ samples had a raised κ / λ ratio, the 6 IgA λ κ / λ ratios were low, and in addition the appropriate κ or λ HLC was raised, with an abnormal HLC ratio, in all sera. With one exception, all the IgG gammopathies also gave appropriately abnormal results. The exception, a sample classified as an IgG3 λ monoclonal protein, was later found to be from a patient with primary biliary cirrhosis and polyclonal hypergammaglobulinemia, but no clear indication of monoclonality by IFE. The IgM samples were also 100% correctly detected, 17/17 IgM κ and 15/15 IgM λ , with corresponding abnormal κ / λ FLC ratios. The IgD κ sample had a high κ / λ FLC ratio. *Summary/Conclusion.* This study demonstrates that it is possible to quantify 8 immunoglobulin analytes in one assay and identify the FLC type and HLC class. This development could significantly simplify the monoclonal protein screening process into one assay format, thereby eliminating the need for gel electrophoresis. The inclusion of related markers e.g. cystatin C (for renal function) and beta 2-microglobulin (tumour mass) would further enhance the utility of this protocol.

A9 - NORMAL RANGES AND REFERENCE INTERVALS OF SERUM FREE LIGHT CHAINS CONCENTRATIONS IN A SPANISH URBAN POPULATION

J.M. Hernandez,¹ M.J. Jimenez-Cobaleda,² H. Muñoz,² R. Caro,² J.A. Quiéizan,¹ M. Latorre, L. Gonzalez,³ B. de la Hoz,⁴ B. Perez-Moneo⁵

¹Dpt. Hematology-Hospital General de Segovia; ²Dpt. Clinical Analysis-Hospital General de Segovia; ³C.Salud Segovia-3; ⁴C.Salud Segovia-1; ⁵C.Salud Segovia-2, Spain

Background. The diagnostic and prognostic importance of measuring serum free light chains in several monoclonal gammopathies is growing. Reference ranges in normal populations has been studied by some authors. However, we do not know the normal values in Spanish populations. The Spanish city of Segovia is conducting a study of population screening for monoclonal gammopathy of undetermined significance (MGUS). **Aims.** We undertook a study to determine the concentration ranges of serum free light chains kappa (SFK) and lambda (SFL) in our population. **Methods.** We analyzed 444 consecutive serum samples obtained between April 1 and May 16, 2010. The method of recruitment of participants in the study was offered by family doctors or by letter. Inclusion criteria: 1) to have lived 50 years on October 1 2008; 2) to live in Segovia. The determination of SFK and SFL and κ/λ ratios was carried out by turbidimetry. We studied the possible presence of serum monoclonal component by capillary electrophoresis and serum immunofixation (IFE). Furthermore, IFE was performed in doubtful cases.

Table. Parameters by gender and age groups with cutoff at 70 years.

	Sex	n	Media±SD	Median (Range)	Age	n	Media±SD	Median (Range)
Free κ chain /mg/L	Men	149	10.24±5.32	8.73 (2.9-35.2)	<70	253	8.87±3.92	8.3 (2.9-31.7)
	Women	282	9.52±4.12	8.68 (3.36-31.7)	≥70	178	11.05±5.12	9.66 (3.6-35.2)
	Men	149	17.45±7.92	15.5 (6.89-50)	<70	253	16.51±7.26	14.5 (6.11-48.9)
	Women	282	17.79±7.73	15 (6.11-61.9)	≥70	178	18.38±8.39	16.37 (8.29-61.9)
Ratio κ/λ	Men	149	0.59±0.23	0.57 (0.21-1.6)	<70	253	0.57±0.24	0.53 (0.21-2.46)
	Women	282	0.62±0.25	0.55 (0.22-2.46)	≥70	178	0.64±0.23	0.6 (0.22-1.6)
			Media±SD	Median (Range)				
Free κ chain /mg/L			9.77±4.58	8.71 (2.9-35.2)				
Free λ chain /mg/L			17.28±7.79	15.1 (6.11-61.9)				
Ratio κ/λ			0.6±0.23	0.56 (0.21-2.46)				

The test proved the non-Gaussian distribution of the series.

There were no significant differences between the values by gender. By contrast, the concentrations of SFK ($p<0.001$), SFL ($p<0.05$) and $R\kappa/\lambda$ ($p<0.01$) were significantly higher in patients older than 70 years. The reference intervals calculated were: sFK → 3.94-20.29 mg/L; sFL → 7.17-35.52 mg/L; $R\kappa/\lambda$ → 0.28-1.31.

Statistical Methods. Descriptive statistics of central tendency and distribution. We evaluated the normality of the series by asymmetry, kurtosis and Kolmogorov-Smirnov tests. To present the reference ranges we performed logarithmic transformation of the values in order to achieve Gaussian distributions; concentrations were calculated with limits of 95% of the determinations. Non-parametric test (Mann-Whitney

U) was used for the comparison of independent samples. **Results.** 13 MGUS were detected among 444 participants, so that the analysis was restricted to 431 samples who had no serum MC. The table shows the descriptive parameters of the series. **Conclusion.** The reference values and intervals obtained in our population are similar to those reported in other countries. Higher values in the elderly group could be due to impaired renal function but data was not available.

A10 - ESTABLISHMENT OF SERUM FREE LIGHT CHAINS REFERENCE INTERVALS, SELECTION AND EVALUATION OF CUT-OFF VALUE FOR κ/λ RATIO

Y. Liang,¹ W.-m. Chen,² Q.-t. Wang,² Y.-h. Zhai²

¹GongTi, BeiJing, China; ²Beijing Chaoyang Hospital affiliate of Capital Medical University, Beijing, China

Background. Suitable reference intervals are important for laboratory tests. As yet, serum FLCs reference intervals and κ/λ ratios have not been established for Chinese people and may differ from other populations such as those reported by Katzmman *et al.* **Aims.** To establish the reference intervals of serum free light chains, to select and evaluate the cut-off value for the ratio of free κ to free λ . **Methods.** Serum samples from 326 healthy donors were measured by free light chain immunoassays and the κ/λ ratios were calculated. The reference intervals were established by nonparametric methods suggested by CLSI document C28-A2. Serum free light chains were measured for samples from 117 plasma cell dyscrasia disease and related disorders and the cut-off value for κ/λ ratio were selected by means of ROC curve. Then the accuracy of the cut-off values was evaluated using another 178 serum samples of plasma cell dyscrasia disease and related disorders. **Results.** For sex subgroups, $Z<Z^*$ and $SD_{larger}<1.5\times SD_{smaller}$. The total mean square is homogenous for all age groups, but there was a statistically significant difference among them ($p<0.001$) by one way ANOVA test. According to CLSI document C28-A2, the sample size should be expanded if different age groups' reference intervals need to be assessed. In this study, the reference intervals for 20 to 86 years of old were 8.9 to 25.5 mg/L for free κ and 12.5 to 33.6 mg/L for free λ . The cut-off values for κ/λ ratio were 0.36 and 1.36. The diagnostic accuracy, sensitivity and specificity of the cut-off values were 93.3%, 95.2% and 88.5% respectively. **Summary/Conclusion.** There was no need to set up serum free light chains reference intervals for different sexes, the reference intervals for age 20 to 86 were different from the manufacturer's, the cut-off values 0.36 and 1.36 for κ/λ ratios were accurate in clinical test.

A11 - BONE MARROW CELL POPULATIONS AND SERUM FREE LIGHT CHAIN LEVELS IN MONOCLONAL GAMMOPATHY PATIENTS

M. Ayliffe, N. Sumar, J. Behrens

Immunology and Haematology Departments, Epsom and St Helier Hospital NHS Trust, Carshalton, Surrey, UK

Introduction. We have previously reported dual populations of plasma cells, some making intact monoclonal immuno-

globulin; others restricted to free light chain (FLC) production only, are found in the bone marrow of a minority of individuals with monoclonal gammopathies. We also showed changes in these cell populations within individual patients during the course of their disease and treatment. The presence of FLC-only cells was associated with shortened survival and we suggested that this might be a cellular mechanism underlying so-called light chain escape. Separate studies have observed sharp rises in FLC concentrations in the serum co-incident with light chain escape and urinary light chain rises. *Aims.* Here we aimed to formally test the connection between marrow plasma cell populations and the level of FLCs in serum taken at the same time, from individual patients. *Methods.* Bone marrow aspirates, collected from patients with paraproteins were examined using an established double immunofluorescence method that counts marrow plasma cells of each heavy and light chain isotype independently to establish the cellular populations present. These results were then compared to the concentration of FLC in serum, collected within 24hrs of the aspirates and assayed by the standard latex-enhanced nephelometric immunoassay. *Result.* We report here the results of the first 30 patients tested. The presence of a subpopulation of cells restricted to FLC production only, did appear to be generally associated with higher serum FLC levels. This observation was consistent for both kappa and lambda-restricted tumours. There were, however, some exceptional patients where serum FLC levels were high without a separate FLC-only cellular population. *Conclusion.* We need to continue to recruit more patients, especially those with high levels of serum FLC, to clarify these observations.

Figure 1.

A12 - IMPACT OF SERUM FREE LIGHT CHAINS ON THE ANION GAP IN MONOCLONAL GAMMOPATHIES

K. van Hoven,¹ L. McBride,² E. Bilotti,² A. McNeill,² L. Schmidt,² D. Schillen,² D. Siegel²

¹The Binding Site, Inc., San Diego, CA, USA; ²Hackensack University Medical Center, Hackensack, NJ, USA

Background. A decreased anion gap is associated with

IgG monoclonal gammopathies, and an increased anion gap is associated with IgA monoclonal gammopathies. The effect of free light chains on the anion gap is unknown. *Aim.* To study the impact of serum free light chains on the anion gap in light chain (LC), IgG and IgA monoclonal gammopathies. *Methods.* The anion gap, $\text{Na}^+ - (\text{Cl}^- + \text{HCO}_3^-)$, was calculated in patients with no evidence of monoclonal gammopathy by serum protein electrophoresis (SPEP), serum immunofixation and serum free light chains to establish a normal range. Then, the anion gap was calculated in patients with monoclonal gammopathies (mostly with myeloma) at presentation or at relapse. Following initial data analysis, patients with monoclonal gammopathies were excluded if serum albumin was <2.5 g/dL, serum Ca^{++} was >11.0 mg/dL, or serum creatinine was >2 mg/dL.

Results. In 40 patients without monoclonal gammopathy, the anion gap range was 9–15. In 37 of these 40 patients, the anion gap range was 9–14, and thus this was established as the normal range. A decreased anion gap was found in 35% of 102 IgG monoclonal gammopathies. An increased anion gap was identified in 36% of 50 IgA monoclonal gammopathies. The anion gap was increased in 25% and decreased in 3% of 60 LC monoclonal gammopathies. With exclusion criteria applied, the anion gap was decreased in 34% of 89 IgG, and increased in 31% of 42 IgA monoclonal gammopathies. In 40 LC monoclonal gammopathies, 17% had an increased and 3% had a decreased anion gap. A poor correlation (strongest r was 0.58) was found between the anion gap and the paraprotein spike on SPEP, as well as between the anion gap and the mean concentration of the involved Ig (IgG 4.64 g/dL ± 0.18 (SEM), IgA 3.27 g/dL ± 0.26 , free kappa 0.5 g/dL ± 0.08 [or 4956 mg/L ± 769 ; $n=20$], free lambda 0.36 g/dL ± 0.08 [or 3607 mg/L ± 809 ; $n=20$]). Importantly, measurable paraprotein spikes were identified in only 23 of 40 (58%) LC monoclonal gammopathies, but free light chains were diagnostic in all: free kappa ranged from 478–14,400 mg/L in 20 kappa light chain processes, and free lambda ranged from 440–17,300 mg/L in 20 lambda light chain processes. In IgG monoclonal gammopathies with a decreased anion gap, the mean clonal serum free light chain concentration was 620 mg/L ± 198 versus 967 mg/L ± 508 in other IgG monoclonal gammopathies ($P=\text{NS}$). In IgA monoclonal gammopathies with an increased anion gap, the mean clonal serum free light chain concentration was 821 mg/L ± 420 versus 431 mg/L ± 177 in other IgA monoclonal gammopathies ($P=\text{NS}$). *Conclusions.* The association of IgG monoclonal gammopathies with a decreased anion gap was confirmed, as was the association of IgA monoclonal gammopathies with an increased anion gap. There was a weak association between LC monoclonal gammopathies and an increased anion gap. No significant impact of free light chains on the anion gap in IgG and IgA monoclonal gammopathies was identified, likely due in part to relatively low serum concentrations of clonal free light chains versus IgG or IgA.

FREE LIGHT CHAINS IN DIAGNOSIS AND PROGNOSIS

B13 - SERUM FREE LIGHT CHAIN MEASUREMENTS IN SCREENING FOR LYMPHOPROLIFERATIVE DISEASES - COMPARISON RECOMMENDED AND RENAL REFERENCE INTERVAL FOR FREE LIGHT CHAIN RATIOS IN PATIENTS WITH RENAL FAILURE

Z. Cermakova,^{1,2} M. Dastych,^{1,2} J. Gottwaldova¹

¹Department of Clinical Biochemistry University Hospital Brno; ²Department of Laboratory Method Faculty of Medicine Masaryk University, Czech Republic

Background. The assay of serum free light chains (sFLCs) is carried out in patients with symptoms of monoclonal gammopathies (MG). Suggestion of monoclonal sFLC production is when the ratio of kappa to lambda is outside the reference interval (0,26-1,65). For patients with renal impairment the recommended renal reference interval is (0,37-3,1). **Aims.** Comparison of the diagnostic sensitivity of using the recommended reference range with using the renal reference range for sFLC ratios in patients with renal failure. **Methods.** We have retrospectively evaluated samples from 334 patients who were newly diagnosed with MG in the time period of 2008-2009 at the University Hospital in Brno. For these samples we carried out: serum electrophoresis (sEPG), urine electrophoresis (uEPG), serum immunofixation (sIFE), urine immunofixation (uIFE), sFLC κ/λ ratio, serum creatinine concentration and calculated glomerular filtration rate (GFR) according to MDRD (Modification of Diet in Renal Disease). The group of tested patients included 216 diagnosed with multiple myeloma (MM) and 118 people diagnosed with MG of undetermined significance (MGUS). **Results.** 39.4% patients were found with reduced GFR in the group of patients with MM. Also, 11.9% of patients showed lowered GFR in the group of patients with MGUS. The highest sensitivity of the conducted tests was reached by a combination of the three tests: sIFE, uIFE, and sFLC κ/λ ratio. 97.2% of patients with MM and 96.6% patients with MGUS were found positive with this method and with the recommended reference interval for sFLC ratio. We used the same combination of tests for the patients with renal failure but using the renal reference interval for sFLC ratio. Although the number of positive results was lower in both groups of patients, the sensitivity of the combination of the three tests was the same. **Summary/Conclusion.** We have proved that the highest sensitivity was reached by the combining all three tests: sIFE, uIFE and sFLC κ/λ ratio. Using the renal reference range in patients with renal failure did not reduce sensitivity for this combination in both groups of patients with MM or MGUS.

B14 - SERUM FREE LIGHT CHAINS IN THE SCREENING OF MONOCLONAL GAMMOPATHIES

A. Miralles, C. Martinez, C. Sempere, G. Galar, B. Alegre
Hospital de Sagunto, Puerto de Sagunto, Valencia, Spain

Background. Plasma cells proliferative disorders may be presented in a variety of ways, and the diagnostic key is often finding a monoclonal gammopathy (MG) in blood and urine. Electrophoresis (SPE) and immunofixation (IFE) in serum (s) and urine (u) are the most frequently used methods. However, with the recent addition of serum free light chains (sFLC) quantification, some authors proposed a new screening panel using only serum. **Aims.** To evaluate in a prospective study the benefits of sFLC quantification in the screening/diagnostic protocol for monoclonal gammopathies (MG). **Methods.** All samples requested for a MG study from December 2008 to May 2010 were included. Samples coming from laboratory findings by SPE (monoclonal band or hypoglobulinemia <0.6 g/dL) that were not previously studied were also included. All controls from the follow-up of a previously known MG were excluded. Serum and recent urine (2nd of the morning) were requested. The study protocol included SPE, sIFE and uIFE in agarose gel, urine proteins, quantification of IgG, A and M, total kappa and lambda, and sFLC by turbidimetry. **Results.** We performed 312 MG screening studies from 293 patients. These requests represent 10% of the total SPE requests in the same period. 53 new MG were identified (7 multiple myeloma (MM), 1 light chain (LC) MM, 4 smoldering MM, 3 AL amyloidosis, 34 MGUS, 4 plasmacytoma). From the 53 MG diagnosed, SPE was able to identify 84.9% of the cases (1AL, 7MM, 4SMM, 33MGUS). The use of SPE + sFLC detected 94.3% (1 AL; 7 MM; 4 SMM; 33 MGUS + 2 AL, 1 LCMM, 5 MGUS). 5.7% (3 cases) were only detected by uIFE. During the follow-up of these patients uIFE became negative for what we considered as transitory MP. From the remaining 259 studies in which a MP was not detected, 42 presented with an altered sFLC ratio of between 1.66–3.27. It was observed that at least 43% of these patients had an altered renal function (creatinine (Cr) $>1,3$ mg/dL). When the recommended sFLC ratio renal range was applied (0.37–3.1) for patients with renal impairment, the number of patients with abnormal ratios was $<7.7\%$. **Discussion.** The use of SPE + sFLC in the screening of a MG identified all patients with a significant abnormality. SPE + sFLC is a simple diagnostic algorithm for MG with high diagnostic sensitivity with the benefit of working only with serum. sIFE and uIFE are laborious techniques not recommendable for initial screening. However, they are necessary to complete the study for the detected MG.

B15 - PREVALENCE OF MONOCLONAL COMPONENTS IN PLASMA DONORS IN CATALONIA

E. Ruiz Ortiz de Arrizabaleta,¹ A. Teniente Serra,¹ A. Marín Sánchez,¹ I. Salvador Corres,¹ M. Sáez Bruguera,² A. Ester Codins,² R. Pujol-Borrell,¹ E. Martínez-Cáceres¹

¹Laboratorio de Inmunología (LIRAD)-BST, Badalona, Spain; ²Banc de Sang i Teixits (BST), Barcelona, Spain

Background. Prevalence of monoclonal gammopathy of undetermined significance (MGUS) is 3% in people >50 years, and the risk of progression to multiple myeloma (MM), Waldenström's macroglobulinemia, AL amyloidosis or lymphoproliferative disorders, 1%/year according to a recent review (Kyle, Leukemia; 2010). Plasma donors of the Banc de Sang i Teixits of Catalonia (BST), are screened for total serum proteins, immunoglobulin (Ig) levels and serum protein electrophoresis (SPE) at the inclusion and every six donations in order to rule out the existence of plasma abnormalities such as the presence of a monoclonal component. This data provides us excellent information to assess the prevalence of MGUS in our population. **Aims.** To analyze the prevalence of monoclonal components in plasma donors in Catalonia (Spain). **Methods.** A total of 8,262 plasma donors were analyzed between January 2006 and December 2009, corresponding to 31,064 donations. Of these, 4,981 (60.3%) were men and 3,281 (39.7%) women. Average age was 43.7 years (28% >50 years and 72% <50 years). Ig levels were measured by nephelometry and SPE by capillary electrophoresis. When suspicious peaks were detected in the SPE the sample were analyzed by immunofixation electrophoresis. Samples with confirmed monoclonal protein (M-protein) and abnormal total Ig levels were analyzed for free light chains (FLC). **Results.** Monoclonal components were suspected in 104 plasma donors, and an M-protein was confirmed in 49 cases (47%), corresponding to 0.59% of all donors. From them, 34 (69.4%) were men and 15 (30.6%) women. 25 (51%) of the 49 donors with a monoclonal component were <50 years old. Monoclonal components were: a) IgG isotype: IgG kappa 22 (44.9%); IgG lambda 13 (26.5%); b) non-IgG isotype: IgM kappa 3 (6.1%); IgM lambda 1 (2%); IgA kappa 4 (8.2%); IgA lambda 3 (6.1%). In 3 donors (6.1%) a biclonal component was found. In 4 (8.2%) of 10 donors an abnormal FLC ratio was detected. **Discussion.** The analysis of monoclonal components in healthy plasma donors of Catalonia shows an incidence of 0.59% (1.04% in donors <50 years and 0.42% in donors >50 years). Most of the monoclonal components were IgG. When monoclonal components are suspected in plasma donors it is advisable to fully characterize them in order to exclude the products from the plasma pool used for Ig production and to refer donors to hematological consultation.

B16 - SERUM FREE LIGHT CHAIN ASSAYS (sFLC) IN PATIENTS WITH MGUS ATTENDING THE HAEMATOLOGY DEPARTMENT AT ST. MARY'S HOSPITAL IN LONDON

S. Adballa

Imperial College Healthcare Trust, London, UK

Introduction. MGUS is defined as a monoclonal gammo-

pathy without any end organ damage with a paraprotein (PP) level of less than 30g/L and clonal bone marrow plasma cells (CPC) of less than 10% (International myeloma working group consensus, Leukaemia 2010). Patients with levels of PP of over 30g/L and those with CPC of over 10% without end organ damage are classified as Smoldering Multiple Myeloma. A third of patients with MGUS have abnormal sFLC ratios. A recent publication from the Mayo clinic has further risk stratified patients with MGUS according to the sFLC abnormalities, type of immunoglobulin (Ig) and levels of Ig into three risk groups (Rajkumar et al. Blood, 2005). The highest risk of progression was found in patients with three risk factors and the risk of progression was increased with an increase of sFLC imbalance. **Aim of study.** To prospectively test the Mayo clinic risk stratification in our patient population with MGUS in a practical clinical setting. **Patients and Methods.** The patients were a cohort of 174 patients with MGUS who were being followed up in the paraproteinaemia clinic at St Mary's Hospital. Patients underwent the usual investigations at diagnosis, but not all the patients had sFLCs measured initially. Bone marrow and skeletal surveys were only carried out routinely on patients with paraproteins of above 10 g/L until 2009 and 15g/L after that date unless there were additional clinical features. Similarly, skeletal surveys were carried out only on selected cases using similar criteria. **Results.** Of the 174 patients, 85 had abnormal sFLC ratios (48.6%). Of these, 59 had overproduction of kappa chains (ratio range: 1.77 to 1066) and 26 had overproduction of lambda chains (ratio range: 0.11 to .25). 4 patients progressed to symptomatic MM after 2, 8, 12 and 13 years of follow-up. All 4 had abnormal sFLC ratios. **Conclusion.** We found a higher rate of sFLC imbalance in our patient population with MGUS than in the Mayo clinic and other studies. Several factors may contribute to this including the assessment of sFLCs not always being at the point of diagnosis. Following the Mayo clinic risk stratification model, patients with low risk disease did not have bone marrow and skeletal survey studies at diagnosis and the period of follow up for low risk patients has also been increased leading to more efficient use of clinician and patient time.

B17 - NORMAL SERUM FREE LIGHT CHAIN RATIO AT PRESENTATION IS MORE COMMON IN IgA-κ MULTIPLE MYELOMA: A SINGLE CENTRE OBSERVATIONAL STUDY

H. Ahmed, S. Simpson, A. Roy

Basingstoke and North Hampshire NHS Foundation Trust, Basingstoke, Hampshire, UK

Background. Several studies have shown that baseline serum free light chain ratio (sFLCr) is an independent prognostic factor in patients with multiple myeloma and other plasma cell disorders. Baseline sFLC correlated with serum creatinine, lactate dehydrogenase, marrow infiltration by plasma cells and light chain type multiple myeloma in some studies. The relationship between baseline sFLCr at diagnosis and the different subtypes of multiple myeloma has not been reported to date. **Aims.** To

analyse the baseline sFLCr in relation to the different subtypes of multiple myeloma and to assess the relationship between the baseline sFLCR and parameters of disease activity. **Methods.** All newly diagnosed multiple myeloma patients in our centre between June 2005 and January 2010 were included. The sFLC measurements were performed by immunonephelometry (**Freelite** assay; The Binding Site Group Ltd, Birmingham, UK). sFLCr was calculated as κ/λ (normal range 0.26-1.65). Clinical and laboratory parameters at the time of diagnosis were recorded. Statistical tests were performed with SPSS software. Categorical variables were contrasted by the Fisher exact test and median differences by the Mann-Whitney U test. **Results.** 48 patients were included in this study. The male to female ratio was 1.4:1 with a median age of 73 years (range 45- 94 years). The majority of the patients (56%) had IgG multiple myeloma, 31% had IgA multiple myeloma and 13% were light chain only subtype. 44 patients had their sFLCr measured at diagnosis. All patients with IgG and FLC multiple myeloma had an abnormal sFLCr at presentation. Interestingly 33% of IgA multiple myeloma patients in our study showed an absolutely normal sFLCr with a further 20% of patients showing only mild elevation of monoclonal FLCs. Normal baseline sFLCr was associated with IgA- κ subtype of multiple myeloma ($p=0.008$). Normal sFLCr correlated with negative Bence Jones proteinuria, heavy bone marrow infiltration (>45% plasma cell infiltration), and the ISS stage II – III. However, there was no observed relation of the baseline sFLCr with serum creatinine, calcium level or serum albumin. The median baseline sFLCr in IgA- κ group was 2.59 (range 0.5–310.6) in comparison with 32.84 (range 1.74-1791.75) in IgG- κ group ($p=0.009$). In contrast, IgA- λ and IgG- λ groups showed no difference in the median baseline sFLCr. **Summary/Conclusion.** Our study has shown, for the first time, that IgA- κ multiple myeloma patients commonly present with normal sFLCr. Our data suggests that sFLCr at presentation may be of less prognostic value in IgA- κ multiple myeloma subtype. This might also have an implication in the assessment of stringent complete response in this group of patients. Further analysis is required to assess the clinical course in this group of patients.

B18 - SERUM FREE LIGHT CHAINS RATIO: AN INDEPENDENT PROGNOSTIC FACTOR FOR OVERALL SURVIVAL AND PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA

M. Sobh, S. Morisset, T. Guilli, S. Ducastelle, F. Barraco, Y. Chelghoum, X. Thomas, A. Praire, F. Nicolini, C. Chapuis-Cellier, M. Michallet

Edouard Herriot Hospital, Haematology, Lyon, France

Background. Some studies have evaluated the prognostic value of the serum free light chain (sFLC) levels expressed as κ/λ ratio (sFLCR), but this factor has not been introduced yet into the international staging system (ISS). **Aims.** To evaluate the impact of sFLCR, measured at diagnosis in multiple myeloma (MM) patients, on the progression free survival (PFS) and overall survival (OS). **Methods.** A total of 118 consecutive patients diagnosed between years 2002

and 2008, and for which we have assessed the sFLCR at diagnosis, were included in this study. There were 73(62%) males and 45(38%) females, median age: 57 years [34-72], 63 IgG, 35 IgA, 2IgD and 18 light chains; 11(9%) in stage I, 13(11%) in stage II and 94(80%) in stage III. Among 55(47%) having FISH analysis done, 28(24%) detected a chromosome 13 deletion. Measurements of sFLC were made using the **Freelite** test from the Binding Site on a BNII, Dade Behring. According to the distribution of the different ratios, we have defined three groups: group1 ($n=25$): patients with $0.13 < \text{sFLCR} < 3.3$ which represents double the normal range(0.26-1.65); group2 ($n=63$): patients with $\text{sFLCR} > 3.3$ and group3 ($n=30$): patients with $\text{sFLCR} < 0.13$. Kaplan Meier and Cox regression analysis were performed to study the PFS and OS in different groups. **Results.** After a median follow-up of 38 months [3.3-93.7], the probability of OS at 5 years for groups 1, 2 and 3 was 75% [56-100], 60% [47-76] and 40% [23-69] respectively; and the probability of PFS at 5 years was 69% [49-96], 43% [31-60] and 29% [15-54] respectively (Figure).The multivariate analysis showed that both OS and PFS are significantly worse with a more abnormal sFLCR. **Summary/Conclusion.** Our study has showed that abnormal sFLCR at diagnosis predicts OS and PFS independently of any other concomitant variables. We suggest that this factor deserves more focus for its validation as a prognostic factor in MM.

Figure.

B19 - THE CLINICAL VALUE OF THE SERUM FREE LIGHT CHAIN ASSAY IN NEWLY DIAGNOSED UNIFORMLY TREATED ELDERLY PATIENTS

M.E. Fernández Redondo,¹ B. Paiva,² J. Martínez-López,¹ J.J. Lahuerta,¹ M.V. Mateos,² J.F. San Miguel,² J. Bladé,³ M.B. Vidriales,⁴ L. Rosiñol,⁴ J. Besalduch,⁴ J. Bargay,⁴ M. Canales,⁴ A. Alegre,⁴ J. García-Laraña,⁴ R. Martínez,⁴ J.F. Tomás⁴

¹Hospital 12 de Octubre de Madrid, Servicio de Hematología, Laboratorio de Biología Molecular, Madrid; ²Hospital Universitario de Salamanca;

³Hospital Clinic de Barcelona, Spain; ⁴Spanish Myeloma Group

Background. The measurement of serum free kappa and lambda light chain (sFLC) concentrations provides a highly sensitive tool for the diagnosis of monoclonal gammopathies. In multiple myeloma (MM), it has been suggested that the sFLC ratio at initial diagnosis is an important predictor of prognosis. However, the majority of these results were obtained retrospectively from non-uniformly treated, younger MM patients (transplant candidates). **Aims.** To evaluate the clinical value of the sFLC assay in newly diagnosed, uniformly treated elderly MM patients. **Material and methods.** A total of 230 symptomatic MM patients were included in this study, all of them uniformly treated according to the Spanish GEM05>65y protocol (two arms of 6 cycles of bortezomib/melphalan/prednisone (n=118) or bortezomib/thalidomide/prednisone (n=112) supplemented with maintenance therapy). Median follow-up was 32 months and the EBMT criteria were used for response evaluation. sFLC levels were measured at diagnosis using standard techniques (Freelite, The Binding Site Group Ltd., Birmingham, UK) performed on a Dade-Behring Nephelometer BNII (Deerfield, IL). sFLC ratios between 0.26-1.65 were considered normal. On the basis of the distribution of sFLC values for and patients, sFLC ratios <0.02 and >10 were classified as highly abnormal. **Results.** Abnormal sFLC ratios were detected at diagnosis in 204 (89%) out of 230 MM patients. In order to gain further insight into the group of newly diagnosed myeloma patients with normal sFLC ratios (n=26; 11%), we have compared the baseline characteristics of abnormal vs. normal sFLC cases. No significant differences were found for patients' age, light chain myeloma subtype, biochemical markers, ISS and the pattern of cytogenetic abnormalities, including the high-risk t(4;14), t(14;16) and/or del(17p). However, we have found that abnormal vs. normal baseline sFLC patients showed significantly different distribution of heavy chain myeloma subtypes (IgG, IgA and B-J: 56%, 31% and 13% vs. 81%, 19% and 0%; respectively, $p=.03$), bone marrow plasma cell (BMPC) infiltration (12% vs. 3.1%; respectively, $p=.01$) and normal PC detected by flow cytometry (patients with >5% normal-PC/total-BMPC: 8% vs. 32%; respectively, $p<.001$). No differences were found between abnormal vs. normal baseline sFLC patients for overall response (87% vs. 88%; respectively) and complete remission (25% vs. 28%, respectively) rates after induction therapy, nor for progression-free survival (PFS; median of 28 months vs. not reached -NR-; $p=.2$) and overall survival (OS; $p=.2$).

In turn, patients with highly abnormal baseline sFLC ratios - <0.02 or >10 - (n=145; 63%) when compared to the rest of cases (n=85; 37%) did showed a significantly decreased PFS (median of 27 vs. 35 months, $p=.03$) and also a trend for decreased OS ($p=.09$). **Conclusions.** In summary, this study confirms the prognostic value of the sFLC assay in elderly MM patients (non-transplant candidates) treated with novel agents. The presence of baseline normal sFLC ratios should be taken in consideration particularly in cases with lower BMPC infiltration and residual normal PC.

B20 - FREE LIGHT CHAINS RATIO AS A MARKER OF THE COURSE AND SURVIVAL IN PLASMA-PROLIFERATIVE DISEASES

Z. Mijuskovic,¹ V. Radović,² J. Pejovic,¹ L. Tukić,³ S. Marjanović³

¹Institute of Medical Biochemistry, Military Medical Academy, Belgrade; ²Institute for research and development, STADA Hemofarm, AD Belgrad; ³Clinic of Hematology, Military Medical Academy, Belgrade; Serbia

Background. The quantification of monoclonal immunoglobulins (Ig) and its fragments are used for the monitoring of plasmaproliferative disease course and effects of therapy. Relatively new laboratory tests in serum for the quantification of kappa (κ) and lambda (λ) free light chains (FLC) and the calculation of the FLC ratio represents additional parameters for monitoring of malignant plasmocytic production. **Aims.** The aim of FLC examination was to evaluate significance of κ/λ ratio as a prognostic factor for progression, remission and survival in different disease groups.

Table. Percentage of FLC ratio in plasmaproliferative diseases groups. From 2004 to 2010 yrs.

Disease Groups (n-number of patients)	κ/λ ratio cut point <0.26 or >1.65 abn. value <0.26-0.03 λ isotype – low RR	κ/λ ratio cut point <0.26 or >1.65 abn. value >1.65-4 κ isotype – low RR	κ/λ ratio cut point <0.03-0.001 abn. value >4-32 intermediate RR	κ/λ ratio abn. value >32 high RR
MGUS (n=31)	5/31 (16%)	16/31 (52%)	10/31 (32%)	0
LCD (BJM) (n=28)	4/28 (14%)	7/28 (25%)	0	17/28 (60%)
LCDD (n=3)	1/3 (33%)	0	1/3 (33%)	1/3 (33%)
AL (n=3)	0	0	1/3 (33%)	2/3 (66%)
NSMM (n=3)	1/33 (33%)	1/33 (33%)	1/33 (33%)	0
MM (n=15)	3/15 (20%)	4/15 (2%)	3/15 (20%)	5/15 (%)

Methods. The concentrations of Ig and FLC were measured by immunonephelometric method on a Siemens BN II analyser, combined with serum protein electrophoresis on Sebia (Capillarys) and immunofixation (Hidrasys) methods. In this examination, 83 patients from 6 different disease groups were investigated during the period of 7 years: 1. Monoclonal gammopathy of undetermined significance - MGUS (n=31); 2. Light chain multiple myeloma-LCMM or Bence Jones myeloma (n=28); 3. Light chains deposit disease-LCDD (n=3); 4. Primary AL

amyloidosis (n=3); 5. Nonsecretory multiple myeloma - NSMM (n=3) and 6. Multiple myeloma - MM (n=15). Cut point intervals for κ/λ ratios was (0.26-1.65). **Results.** According to the ISS model of stratification risk of disease progression, values <0.26 to 0.03 for λ and >1.65 to 4.0 for κ -isotype represents low relative risk (RR) of disease progression. Values inside intervals <0.03 to 0.001 as well as >4.0 to 32 indicate intermediate and >32 are connected with high RR of disease progression. Results showed that in MGUS group 10/31 (32%) patients had elevated κ/λ ratios of intermediate RR for progression. Patients with worse prognosis were from LCMM group in which 17/28 (61%) had high RR (table). Among the LCMM group 5/28 (18%) patients died during the period of 24-36 months. In LCDD and AL, rare disease groups, 2/3 patients died inside 12-28 months. **Summary/Conclusion.** Results confirmed concordance with ISS model of stratification risk of disease progression in MM. sFLC ratio didn't show additional diagnostics aid in MM group, compared with the intact immunoglobulin concentrations.

B21 - VALUE OF SERUM FREE LIGHT CHAIN ANALYSIS IN IgD AND IgE MULTIPLE MYELOMA

K. van Hoeven,¹ A. McNeill,² E. Bilotti,² L. McBride,² L. Schmidt,² D. Schillen,² D. Siegel²

¹The Binding Site, Inc., San Diego, CA, USA;

²Hackensack University Medical Center, Hackensack, NJ, USA

Background. IgD and IgE are the rarest myeloma subtypes. IgD myeloma has been associated with hypogammaglobulinemia and renal impairment at presentation and an aggressive course. **Aim.** To explore the value of serum free light chain (sFLC) analysis in conjunction with other laboratory tests in diagnosis and monitoring of IgD and IgE myeloma. **Methods.** Data were analyzed from electronic laboratory reports of patients with IgD and IgE myeloma treated between 2003 and 2007. **Results.** Among 7 IgD myeloma patients (5 men, 2 women), median age was 62 yr (range 41-72). Baseline characteristics are shown (table). Serum protein electrophoresis (SPEP) demonstrated an M-protein (1.1 g/dL) in only 1 patient; in the other 6 patients, SPEP was non-diagnostic (3 normal, 2 hypogammaglobulinemia, and 1 faint non-quantifiable band). Initial serum and urine immunofixation in two patients yielded light chains only, but subsequent studies demonstrated IgD myeloma. Six patients had increased IgD levels; the other patient demonstrated an IgD M-protein by immunofixation only. Baseline sFLC ratios were abnormal in the 6 patients for whom data were available. Only 2/6 patients had baseline serum creatinine >2.0 mg/dL. These 2 patients had the highest clonal sFLC concentrations (kappa 81,200 mg/L, lambda 3,600 mg/L). IgD has a short half-life (2-days), thus IgD and sFLC tended to show similar changes while monitoring treatment response. In the two patients with significant renal impairment, sFLC decreased during treatment and mirrored improved renal function. In one patient with IgE kappa myeloma (SPEP M-protein 0.81 g/dL; IgE $>50,000$ mg/dL [normal ≤ 114 mg/dL], free kappa 42.8 mg/L, free lambda 10 mg/L), sFLC concentrations were

below the measurable disease threshold of ≥ 100 mg/L at presentation and during follow-up. **Conclusion.** sFLC analysis was a valuable clinical tool in IgD myeloma. IgD myeloma may be under-diagnosed given that it may be confused with light chain myeloma.

Table.

Baseline (serum)	Median (range)	Normal range
IgD, mg/dL (n=5)	123 (12.93-842)	0.13-15.27
Creatinine, mg/dL (n=6)	1.45 (0.8-7.50)	0.5-1.3
β_2 -microglobulin, mg/dL (n=7)	3.94 (2.37-27.8)	≤ 2.51
Uncorrected Ca ⁺⁺ , mg/dL (n=6)	9.1 (6.8-9.7)	8.6-10.2
Albumin (sPEP), g/dL (n=7)	3.80 (2.60-4.50)	3.5-4.7
Globulin, g/dL (n=5)	2.9 (1.9-4.1)	2.2-4.2
Free light chain clone		
- κ , mg/L (n=3)	1310 (143-81,200)	3.3-19.4
- λ , mg/L (n=3)	987 (163-3600)	5.7-26.3
International stage (n=7)	stage 1=2; stage 2=2; stage 3=3	Not applicable

B22 - SMOLDERING MULTIPLE MYELOMA WITH IgE λ PLUS λ FREE LIGHT CHAINS. A DIAGNOSTIC CLINICAL CASE

B. Cabanas López de Vergara, M.A. Rodríguez López, R. Ocampo Martínez, J.P. Plaza Villa

Complejo Hospitalario Universitario de Vigo, Calle de Pizarro, Vigo, Spain

Background. Most multiple myeloma (MM) patients are characterized by the secretion of a monoclonal protein (MP) of the IgG or IgA type, and less frequently by the light chain type (Bence Jones). IgD, IgM, and IgE are uncommon MP found in patients sera (1%, 0.2%, and 0.02%, respectively). **Methods/Clinical presentation.** An 82-year old male was admitted with respiratory problems and congestive cardiac failure. Although the protein analysis results did not show any clear MP, a suspicious peak in the gamma fraction was observed and, consequently, a 24h urine immunofixation (uIFE) was performed. The uIFE results were negative but the concentration of the λ free light chain in the serum (sFLC) was >289 mg/dL. Further laboratory investigations for the diagnosis of a MP were performed. Serum immunofixation (sIFE) confirmed the increased levels of λ sFLC. While the concentrations of immunoglobulins IgG, IgA, and IgM were of 600, 172, and 27mg/dL, respectively, the IgE concentration was of 328,500 kU/L. sIFE against IgE antiserum confirmed the MP result. Therefore, the patient presented a λ type IgE MP and a free λ sFLC. No numerical or structural chromosomal alterations were found at the time of diagnosis and the FISH assay for the genes Rb, p53, and IgH did not show any alteration. There was 16% bone marrow infiltration by plasma cells and PCR detected monoclonal IgH. Subsequent follow-up of the uIFE continued to be negative, while the sIFE continued to be positive for IgE, and the sFLC ratios continued to be altered (0.196; 0.204; 0.183). **Conclusions.** IgE MM is believed to have a greater clinical malign course than other forms of monoclonal gammopathy. There is a need for sensitive techniques that detect monoclonality and prompts the clinical tests towards a definitive diagnostic.

In this case, the altered concentration of sFLC and the abnormal κ/λ ratio led to the diagnosis of smoldering MM with IgE λ plus FLC λ . Furthermore, the sFLCs were found to be most useful simple test for patient follow-up because of the scarcity of monoclonal IgE tests.

B23 - ROLE OF KAPPA FREE INDEX IN PREDICTING CONVERSION OF CLINICALLY ISOLATED SYNDROMES TO MULTIPLE SCLEROSIS

L.M. Villar, M. Espiño, J.C. Álvarez-Cermeño

Hospital Ramon y Cajal, Madrid, Spain

Background. It has been recently described that local production of free kappa chains within the Central Nervous System calculated by kappa free index may be a useful tool in the diagnosis of multiple sclerosis (MS). Most MS patients initiate with a clinically isolated syndrome (CIS). However some CIS are auto-limited and do not develop MS. The finding of reliable markers that predict the conversion of CIS to MS is of great importance since it has been widely shown the importance of early treatment in MS. **Aims.** To investigate the role of the kappa index in predicting the conversion of CIS to MS. Comparison with quantitative and qualitative measures of intra-theal immunoglobulin synthesis. **Methods.** Patients: 96 with CIS consecutively recruited at the MS unit from Hospital Ramon y Cajal (Madrid, Spain) from 2001 to 2009. Control group: 20 patients with other neurological diseases. Baseline studies. We studied IgG, IgM, albumin, free kappa lambda chains and IgG and IgM oligoclonal bands in serum and CSF of all patients. A magnetic resonance imaging study was also performed in every CIS patients within 3 months from disease onset Follow-up study: Patients were examined every 6 months and at the time of relapse. Statistical differences were studied by an Anova test, with a Dunns Post Hoc analysis. **Results.** 36 patients converted to clinically definite MS during follow-up (Group 1) and 60 did not. We divided patients that did not converted to MS as follows: Group 2 included 35 patients with IgG oligoclonal bands and at least two T2 MRI lesions, which show a high probability of conversion to MS. Group 3 included twenty five CIS that did not show oligoclonal IgG bands and did not fulfil Barkhof-Tintore MRI criteria and have a very low probability of conversion to MS. Group 1 and 2 patients showed similar increase of kappa free index ($p<0.0001$) when compared with group 3 patients. A moderate increase of kappa free index could also be observed in some patients with other neurological inflammatory diseases, although this group did not show statistical differences in the kappa index with respect to group 3 values. **Discussion.** Kappa free index may be a useful biomarker to predict conversion to MS in patients with clinically isolated syndromes.

FREE LIGHT CHAINS AND DISEASE MONITORING

C24 - SERUM FREE LIGHT CHAINS ADD TO M-PROTEIN BY IMMUNOFIXATION IN RESPONSE ASSESSMENT IN NEWLY DIAGNOSED PATIENTS WITH MYELOMA RECEIVING NOVEL THERAPIES AND MAY HOLD UNIQUE, A PRIORI PROGNOSTIC POWER PRIOR TO HDC/SCT

S. Mori, B. Crawford, J. Roddy, G. Phillips, P. Elder, C. Hofmeister, Y. Efebera, C. Greenfield, K. Rose, D.M. Benson Jr

The Ohio State University Comprehensive Cancer Center Columbus, OH, USA

Background. The care of patients with multiple myeloma (MM) has evolved rapidly with the advent of novel treatments, including bortezomib and the immunomodulatory agents, thalidomide and lenalidomide. In addition, the wide-spread availability of technology to measure free light chains (sFLC) in serum has provided an additional biomarker for screening, prognosis, and monitoring of patients (pts) with oligo/non-secretory MM. Whether novel agents will obviate the need for high dose chemotherapy (HDC) and autologous stem cell transplantation (SCT) remains controversial, and most newly-diagnosed, transplant eligible patients with MM now receive an induction regimen incorporating a novel agent prior to HDC/SCT. Moreover, specific roles for sFLC in response assessment and prognosis following HDC/SCT remain to be defined. **Aims.** 1.To determine if sFLC contributes to response assessment in newly diagnosed MM undergoing induction therapy 2.To determine if sFLC holds prognostic value for pts with MM undergoing HDC/SCT. **Methods.** Under an IRB-approved protocol, data were collected on n=73 pts with MM who received novel agent-based induction therapy prior to stem cell mobilization and HDC/SCT. Pt demographics and disease characteristics at diagnosis and following induction were collected specifically; M-protein measured by immunofixation (IFE), serum FLC, kappa/lambda ratio, as well as bone marrow plasmacytosis at diagnosis and post-induction therapy. Linear regression models were created with either one or two primary predictor variables using residual disease burden (as defined by bone marrow plasmacytosis) post-induction as the outcome variable. Kaplan-Meier analyses of PFS and OS post-HDC/SCT were performed. **Results.** M-protein IFE correlated with bone marrow plasmacytosis and diagnosis ($p=0.001$) and post-induction therapy ($p<0.001$). Involved sFLC also correlated with bone marrow plasmacytosis at diagnosis ($p=0.02$) and post-induction ($p=0.03$). 16 individual, multivariable-regression models were created to determine residual marrow plasmacytosis with M-protein IFE or involved sFLC serving as the primary predictor. Statistical significance was observed in 6 of the 8 models regarding M-protein as the primary predictor, whereas statistical significance was observed in only 2 of the 8 models regarding involved sFLC as the primary predictor. 8 additional linear regression models were created using

M-protein IFE and involved sFLC as co-primary predictors. In these models, the change from diagnosis to post-induction of both M-protein and involved sFLC performed best at predicting marrow plasmacytosis. A clear OS survival advantage was observed post-HDC/SCT in patients in whom involved sFLC fell by more than 50% during induction ($p=0.009$). Also, in pts in whom involved sFLC normalized during induction, significantly better PFS ($p=0.009$) following HDC/SCT was also observed. Similar findings were not seen with M-protein by IFE change or pre-HDC/SCT marrow plasmacytosis. **Summary/Conclusion.** As independent predictors of disease burden post-induction, M-protein by IFE appears to outperform involved sFLC; however, the addition of involved sFLC to M-protein by IFE significantly improves test performance at estimating residual marrow plasmacytosis post-induction therapy. In addition, involved sFLC may carry a unique, a priori prognostic significance for estimating PFS and OS following consolidative HDC/SCT. These findings suggest potential novel applications for sFLC in the management of pts with MM.

C25 - SERUM FREE LIGHT CHAINS ANALYSIS CAN DETECT EARLY RELAPSE/PROGRESSION IN INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA

M. Sobh, S. Morisset, T. Guilli, S. Ducastelle, F. Barraco, Y. Chelghoum, X. Thomas, A. Praire, F. Nicolini, C. Chapuis-Cellier, M. Michallet

Edouard Herriot Hospital, Haematology, Lyon, France

Background. Serum free light chains (sFLC) analysis has proved its usefulness in disease diagnosis and early treatment responses in multiple myeloma (MM). The sFLC ratio (sFLCr) has been included as one of the necessary criteria for achieving a "stringent complete response" in the proposed international response criteria. In contrast, performing this analysis during patients' follow up and after different treatments, in a daily practice setting, is still not very well defined yet. **Aims.** The main objective of this study was to assess the importance of sFLC analysis during MM patients' follow-up especially for relapse/progression detection alongside traditional serum protein electrophoresis (SPE). **Methods.** We have analysed 174 MM patients diagnosed at the Edouard Herriot University Hospital between years 2002 and 2008, for which a concomitant measurement of sFLC and SPE was done during follow-up. The sFLC analysis was performed using the **Freelite** test from the Binding Site on a BNII, Dade Behring, and for SPE, analysis was done using a Paragon CZE 2000, Beckman Coulter. There were 92 (53%) males and 82 (47%) females, median age at diagnosis 57 years (34-72). One hundred and twenty patients (69%) had IgG (87 κ &33 λ), 52 (30%) IgA (41 κ &11 λ) and 2 (1%) IgD (1 κ &1 λ) monoclonal proteins. Twenty six (15%) patients had renal insufficiency. We were interested to monitor the behaviour of sFLC and SPE in a way to detect early relapse or progression independently of treatment type. Slopes of the increase period corresponding to each measurement were compared using the student t-bilateral test with R statistical software (version 2.9.2). **Results.** After monitoring all patients, we observed 117 (67%) patients with relapse

or disease progression and 57 (33%) patients were still in response to treatment. As our main objective was to detect relapse or progression, the 57 patients in response were excluded from the analysis. Among the 117 patients, in 77 (66%) cases, relapse or progression was detected by concomitant increase of both sFLC and the intact immunoglobulin level (Ig) with a significant earlier increase for sFLC. In 17 (15%) patients, the relapse or progression was characterised by the increase only of sFLC without any increase of the Ig. Contrarily, in 5 (4%) patients there was only an increase of the Ig without increasing the sFLC. Finally, in 18 (15%) patients, the relapse or progression was revealed by the increase of Ig faster than the concerned sFLC. When comparing slopes of increasing sFLC comparing to increasing Ig, we found a very high significant difference ($p<0.001$), thus showing that sFLC have a faster detection of relapse or progression. (Figures of different profiles will be communicated). **Summary/conclusion.** We have observed that in 81% (66%+15%) of patients, sFLC analysis enabled an earlier detection of relapse or progression compared to SPE, that could be very important for early treatment intervention especially for high risk patients. Since there are no uniform recommendations for the use of this analysis during follow-up, we recommend its concomitant use with SPE, while waiting for guidelines.

C26 - SERUM & URINE FREE LIGHT CHAIN MONITORING COMPARED TO CONVENTIONAL SPE: AN UPDATE ON USEFULNESS AND PITFALLS IN "REAL LIFE HAEMATOLOGY"

W. Willenbacher,¹ G. Gastl,¹ W. Prokop,² E. Willenbacher,¹

¹Innsbruck University Hospital, Internal Medicine V
Haematology & Oncology, Innsbruck, Austria

Introduction. Serum free light chain analysis (sFLCA) is a tool to monitor myeloma disease activity and treatment response, as well as to stratify myeloma patients (pts) and to define risk groups and has been incorporated into recent diagnostic guidelines.¹ Either, the ratio of the free kappa/lambda light chains (FLC_o), the absolute value of the involved light chain (FLC_i) or the difference of involved and uninvolved light chain (FLC_d) may be used. While sFLCA is recommended in guidelines, urine free light chain analysis (uFLCA) at the moment is not. To analyze whether results from sFLCA and uFLCA would potentially have translated into altered clinical decision making and timing of treatments, compared with classical paraprotein measurements (PPM) in a cohort of myeloma pts, we analyzed all measurements routinely performed at Innsbruck University Hospital between MAR 03 and OKT 08 and correlated them with individual patients clinical courses. **Patients, assays and treatment lines.** 187 pts. (109 m, 78 f) out of 235 pts. identified were deemed eligible. Myeloma subtypes were IgG (57.2%), IgA (21.9%), FLC only (13.9%), IgM (3.2%), oligo and nonsecretory (2.6 %, incl. 2 pts. completely asecretory), and IgD (1.0%). 4 pts. were complete immunoglobulin only secretors. According to mSMART 15% were high risk, 61% standard risk and 23.5% of unknown category. In this cohort 3,202 sFLCA, 1,136 uFLCA and 2,583 PPM were performed (range 2-89, median 12). The measurements were correlated with 167

treatment times applied in this pts. (49 auto-transplants, 3 allo-transplants, 7 auto/allo procedures, 68 regimes containing novel agents and 40 conventional chemotherapeutic approaches. **Results.** sFLCA showed a significant advantage in detecting any of the predefined clinical endpoints (Table 1). By using PPM only, ~ 40% of events would have been missed during the observation period. A median of 13% of the applied therapies proven to be ineffective could have been stopped and altered earlier using the results of sFLCA. While the use of sFLC_i and sFLC_D resulted in comparable rates of false positive and negative results (Table 2), in comparison to PPM, sFLC_Q is more sensitive to effects of immunoparesis, changes of the uninvolved FLC concentration and renal function resulting in both more false positive as well as false negative results. sFLCA detected relapses with a median of 3 months prior to PPM, therapeutic effectiveness with a median of 2 therapy cycles earlier than PPM and therapeutic failure with a median of 1 antecedent cycle of therapy. **Discussion.** This analysis proves sFLCA is a useful tool in monitoring myeloma pts, clinical courses and the therapeutic effectiveness of myeloma treatment approaches, even in the setting of “*real life medicine*”. For monitoring purposes sFLC_i and sFLC_D should be used preferably due the higher false positive/negative potential of sFLC_Q. By using sFLCA in a structured diagnostic pathway, treatment effectiveness could be judged earlier and altered if necessary. Thus this analysis shows a potential clinically significant benefit to myeloma pts. An update on this data and an analysis of the techniques pitfalls from high dose hook effects to immunoparesis, light chain escapes, and treatment effects will be given.

Table 1. sFLCA vs. PPM.

Clinical events	sFLC _i	sFLC _Q	sFLC _D	sPPM	p (vs PPM)
Antecedent relapse	51	53	54	36	0.1
Antecedent remission	43	46	48	40	0.1
Antecedent therapy effectiveness	31	33	34	11	0.1
Antecedent therapy failure	18	15	16	4	0.1
Combined clinical endpoints (total number 154)	143	147	152	91	0.01
% detected	92.9	95.4	98.7	59.1	0.01

Table 2. sFLCA vs. PPM. Frequency of false negative.

Result	FLC _i	FLC _Q	FLC _D	sPPM
False pos. (relapse/effectiveness)	1	8	1	
False neg. (relapse/effectiveness)	1	5	1	2
False pos. remission		1		
False neg. remission	1		1	3
	3	14	3	5

References

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C27 - MONITORING PATIENTS WITH MONOCLONAL PLASMA CELL DISORDERS (PCDs) AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION BY USING SERUM FREE LIGHT CHAINS AND SERUM PROTEIN ELECTROPHORESIS

M. Dogaru,¹ D. Coriu,^{1,2} S. Badelita,² M. Crisan¹

¹Fundeni Clinical Institute, Bucharest, Romania;

²University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

Serum free light chains are important markers for monitoring patients with monoclonal plasma cell disorders (PCDs). Automated immunoassays that measures serum free light chains (FLCs) have been recently introduced in our lab and this report presents our experience. **Aims.** to analyse the efficiency of serum FLC in comparison with serum protein electrophoresis (SPE) in monitoring patients after autologous peripheral blood stem cell transplantation (PBSCT). **Methods.** We analyzed serial samples from 10 patients with monoclonal plasma cell disorders (PCDs) undergoing autologous stem cell transplantation. All samples had been analyzed by scanning densitometry of electrophoresis gels and FLCs. The FLC assay (**Freelite**; Binding Site) was performed on a Dade Behring, BN ProSpec automated nephelometer. Data were analysed based on previously established normal ranges. A κ/λ ratio less than 0.26 (positive for λ FLC) or greater than 1.65 (positive for κ FLC) were considered abnormal. SPE was performed on a SEBIA electrophoresis automated analyzer. **Results.** In this study we presented in dynamics the evolution of 7 patients with intact immunoglobulin multiple myeloma (IIMM) (2 IgG, κ ; 3 IgG, λ ; 1 IgA, κ ; 1 IgA, λ) and 2 patients with light chain multiple myeloma before and after autologous PBSCT. All patients had previously been given cytotoxic chemotherapy (VAD or velcade). In two of the patients with IIMM both FLC and protein electrophoresis fell towards normal range after autologous PBSCT and both reported a relapse after 23 month and 9 months respectively. In one patient FLC fell in the normal range after induction chemotherapy but SPE continued to be abnormal. The patient has undergone autologous PBSCT and will be under our observation. In one patient FLC became normal after transplantation but the SPE stayed abnormal. In one patient SPE became normal before FLC but FLC were the first to signal the relapse after 20 months from transplantation. In one patient (IgG λ) the outcome was poor. SPE did not normalize and although the κ/λ ratio fell in the normal range, the concentrations of free λ were above the normal range. In one patient SPE and FLC fell in the normal range at the same time but FLC was the first to signal the relapse after 16 month from transplantation. For patients with light chain multiple myeloma, classical SPE failed to demonstrate monoclonal protein and FLC assay helped us to identify the monoclonal light chain in these cases. After autologous PBSCT the serum free light chains concentrations fell but they continued to be out of the normal range even after 3 month. These patients continue to be under our observation in the future. **Conclusions.** With regard to the choice of method for monitoring patients with PCDs that undergone autologous stem cell transplantation, FLC assay has proven to

be more efficient than SPE in indicating whether autologous PBSCT is effective or not.

C28 - NORMALIZATION AND MAINTENANCE OF NORMAL LEVELS AND RATIOS OF SERUM FREE LIGHT CHAIN (sFLC) OVER 1 YEAR POST-TRANSPLANT ARE ASSOCIATED WITH LONGER SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA (MM)

C.K. Lee,¹ M. Berg,² J. Ryder,² H. Myint,² J. Gutman,² W. Robinson²

¹UCHSC Cancer Center, Colorado, US; ²University of Colorado, Aurora, CO, USA

Background. The role of sFLC assay for predicting survival has been uncertain for patients with MM following autotransplant-based therapy. **Aims.** We sought to see whether survival improves by achieving and maintaining normal level/ratios of sFLC for at least a year after autotransplant. **Methods.** We followed a cohort of MM patients with serial sFLC assays before and after transplant at least once month for a minimum of one year. Between September 2006 and July 2009, patients (n=59; median age 60, range 37-77) were treated with induction chemotherapy, autotransplant following high-dose melphalan-based ablative therapy and post-transplant maintenance. Polyclonal antisera-based FLC test of the Binding Site was used for sFLC assay. **Results.** Twenty six patients had IgG, 14 IgA, 15 FLC only and 4 nonsecretory MM. At diagnosis, six patients (10%) had a normal sFLC level/ratio. During the first year follow-up after transplant, 16 of the 53 patients (30%) showed abnormal sFLC related to new mono- or oligoclonal immunoglobulin (Ig) in serum, with a median time of onset of 44 days from the 1st autotransplant, and 7 of 23 (30%) in 64 days following the 2nd autotransplant. These new clonal Ig(s) disappeared spontaneously in a median of 51 and 48 days, following the 1st and 2nd autotransplant, respectively. Changes in sFLC level/ratio in the presence of new clonal Ig were not taken as indicator of relapse for the current study. Of the 53 patients, 40 achieved a normal level and ratio of sFLC in a median of 143 days (range 31-949), with a 3-year cumulative incidence of 67%. Thirty five of the 40 patients (88%) remained normal on the sFLC assay through the end of the study. Forty four of the 53 patients are currently alive, with an estimated 3-year overall survival of 78%. Thirteen patients relapsed, with an estimated 3-year event free survival (EFS) of 64%. Patients (n=35) who became and remained normal on the sFLC assay for at least one year post-transplant had a 3-year overall survival of 68% whereas those (n=18) who remained or converted to abnormal had 50%, Logrank $p < 0.01$. An estimated 3-year EFS was 59% for the 35 and 50% for the 18 patients, respectively, Logrank $p = 0.09$. **Summary/Conclusion.** The current study suggests that survival of MM patients may improve by normalization and maintenance of normal level/ratio of sFLC at least over a year following autotransplant based therapy. Further study is necessary to confirm the findings.

C29 - FREE LIGHT CHAIN RECOVERY AFTER REDUCED INTENSITY ALLOGENIC TRANSPLANT (RIC) FOR MULTIPLE MYELOMA (MM) - EFFECT OF CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD) & PROGRESSION

V. Singh, R. Cornell, M. Sala, S. Paruchuri, A. Saad, J. Palmer, P. Hari

Department of Neoplastic diseases, Medical College of Wisconsin, Milwaukee, WI, USA

Introduction. RIC allogeneic transplantation depends on donor derived allo-immunity to prevent progression or relapse of MM. Donor T cell mediated graft vs. MM effect is central to this. Also after RIC, recipient B cell function is donor derived. We hypothesized that recovery of B cell function in this setting is influenced by the competing effects of recurrence of myeloma clone vs. the impact of graft vs. MM. The recovery of donor B cell function could be delayed by graft vs. host effect (GVHD) which also is associated with the graft vs. MM effect. We studied alternate (uninvolved) free light chain recovery as a marker for donor derived immune function after RIC for MM. The outcomes of 47 recipients (matched siblings donors =45) of RIC for multiple myeloma were analyzed. Conditioning regimen did not involve anti-myeloma chemotherapy and was primarily immunosuppressive. Median follow up was 2 years.

Table 1.

FLC response group N=47	Median PFS	TRM 7	DFS 23	Relapse death 10	Relapse No Rx 1	Relapse an alive vs Rx 6
Never (12)	4.6 mo	6	0	6	0	0
Early recovery (10)	11.8 mo	1	4	3	0	2
Late recovery	NR @ 5 yrs P=0.0001	0	19	1	1	4

Methods & Results. Serum free light chain assay was performed pre-transplant and at 3-monthly intervals after transplant. Progression free survival, treatment related mortality, overall survival and myeloma progression were analyzed. The light chain associated with MM clone was designated as involved (IFLC) and the complementary light chain designated as the uninvolved (UFLC). All patients, except those with progressive MM, had suppression of both light chains immediately after RIC. Based on pattern of recovery of uninvolved free light chains we identified 3 clear patterns. First group – those who did not have any UFLC recovery, secondly those whose UFLC returned to normal levels within 6 months of RIC (early recovery) and a third group were the UFLC recovery was delayed beyond 6 months (late recovery). OS and PFS of whole group at 4.5 years was 54% (CI 36-72%) and 38% (CI 15- 61%) respectively. TRM was 14.5%. Median PFS was significantly superior in the late recovery group ($p=0.0001$). A landmark analysis of 6 month survivors after RIC confirmed these findings (Figure 1). **Conclusion.** Recovery of uninvolved light chain serves as

a marker for the competing interplay of cGVHD and myeloma clone recurrence which follows RIC allo-transplantation. The effect of cGVHD likely explains the early suppression of uninvolved FLC and paradoxically better anti-MM effect in the late recovery group. This phenomenon may also operate in patients with lymphoproliferative disorders undergoing RIC allografts.

Figure 1.

C30 - CAN LIGHT CHAIN ESCAPE PHENOMENON BE OBSERVED WITHOUT PRIOR HAEMATOLOGICAL THERAPY?

J. Granátová,¹ K. Valentová,¹ M. Bolková,¹ J. Haber,² V. Hanzal,³ L. Hachová⁴

¹Dpt. of Clinical Chemistry, Thomayer Teaching Hospital, Prague, Czech Republic; ²Ist. Dpt of medicine - Dpt of haematology, General Teaching Hospital, Prague, Czech Republic; ³Dpt of Nephrology, IKEM, Prague, Czech Republic; ⁴Dpt. of Clinical Chemistry, General Teaching Hospital, Prague, Czech Republic

Background. Light chain escape (LCE) is characterized as a change in tumor biological behavior in patients with multiple myeloma (MM). At the diagnosis, complete monoclonal immunoglobulin molecules (mIg) together with monoclonal free light chains (mFLC) are present, however only a significant increase of mFLC is observed in relapse or disease progression together with end-organ impairment while mIg concentration is usually stable or decreasing. Serum FLC monitoring is recommended for early diagnosis of LCE in patients with MM. **Aims.** So far, LCE phenomenon has been described only in patients after haematological therapy. A patient with MM fitted LCE phenomenon criteria is demonstrated without prior therapy. **Methods.** A 52 year-old man presented with monoclonal IgA- κ in low concentration (6,5 g/L) at the diagnosis, mFLC- κ in serum and urine with pathological κ/λ ratio on the background of diabetes mellitus and hypertension for many years with serious organ complications including renal impairment (eGFR 0,72 ml/s/1,73

m²), proteinuria and vertebral pain. Smoldering myeloma was diagnosed not requiring chemotherapy. **Results.** In 6 months, the mFLC- κ serum concentration doubled, bone marrow monoclonal plasma cell count rose from 10 to 12%, serum beta-2-microglobulin by 30% and mIgA- κ concentration slightly decreased (to 5,5 g/L). Renal impairment and anaemia were stable, serum calcium was not increased and no changes on bone X-ray were observed. Therefore, the criteria for starting therapy were not attained. In the next 2 months, he developed heart and renal impairment, multiple osteolytic lesions and progressive anaemia. mFLC- κ serum concentration rose 5 times, κ/λ ratio 7,5 times, plasma cell count to 44% with immunophenotypical changes, total protein in urine increased 4 times with impairment of renal function, however mIgA- κ became undetectable. Light chain deposition disease was noted in the renal biopsy and 2 weeks later a MM was diagnosed with mFLC- κ alone and clinical stage III in ISS and IIIB in Durie-Salmon classification. In the second course of chemotherapy (cyclophosphamide + dexamethazone) bleeding from peptic ulcers appeared. After an emergency gastric resection, multiorgan failure developed (hepatorenal and cardiovascular together with heavy neutropenia after chemotherapy) and the patient died from terminal bronchopneumonia in intensive care, 10 months after the diagnosis. No novel (biological) therapy (except for anaemia correction) was introduced before the relapse but no causative factor for the progression was found. Retrospectively, the hematologists favoured the diagnosis of multiple myeloma with biclonal immunoglobulin production (mIgA- κ and mFLC- κ) with an aggressive time course instead of smoldering myeloma. **Summary/Conclusion.** LCE as an atypical relapse can be observed in patients with MM not only after hematological therapy, but also in disease progression. For early diagnosis a reflective attitude is necessary. In our knowledge, SPE with serial FLC measurements, renal function and urine protein assessment (if total protein in urine is present) are recommended.

C31 - MONITORING MYELOMA PATIENTS ON LENALIDOMIDE - EFFICACY AND INCIDENCE OF LIGHT CHAIN ESCAPE

A. Mahdi,¹ D. Andrews,¹ N. Evans,² M. Moody,³ C. Poynton⁴

¹Haematology, University Hospital of Wales, Cardiff, UK; ²Dept Pharmacy, University Hospital of Wales, Cardiff, UK; ³Medical Biochemistry, University Hospital of Wales, Cardiff, UK; ⁴Dept Haematology, University Hospital of Wales, Cardiff UK

Background. Lenalidomide is a recently introduced IMiD derivative with a broad range of anti-neoplastic activities. Effective monitoring of its efficacy has additional implications in view its high cost, its potential for long term maintenance use (about £60,000 per annum per patient) and that its approval for UK use is third line. This therapy may select advanced refractory patients who are susceptible to the development of light chain escape from paraproteinaemia (LEPP). Several authors have recently suggested that lenalidomide may be causally associated with the deve-

lopment of LEPP. *Aims.* We aimed to review the patients in our institution who have received lenalidomide for myeloma since its approval in Wales in 2008, in order to determine the efficacy, method of monitoring, incidence of LEPP and outcomes. *Methods.* Retrospective analysis of laboratory paraprotein and LEPP measurements, pharmacy records of prescriptions for lenalidomide and individual patient medical records were assessed. *Results.* 15 patients received lenalidomide for progressive myeloma from 2008-2010. All patients had previously been treated with thalidomide. There were 10 males and 5 females. Median age was 59 (38-79). Median number of prior treatments for the myeloma was 3 (2-6). 8 patients had received high dose melphalan with autologous stem cell rescue previously (one had a double autograft). A total of 117 cycles (3 weeks on and one week off) of lenalidomide were prescribed (range 2-13) and a total of 143 serum free light chain (sFLC) measurements were made (on average, 1.2 tests per cycle). Only 2 patients had light chain only myeloma when first diagnosed but 8 patients developed LEPP prior to starting lenalidomide and 1 patient reverted to whole paraprotein secretion from LEPP while on lenalidomide. The presence of LEPP in this patient group correlated highly with the number of prior treatments. No patient developed LEPP since starting Lenalidomide. sFLC measurements appeared highly concordant with the clinical outcome and an effective means of monitoring the clinical course. 4 patients had the lenalidomide stopped due to lack of response or toxicity. There were 2 patients who progressed and died, 3 partial responses and 10 patients are now in plateau phase again. One of these patients has reverted from LEPP to monoclonal immunoglobulin production during lenalidomide treatment. *Summary/Conclusion.* Lenalidomide appears highly effective in patients with advanced myeloma and the majority of patients receiving the drug will inevitably have been heavily pre-treated. These patients have a high incidence of (LEPP) so that sFLC measurements are crucial both for monitoring their progress and for early cessation if the drug is not effective. A preliminary guideline on the optimal timing and frequency of monitoring would be useful. There is no evidence from this group of patients that lenalidomide enhances the development of LEPP.

C32 - A SINGLE CENTRE EXPERIENCE OF CLONAL EVOLUTION IN RELAPSED MYELOMA MANIFESTED BY LIGHT CHAIN ESCAPE

H. McCarthy, M. Lasebai, R. Hall

Royal Bournemouth Hospital, Bournemouth, UK

Background. The majority of myeloma patients secrete an intact immunoglobulin (Ig) molecule accompanied by variable isotypic light chain secretion. Following prolonged therapy clonal evolution can occur with acquired genetic changes such as secondary Ig translocations, Myc dysregulation and p53 inactivation. Light chain escape (LCE), characterised by a shift from intact Ig secretion to free light chain only, is another manifestation of clonal evolution recognised by haematologists but rarely reported. Such biological change often manifests by the development of significant organ dysfunction including renal

impairment or new bony lesions. *Aims.* The aim of this study was to investigate the prevalence and clinical features of LCE myeloma. *Methods.* We retrospectively studied a patient cohort treated in our institution from 2005 to 2010. For identified cases, patient characteristics, number of lines of treatment, response to treatment and mode and speed of disease progression were recorded. Serum free light chains (FLC) were measured using the **Freelite** assay and correlated with other laboratory results such as paraprotein level (intact Ig) and serum creatinine.

Results. We identified 7 cases of LCE myeloma in our cohort of 155 patients. The prevalence of LCE within our patient's cohort was 4.5%. The median time from diagnosis to LCE was 14.5 months. In all cases the LCE was manifested by renal deterioration. The median number of therapies received prior to the onset of LCE was 2, ranging from 0 to 5. Interestingly, one case demonstrated LCE prior to receiving any therapy. *Conclusion.* Our study demonstrates that LCE myeloma is more common than previously reported, and frequently characterised by renal deterioration. Early detection facilitated by serial serum FLC measurements could allow early intervention to prevent unwanted complications. Furthermore, our data demonstrates that LCE can present early in the disease course prior to initiating anti-myeloma therapy. Further large studies on the prevalence and molecular mechanisms underlying this phenomenon are warranted.

C33 - PROGRESSIVE CLONAL SERUM FREE LIGHT CHAIN ABNORMALITIES PRIOR TO RELAPSE OF OLIGOSECRETORY MULTIPLE MYELOMA (MM)

K. van Hoeven,¹ E. Bilotti,² L. McBride,² A. McNeill,² L. Schmidt,² D. Schillen,² D. Siegel²

¹The Binding Site, Inc., San Diego, CA, USA;

²Hackensack University Medical Center, Hackensack, NJ, USA

Background. Serum free light chain (sFLC) analysis is a sensitive technique for detection of clonal light chain disorders. An increased clonal FLC with an abnormal sFLC ratio in the absence of intact immunoglobulin M-protein or other abnormality is a phenomenon known as FLC monoclonal gammopathy of undetermined significance (MGUS). Progression of FLC MGUS to light chain or oligosecretory MM has been described in stored serum samples (Dispenzieri et al. Lancet 2010; Weiss et al. Blood 2009). Progressive clonal sFLC abnormalities in the setting of relapsed oligosecretory MM represent a similar phenomenon in which sFLC abnormalities may be the first measurable disease manifestation. *Aim.* To assess temporal changes in laboratory results prior to relapse of oligosecretory MM. *Methods.* Clinical and laboratory records were analyzed in a patient with oligosecretory MM. *Results.* A 59-year old woman received pulsed dexamethasone followed by tandem melphalan 200 mg/m² autologous stem cell transplantation (ASCT) for newly diagnosed MM in 1999. Sustained remission was achieved through 2005, with no M-protein evident by serum protein electrophoresis (sPEP), serum and urine immunofixation (IFE), or urine protein electrophoresis (uPEP), and a normal sFLC ratio. In 2006 (at months 74

and 80 post-ASCT), free lambda concentrations began to slowly increase and the sFLC ratio became abnormal (graph). In early 2007 (month 86), sIFE and uIFE became positive for lambda light chains, the uPEP showed 16 mg M-protein/day, and lambda markedly increased by sFLC analysis. MRI showed multifocal involvement, and a pre-treatment sPEP became positive (M-protein 0.24 g/dL). The patient underwent another ASCT and a rapid treatment response was achieved. **Conclusion.** Progressive increases in clonal sFLC may be the first manifestation of a monoclonal protein prior to relapse of oligosecretory MM, just as FLC MGUS is an early indicator of risk of progression to light chain MM and related disorders.

Figure 1.

C34 - SERUM FREE LIGHT CHAINS IN MONITORING A LAMBDA LIGHT CHAIN MULTIPLE MYELOMA PATIENT'S TREATMENT

C. Bermudo, J.L. Garcia, J.A. Martin Ruiz, R. Duro Millán, M.M. Iglesias, F.F. Romero

Hospital Universitario Virgen Macarena - Clinical Biochemistry Unity, Seville, Spain

Background. An excess of free light chains (FLC) consists of homogeneous populations of kappa (κ) or lambda (λ) immunoglobulin light chain molecules produced by malignant B-cell clones. These patients are difficult to diagnose as they frequently show no abnormality when tested by serum protein electrophoresis (SPE). The FLCs are very important markers for monitoring patients with multiple myeloma (MM) and other monoclonal gammopathies (MG). The serum κ/λ FLC ratio allows us to detect a relapse of the patient before any symptoms are present or could be detected by other diagnostic tests.

Methods/Case report. A 66 year old man was diagnosed in May 2008 with light chain lambda MM (LCMM) with orbital plasmacytoma. In June 2008, he began treatment with six cycles of VAD (Doxorubicin, Vincristine and dexamethasone) and one month later the κ/λ FLC ratio was normal while SPE and serum immunofixation (sIFE) were negative. In late October 2008, after the first chemotherapy cycles, the κ/λ FLC ratio was abnormal (0.002) while SPE and sIFE were negative. This value of the ratio predicted a relapse in the patient's condition. Due to the

partial response of the disease, the patient began a treatment of five cycles of Bortezomib and dexamethasone from January 2009 to May 2009 and received twenty sessions of radiotherapy in May 2009. These treatments improved ocular motility and the associated symptoms but the κ/λ FLC ratio remained altered (0.01) until June 2009. Then the patient started a new cycle of treatment with Lenalidomide and dexamethasone improving the patient's condition. The κ/λ FLC ratio was normal during this new treatment and this ratio did not predict a relapse of the patient. Currently (January 2010), the SPE and IFE are negative, the κ/λ FLC ratio is normal (0.73) and the patient is clinically well. **Summary/Conclusions.** This case is a good example of the utility κ/λ FLC ratio in the monitoring of MM and it can predict future relapses in the patient. The value of the κ/λ FLC ratio helps determine if the patient responds to chemotherapy or the patient has a relapse and we need to change treatment. This is due to the high specificity of FLCs and the high sensitivity of this assay that enables an early identification of residual activity that otherwise could not have been detected.

C35 - SERUM FREE LIGHT CHAIN (sFLC) ASSAYS IN PATIENTS WITH UNQUANTIFIABLE LOW-LEVEL PARAPROTEIN (LLPP) AND TRANSIENT PARAPROTEINAEMIA (TP)

S. Abdalla

Imperial College Healthcare Trust, London, UK

Introduction. With increasing awareness and screening for paraproteinaemias, a significant number of patients are referred to our hospital with low levels of paraproteins. It is often difficult to assess their relevance below 1g/L in relation to further investigations such as skeletal surveys or bone marrow biopsies. There are no studies specifically addressing this group of patients. The sFLC assay has an established role in the monitoring and management of plasma cell dyscrasias and in relation to MGUS, a specific place in risk stratification. We, therefore, took this opportunity to look at the role of the sFLC assay in defining the need for further investigations and follow up in this group of patients, taken together with other clinical and laboratory findings. **Patients and methods.** The population was a group of 79 patients with persistent LLPP and 17 patients with transient paraproteinaemia. They were referred for further investigations to the paraproteinaemia clinic at St Mary's Hospital, in Paddington. All had a PP reported as being too low to quantify, with an upper limit of of ~ 1g/L in our laboratory. The patients were investigated with the usual laboratory tests for evaluating patients with paraproteinaemia and included sFLC assays. Few patients had skeletal surveys or bone marrow biopsies. **Results.** Results for M-proteins were available in 73 patients. The M-proteins for the LLPP group were IgG 36 (50%), IgA 14 (19%) IgM 12 (16.5%), biclonal 6 (8%) and BJP 4 (5.5%). For the TP group the proportions were: IgG 11 (69%), IgM 3 (19%), IgA 1 (6%) BJP 1 (6%), characterised as κ 8 (50%) and λ 8 (50%). None of the patients had evidence of end-organ damage due to the paraproteinaemia and none had immunoparesis. sFLC assay results were available in 40 patients with LLPP and 6 patients

with TPP. The sFLC ratio was abnormal in 12 out of 40 LLPP (ratio range: 0.87 to 4.46), increased κ in 10 (range 20.1-157 mg/L and κ/λ ratio range: 1.67-4.46) and increased λ in 2 (237 and 321 mg/L: κ/λ ratios 0.087 and 0.25). None of the TP group was abnormal. Ten of the original 73 patients with LLPP developed higher levels of quantifiable PP from 2-10g/L between 2 and 5 years after the initial detection of the paraproteins. 3 of these patients had abnormal sFLC ratios. *Conclusion.* Many of the LLPP patients had abnormal sFLCs but, interestingly, none of the TP patients. Over the limited period of the study sFLC abnormalities did not relate to clinical outcome. Further studies with longer follow up periods are needed to draw firm conclusions.

FREE LIGHT CHAINS IN RENAL AND OTHER DISEASES

D36 - MONITORING OF SERUM FREE LIGHT CHAINS IN THE TREATMENT OF LIGHT CHAIN DEPOSITION DISEASE WITH HIGH CUT-OFF HAE-MODIALYSIS

C. Bermudo, J.L. Garcia, J.A. Martin Ruiz, R. Duro Millán, M. Merchan Iglesias, F.F. Romero

Hospital Universitario Virgen Macarena - Clinical Biochemistry Unity, Seville, Spain

Background. Light chain deposition disease (LCDD) is a rare entity where serum free light chains (sFLCs) are precipitated on the basement membranes of cells in the kidney and other organs. Severe renal failure is the common presenting feature. This disease does not have a clearly defined treatment and patients have an adverse prognosis. We present the case of a patient with LCDD and severe renal failure, treated by chemotherapy and sFLC haemodialysis with Theralite High Cut-Off technology. This enables an efficient and direct removal of sFLCs. *Case report.* A 57 year old man was admitted to the hospital due to very intense bone pain. He was diagnosed with LCDD. The κ/λ FLC ratio was abnormal at 43. The serum kappa FLC was 289 mg/l and the serum lambda FLC was 6.74 mg/l. While on analgesics, the intense bone pain improved but acute renal failure was detected (creatinine was 9,70 mg/dl) in the context of LCDD. The patient began treatment with Bortezomib and haemodialysis (daily six-hour periods over six days) with high cut-off membrane in order to remove FLCs in serum. We measured pre- and post-haemodialysis sFLC, the κ/λ ratio and the creatinine in serum. The percentage of serum kappa FLC removed was 61% and the levels of kappa sFLC decreased from 90mg/dL to 59mg/dl during the treatment. The percentage of κ/λ FLCs ratio reduction was 57% (from 11.1 to 6.3 during treatment). This produced an improvement in the patient's renal function with a decrease of 88% in the creatinine serum levels (from 5.9 mg/dL to 0.7 mg/dL). *Discussion.* A combination of the efficient and direct removal of the toxic excess of serum FLCs using high cut-off membranes with Bortezomid allowed us to reduce the serum FLCs levels. The determination of the rate of reduction with sFLC was a useful tool that allowed us an accurate and rapid monitoring of serum FLCs levels to guarantee an optimum treatment of the patient.

D37 - FREE LIGHT CHAIN REMOVAL WITH HIGH CUT-OFF HAEMODIALYSIS IN PATIENTS WITH CAST NEPHROPATHY

G. Martin Reyes,¹ N.M. Barbosa de Carvalho,² A. Torres de Rueda,¹ R. Toledo Rojas,¹ M. Martín Velásquez,¹ C. Girona Gallegos,¹ E. Sola Moyano,¹ M. González Molina¹

¹Hospital Universitario Carlos Haya, Avenida de Haya Carlos, Malaga, Spain; ²The Binding Site, Barcelona, Spain

Background. Renal failure is a major cause of morbidity and mortality in patients with multiple myeloma (MM)

and is caused by monoclonal free light chains (FLC). At initial presentation, up to 50% of patients have renal impairment and 12 to 20% have acute renal failure (ARF). A new generation of dialysers with large pores is now available and allow large amounts of FLC removal. We present 4 clinical cases which were treated with chemotherapy and FLC haemodialysis (HD) with high Cut-Off (HCO) technology (HDHCO). **Aims.** To study the effectiveness of HCO membranes for dialysis of FLC in patients with cast nephropathy. **Methods.** HCOHD was used together with MM chemotherapy in 4 patients in order to improve renal function and patients' haematological condition. Serum FLC kappa (κ) and lambda (λ) were measured pre- and post-haemodialysis in the follow-up of the therapy together with renal function parameters. **Case 1:** A 54 year old man was admitted with ARF, presenting at the time of diagnosis with a creatinine (cr) of 8.9 mg/dL. The patient was diagnosed with a k light chain MM. **Case 2:** A 52 year old woman with a k light chain MM presented with cr levels of 6 mg/L. **Case 3:** A man of 79 years, diagnosed with IgAk MM, presented with osteolytic bone lesions and renal function deterioration (cr=3.4 mg/L). **Case 4:** A 70 year old woman with ARF. The patient was diagnosed with IgG k MM with 80% plasma cells in the bone marrow and k FLC of 11,600 mg/L. Follow up data is shown in the Table. **Discussion.** The use of HCO membranes for the dialysis of FLC allowed a very efficient elimination of FLC. With the demonstrated survival data we estimate that HCOHD is a suitable treatment for cast nephropathy. Nowadays, we will use HCOHD in patients presenting with renal failure and sFLC levels higher than 1000 mg/L.

Table1. Pre-dialysis value of: calcium, magnesium, albumin and creatinine and Pre and post-dialysis value of kappa serum free light chain. Numbers (1, 2, 3...) refers to successive dialysis sessions (days). In patient 4, between dialysis session n° 8 and n° 9 passed a 5 days period.

Patient 1	1	2	3	4	5	6	7	8	9	10	11
Calcium	9.3	9.3	9	9.1	8.9	8.9	9.4	9.3			
Magnesium	8.2	7	7	6.2	5.4	5.5	5.7	5.8			
Albumin	2.9	2.7	2.7	2.8	2.8	3	3.2	3			
Creatinine	1.9	1.7	1.9	1.9	1.8	2.1	2	1.8			
κ sFLC pre	12600	12100	11400	10600	6460	6680		6140			
κ sFLC post	9910	6900	5250	4420	3840	3430	4680	3920			
Patient 2	1	2	3	4	5	6	7	8	9	10	11
Calcium	8.7	8.4	7.9	7.7	9.7	8.3					
Magnesium		2.3		1.7	2.2	1.9					
Albumin	3.2	3.8	3.4	3.5	4.3	4.4					
Creatinine	5.9	4.4	4.4	2.7	2.7	3.5					
κ sFLC pre1	146	115	99.1	97.6	109						
κ sFLC post1	57.5	56	51.3	54.3	23.7						
Patient 3	1	2	3	4	5	6	7	8	9	10	11
Calcium	8.1	8.7	9.4	7.8	10	9.7					
Magnesium	1.8		2.1	1.8	2.8	2.1					
Albumin	2.6	3	3.1	4	4.5	4					
Creatinine	1.6	2.6	2.1	1.8	1.1	1.1					
κ sFLC pre	1080	725	638	577	571	393	528	717	779		
κ sFLC post	184		130	135	146	127		131	121		
Patient 4	1	2	3	4	5	6	7	8	9	10	11
Calcium	6.9	7	7.1	6.5	6.3	6.6	6.8	6.4	7.1	7.5	7
Magnesium		2.3		2.1	2.1	2	2.2	2.3	2		
Albumin	1.9	2.2	2.2	2.4	2.4	2.3		2.7	30	3.3	3
Creatinine	6.5	4	3.9	2.7	3.2	1.7	2	2.5	2.2	2.1	4.7
κ sFLC pre	13000	12900	8140	3890	2190	1730	2300	714	4428	1390	2010
κ sFLC post	8240	5280	3300		1220	708	770	400	993	666	

D38 - FAILURE TO REMOVE POLYMERISED IMMUNOGLOBULIN FREE LIGHT CHAINS BY HIGH CUT-OFF HAEMODIALYSIS

D. White,¹ N. Gardner,¹ S. Harding,¹ F. Provot,² J.B. Beuscart,² M. Cook,³ S. Stringer,⁴ P. Cockwell,⁵ C. Hutchison⁵

¹The Binding Site Group Ltd, Birmingham, UK;

²University Hospital of Lille, Lille France; ³Department of Haematology, Queen Elizabeth Hospital, Birmingham, UK; ⁴Department of Immunity and Infection, University of Birmingham, UK; ⁵Department of Nephrology, Queen Elizabeth Hospital, Birmingham, UK

Background. Serum free light chains (FLC) are responsible for the renal injury in cast nephropathy in patients with multiple myeloma (MM). Recently the removal of FLC by high cut-off haemodialysis (HCO-HD) has been described as an adjuvant therapy to complement effective chemotherapy in the management of cast nephropathy. Together these treatments allow a rapid reduction in serum FLC concentrations, which in turn enables renal recovery to occur. The effective removal of FLCs by HCO-HD is dependent on the molecular weight of the FLCs in relationship to the molecular weight cut-off of the dialysis membrane. Here we describe two patients in whom HCO-HD was unable to effectively remove FLCs. **Aims.** The aim of this investigation was to determine why HCO-HD could not remove FLCs in two patients. **Methods.** Patient sera were fractionated by size exclusion chromatography on a Superdex S200 (1.6x70cm) column under native conditions. Free light chain concentrations in sample fractions were determined on a Siemens BN II nephelometer. Fractions were also analysed by SDS-PAGE gels and were further characterised by Western blotting using isotype specific antisera. **Results.** Patient one, a 61 year old man, demonstrated failure to clear λ FLC from serum following six sessions of HCO-HD (median 7% reduction per session between pre- and post-dialysis samples). Size exclusion chromatography of the patient's serum and subsequent analysis demonstrated that the majority of λ FLCs were high molecular weight polymers (>150kDa) and only the minority were present in the serum as dimers (50kDa). Patient two, a 51 year old lady, received 5 sessions of HCO-HD and also failed to demonstrate a significant reduction in serum κ FLC concentration as assessed pre- and post-dialysis (median reduction 10.5%). Evaluation of the patient's serum demonstrated κ FLCs at three molecular weights: ~25kDa (monomer), ~50kDa (dimer) and the majority at >100 kDa (polymer). Analysis of dialysate fluid, from HCO-HD sessions, demonstrated that only κ monomers (25kDa) were present. **Summary/Conclusion.** The presence of a high proportion of FLC as polymers, prevents efficient removal of FLCs by HCO-HD.

D39 - BETA 2-MICROGLOBULIN, KAPPA AND LAMBDA LIGHT CHAINS REDUCTION BY HAEMODIALYSIS

L. Bârsan,¹ S. Stancu,² M. Lipan,¹ V. Decu,¹ C. Talangescu,¹ C. Sântimbrean,³ M. Săndulescu,³ G. Mircescu^{1,2}

¹"Dr Carol Davila" Teaching Hospital of Nephrology, Bucharest, Romania; ²"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; ³"Dr Carol Davila" Fresenius Nephrocare Dialysis Center, Bucharest, Romania

Background. Beta 2 microglobulin (β_2 M) is a recognized surrogate marker of middle-molecule uraemic toxins and a key component in dialysis-associated amyloidosis. Recently, it was associated with the clinical outcome in dialysed patients. Free immunoglobulin light chains (FLC) κ and λ are also markers of middle molecule uraemic toxins. They have been less investigated than β_2 M, but seem to interfere with essential neutrophil functions and to participate in the disturbed immune function of CKD patients. **Aim.** To compare β_2 M, κ and λ light chains reduction by haemodialysis. **Methods.** 87 haemodialysis (HD) patients were studied: 1% diabetics, 59% male; median age 61 years (range 53-72); median HD period 7 years (range 4-10); 23% haemo-diafiltration; 77% high flux HD; median CRP 6mg/L. Investigated parameters (pre- and post dialysis) were: urea, β_2 M, FLC κ and λ , serum albumin; body weight, total body water (TBW), extracellular body water (ECW - measured with BCM, Fresenius Medical Care) and ultrafiltration volume. Solute concentrations were normalized to pre- and post-dialysis albumin levels. Solute depletion was quantified either by reduction ratio or quantitatively (no rebound; mono-compartment model as total body water for urea; and extracellular water for β_2 M and FLCs). **Results.** Dialysis reduced all investigated parameter levels but the slope was different in case of λ light chains from κ and β_2 M. Estimated by reduction ratio, β_2 M depletion (68.2%) was not different from urea (70.9%) but significantly different from κ and λ light chains (49.2% and 25.5%). When examined quantitatively, κ and λ FLCs are excreted in significantly larger amounts than β_2 M (1100 and 803, vs. 212mg/session; $p < 0.05$). β_2 M and urea had similar percentage reduction (68.2% vs. 70.9%), while κ and λ FLCs were less (49.2% and 25.5%; $p < 0.05$). Albumin normalized pre- and post-dialysis; β_2 M and κ levels were closely correlated (r^2 0.91 and 0.23; $p < 0.05$), but not λ FLCs (r^2 0.00 and 0.08; $p > 0.5$), suggesting analogous distribution volumes and similar equilibration constants for β_2 M and κ , but not for λ . A model including the percentage reduction in body weight and pre-dialysis β_2 M concentration predicted the reduction ratio in 17% of cases. A significant component was the percentage reduction in body weight, suggesting a dominant role for ultrafiltration as the mechanism of β_2 M reduction ratio. **Conclusion.** β_2 M, κ and λ light chains are effectively depleted by dialysis. While β_2 M seems to have a similar distribution volume and excretion by dialysis as urea, κ and λ light chains perform differently. A mono-compartment model is more fitted to β_2 M than to κ and λ FLC dialysis excretion. More studies are necessary to validate κ and λ light chains as surrogate markers of middle molecule removal.

D40 - INTERNALIZATION PATHWAY OF AMYLOIDOGENIC SERUM FREE LIGHT CHAINS

D. Ricotta,¹ G. Di Noto,¹ A. Dossi,¹ L. Paolini,¹ A. Radeghieri,¹ B. Amoroso,² L. Caimi¹

¹Biomedical Science and Biotechnology Dept., Brescia, Italy; ²Clinical Research Direction, The Binding Site Rome, Italy

Background. Primary (AL) Amyloidosis is a disease of protein misfolding. During plasma cell dyscrasia, such as micromolecular myeloma, plasma cells produce a great amount of monoclonal immunoglobulin free light chains (kappa or lambda FLCs) that may accumulate in different peripheral districts and determine tissue damage. It has been shown that FLCs induce oxidative stress and FLCs internalization in cardiac fibroblasts enhances sulfatation of secreted glycosaminoglycans (GAGs) suggesting that the intracellular trafficking pathway of FLCs could be correlated with the amyloidogenic potential of monoclonal immunoglobulins. **Aims.** The mechanism of internalization and trafficking of FLCs is not well understood, so we aimed to characterize this pathway. **Methods.** In order to identify a correlation between the FLC phenotype and their pathogenicity, we have investigated the internalization rate of different lambda and kappa FLCs (belonging to hospitalized patients) in different cellular lines. The mechanisms of internalization in different cellular subtypes might be an important key in the study of different pathological conditions strictly correlated to FLCs metabolism (systemic and not only kidney related). **Results Summary/Conclusion.** Our results highlight that the monoclonal FLCs of every patient behaves in a different way in different cellular lines and we could also show that not only epithelial cells are able to process paraproteins. Considering these data we could hypothesize that the interaction of FLCs with peripheral districts depends on high specificity receptors expressed on certain cellular lines and/or that each patient has an individual clinical pattern due to the paraprotein molecular structure.

D41 - MEASUREMENT OF SERUM FREE LIGHT CHAINS IN RENAL TRANSPLANTATION

M. Sánchez-Castañón, M. Gago, G. Fernandez-Fresnedo, C. Gómez, J. Ruiz-Criado, M. Lopez-Hoyos, M. Arias

Hospital Universitario Marqués de Valdecilla - Immunology Services, Santander, Spain

Background. Plasma cell dyscrasias can cause renal disease. Very sensitive methods have been introduced recently to quantify serum free light chains (sFLC). Renal function may influence the variability of these methods as shown in chronic kidney disease patients, but it has not been addressed in renal transplant patients. **Aims.** To evaluate the association between polyclonal sFLC concentrations and renal function in a population of renal transplanted patients. **Methods.** 102 kidney allograft recipients and 53 CKD patients classified according to KDOQI stages were studied. None of the patients had monoclonal gammopathies. The levels of polyclonal sFLC were quantified by nephelometry. **Results.** Both serum κ and λ FLC concentrations rose progressively

through each stage of KDOQI in transplant and non-transplant patients ($p < 0.0001$). In transplanted patients sFLC concentrations correlated (Spearman coefficient) significantly with serum creatinine, serum cystatin and estimated glomerular filtration rate: 0.723, 0.797 and -0.711 for skFLC and 0.705, 0.759 and -0.694 for sλFLC ($p < 0.0001$ in all cases). Spearman correlation coefficients in non-transplant patients were: 0.559, 0.848 and -0.766 for skFLC and 0.702, 0.875 and -0.855 for sλFLC ($p < 0.0001$ in all cases). *Discussion.* sFLC must be interpreted cautiously due to their clear association with renal function. Renal transplantation does not induce changes different from those dependent on renal function. The role of sFLC in the progression of the renal impairment should be interpreted in prospective studies.

D42 - CONVENTIONAL HIGH EFFICIENCY HAEMODIAFILTRATION AS AN ADJUNCTIVE TREATMENT OF KAPPA LIGHT CHAIN NEPHROPATHY: A CLINICAL CASE REPORT

L. Chenine-Khoualef, A. Rodriguez, H. Leray-Moragues, A. Gontiers, M. Morena, C. Cognot-Pons, G. Cartron, B. Canaud

Nephrology, Dialysis and Intensive Care - Hemato-oncology - Immunology - Lapeyronie and Saint Eloi University Hospital - CHU Montpellier, France

Introduction. The incidence of multiple myeloma is 3-4%; approximately 10% of all haematological malignancies. Renal disease is a common manifestation of multiple myeloma (up to 50% of patients) with a poor prognosis. One-year survival was 80% in patients with creatinine (Cr) < 130 compared with 50% in patients with a Cr > 200 . Prognosis is worse in patients who require dialysis with a median survival of 4 months. We report here a clinical

case showing that conventional high efficient on-line haemodiafiltration with high flux polysulfone membrane was able to remove significant amount of kappa light chains and contributed to kidney recovery in a multiple myeloma patient. *Patient and results.* A 40 year old male was admitted to our intensive care unit with acute renal failure. Over the last 4 months, he presented with rapid deterioration. The results showed elevated serum Cr of 500 $\mu\text{M/L}$ and urea 24.6 mM/L , with a mild anemia and haemoglobin of 11 g/dl . Protein electrophoresis showed an abnormal IgG peak. Routine imaging found bilateral enlarged kidneys with interstitial oedema on echography and interstitial pulmonary oedema on chest X-ray. Serum free light chains were kappa 78,000 mg/L , lambda 12.5 mg/L . A kidney biopsy was showed typical cast cell nephropathy with tubulopathy and protein cylinders staining for light chains (kappa+++, lambda+). Multiple myeloma with light chains kappa and focal AL amyloidosis was diagnosed. Multiple myeloma treatment consisted of a short induction therapy with dexamethazone and Bortezomib, completed by melphalan, Bortezomib and dexamethazone. Renal replacement therapy consisted of high efficiency post-dilutional haemodiafiltration (HDF) daily for 3 months. We noticed after this period partial recovery of kidney function allowing interruption of dialysis. After almost 18 months the patient is considered in remission from its multiple myeloma and kidney function is stable with a GFR 45 mL/min/1.73m^2 . In our treatment schedule, daily HDF reduced circulating light chain immunoglobulins kappa by 45% and lambda by 20%. *Conclusion.* HDF combined with standard chemotherapy represents an appealing adjunctive treatment to reduce kidney damage in multiple myeloma free light chains. Prospective studies assessing the role of HDF as adjunctive therapy are required to define the exact place of this method in care of multiple myeloma patients with renal involvement.

FREE LIGHT CHAINS AND NON-PLASMA CELL DISORDERS

E43 - SERUM FREE LIGHT CHAIN LEVELS AS A MARKER OF DISEASE ACTIVITY FOLLOWING TREATMENT WITH B-CELL DEPLETION IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

T. Guillemette, N. Bardin, J.-M. Cournac, J. Mancini, R. Jean, N. Schleinitz, G. Kaplanski, J.-M. Durand, J.-R. Harlé, L. Chiche, J. Boucraut

Hôpital la Conception, Marseille, France

Background. Increased free light chain (FLC) levels have been reported as useful in various autoimmune conditions. B-cell depletion therapy with rituximab is commonly used although there is no agreement on how to monitor the response to this biotherapy. **Aims.** We investigated how FLC concentrations correlate with disease activity upon B cell targeted therapy in SLE patients. **Methods.** Patients with active SLE treated with rituximab, without significant renal failure, were retrospectively identified. Quantitative determination of IgG, IgA, IgM and serum FLCs was performed in serum samples by immunonephelometry, before (T1) and after (T2) rituximab (median delay 104 days). **Results.** Eleven Caucasian patients with SLE were identified with a median age of 36 years (20-59). In SLE patients with active disease at baseline, 7/10 had abnormal serum FLC levels, with 7 patients having both increased kappa levels (mean 32.8 ± 24.2 mg/l; maximum 91.2 mg/l) and increased lambda levels (mean 39.3 ± 31.6 mg/l, maximum 116 mg/l). Conversely, the kappa/lambda ratio was normal for all patients (mean 0.897 ± 0.257). Kappa FLC levels were correlated with serum level of IgG ($r=0.73$, $p=0.025$). FLCs were not correlated with anti-DNA levels. A significant negative correlation was observed between SLE disease activity assessed by complement C3 fraction and kappa levels ($r=-0.929$, $p<0.001$) or lambda levels ($r=-0.854$, $p=0.003$) and by lymphopenia and lambda levels ($r=-0.733$, $p=0.025$). A correlation was also observed with complement C4 fraction ($r=-0.7$, $p=0.036$) and with total complement activity ($r=-0.703$, $p=0.035$) for kappa. No correlation was observed between IgG, A or M levels and neither complement C3 fraction consumption, complement C4 fraction, nor total complement activity. Analysis of all samples pooled from 11 patients ($n=36$), before and after rituximab-induced B cell depletion was also performed. Over time, kappa and lambda FLC concentrations decreased significantly after rituximab treatment: 32.8 ± 24.2 mg/l for kappa at T1 vs 16.4 ± 4.6 mg/l at T2 ($p=0.017$), and 39.3 ± 31.6 mg/mL for lambda at T1 vs 20.8 ± 6.6 mg/l at T2 ($p=0.015$). Total concentrations of IgG, A and M decreased after administration of rituximab, but remained within the normal range, except for IgM in 3 patients. There was a good correlation between kappa and lambda FLC levels and serum level of IgG ($r=0.65$, $p<0.001$ and $r=0.771$, $p<0.001$, respectively) but still no correlation between serum level of IgG and SLE disease activity assessed by complement C3 fraction consumption. Conversely, there was still a strong negative correlation between kappa FLC levels and

complement C3 fraction consumption ($r=-0.543$, $p=0.003$). Again, no correlation was observed between neither FLC levels nor IgG level, and anti-DNA antibodies levels. **Summary/Conclusion.** In the absence of renal failure, increased FLC levels without abnormal kappa/lambda ratio is a common feature of SLE patients with active disease and correlates well with disease activity.

E44 - FREE LIGHT CHAINS: A PREDICTOR OF EMERGENT MEDICAL CARE UTILIZATION IN LUPUS

M. Jolly, A.R. Mikolaitis, J.A. Block, W. Sequeira
Rush University, Chicago, USA

Background. Systemic Lupus Erythematosus (SLE) is associated with disease flares, organ damage and poor quality of life. In addition frequent physician visits and investigative work up for disease surveillance and monitoring of treatment side effects is required. Patients may also need emergent care and hospitalization resulting in significant health care resources utilization and economic burden. Potential biomarkers that predicts emergent use of medical care (Emergency room (ED) visits or hospitalizations) may serve as a screening tool to identify SLE patients at risk and allow for timely intervention to improve patient health. **Aims.** To determine the relationship between serum total free light chains (FLC) and subsequent (a) ED visits (any cause vs. SLE related), (b) Hospitalization (any cause vs. SLE related), and (c) Combined Outcome-ED visit and/or hospitalization (SLE related), (d) Deaths, among SLE patients. **Methods.** FLC kappa and lambda was measured on a Siemens Dade Behring BN II nephelometer in 211 patients sera. Data from 57 SLE subjects was retrospectively compared for subsequent ED visit/s, hospitalization/s and death. Correlation analysis (Spearman's), Mann Whitney test and Odds ratio estimates were utilized. A p value ≤ 0.05 was considered significant. **Results.** Twenty one percent of patients were Caucasian, 58% African-American, Hispanic 14%, Asian 2%, and Other 5%. Ninety one percent were women. Mean age was 43.8 ± 12.7 yrs. 33% of subjects had visited ED, while 34% had been hospitalized. Total FLC correlated with SLE related and any cause ED visits ($r=0.5$, $p=0.001$ and $r=0.39$, $p=0.02$ respectively). There was also a significant correlation with SLE related hospitalisation ($r=0.40$, $p=0.01$). Total FLC values correlated with "any cause" ED visit ($r=0.39$, $p=0.02$) but not with "any cause" hospitalization or death. Total FLC values correlated with "SLE related" ED visit ($r=0.50$, $p=0.001$) and "SLE related hospitalization" ($r=0.40$, $p=0.01$). Total FLC (Median, IQR mg/L) values among those with "SLE related" ED visit (70.6, 21.5 mg/L) or "SLE related" hospitalization (70.6, 29.1 mg/L) were significantly greater as compared to those without ED visit (42.3, 29.1 mg/L) or hospitalization (44.6, 29.1 mg/L). There were no significant differences in TFLC levels among those with "non-SLE related" ED visit or "non-SLE related" hospitalization from those without ED visit and hospitalization respectively.

Total FLC correlated with the combined outcome measu-

re (SLE related ED visit or/and SLE related hospitalization) (r 0.55, $p=0.001$). Total FLC (Median, IQR) among those with this combined outcome (67.9, 27 mg/L) was significantly greater than those without an ED visit and hospitalization (42.6, 24.8 mg/L). The calculated odds for experiencing the combined outcome were 7.8 times higher if Total FLC levels exceeded 65 mg/L. *Summary/Conclusion.* Total FLC may predict subsequent need for emergent medical care in SLE, and may be useful in identifying those at risk. Timely intervention in those identified at risk has the potential to improve health outcomes and reduce health care utilization.

E45 - SYSTEMIC LUPUS ERYTHEMATOSUS AND FREE LIGHT CHAINS

J. Jiménez Jiménez, N.M. Barbosa de Carvalho, M. Crespo Echeverría, R. Jañez Carrera, C. Hernando de Larramendi

¹Hospital Severo Ochoa, Clinical analysis service, Leganes, Spain; ²The Binding Site, Barcelona, Spain

Background. Systemic lupus erythematosus (SLE) is a disease of unknown etiology and is the prototype autoimmune disease. It is characterized by B cell polyclonal hyperactivity with a wide spectrum of antibodies. The majority of patients present with disease flares punctuated by periods of inactivity. *Aims.* To evaluate serum free light chains (sFLC) as a disease activity marker. *Methods.* 40 patients with SLE were studied (33 females and 7 males). The average disease period was 8 years (1 to 24 years). Patients had at least 4 of the 11 disease criteria devised by the American Rheumatologic College. Patients' ages were 28 to 54 years; 35 had inactive disease and 5 were in flare. 15 serum samples from patients that were not affected by any rheumatological disease were used as a control group. Anti-nuclear and anti-dsDNA antibodies, CRP, β_2 -microglobulin, C₃, C₄, immunoglobulins, kappa (κ sFLC) and lambda (λ sFLC), haemoglobin and erythrocyte sedimentation rate (ESR) were assessed in all samples. *Results.* The mean results obtained in the control group for κ sFLC and λ sFLC were: 12.18 (± 2.11) and 14.08 (± 4.85) respectively and for SLE patients; 17.40 (± 10.66) and 23.47 (± 6.17); significantly different for both κ sFLC ($p < 0.02$) and λ sFLC ($p < 0.0004$). For inactive patients, the results for κ sFLC were 16.04 (± 6.67) and λ sFLC 21.0 (± 5.48) and correspondingly for the flare group were 20.84 (± 16.49) and 29.97 (± 2.55), presenting a significant difference for λ sFLC ($p < 0.0008$). ROC curves for the usual SLE follow-up parameters gave higher area under the curve for κ sFLC+ λ sFLC: 0.842 (0.105), λ sFLC: 0.833 (0.130) and ESR: 0.750 (0.153). Sensitivity (Se) and specificity (Sp) obtained for κ sFLC+ λ sFLC Se - 100%, Sp - 71.4%; ESR Se - 100%, Sp - 25%; κ sFLC Se - 66.7%, Sp - 62.5% and λ sFLC Se - 100%, Sp - 63.5%. When cut-off points were marked at 9.9 mg/L for κ sFLC and 16.2 mg/L for λ sFLC, values of Se and Sp respectively were: 100% and 87.5% for κ sFLC and 100% and 75% for λ sFLC. In a patient with serial determinations of sFLC we observed a 50% decrease in sFLC concentrations when she was in a period of inactivity compared with a flare period.

Conclusion. We believe that sFLCs could be included as an SLE follow-up parameter, presenting a clear advantage over other parameters. It has a short half-life that reflects quicker changes produced by the disease so a patient's base-line sFLC is useful. However, this issue should be studied in larger prospective studies.

E46 - SERUM FREE LIGHT CHAINS AND DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

M. Jolly,¹ R. Aggarwal,² S. Francis,¹ A.R. Mikolaitis,¹ T. Niewold,³ S. Chubinskaya,¹ J.A. Block,¹ W. Sequeira¹

¹Rush University, Chicago, ILL, USA; ²University of Pittsburgh Medical Center, Pittsburgh, Penn, USA

Background. Systemic Lupus Erythematosus (SLE) is associated with significant morbidity and mortality. We previously reported greater total FLC (tFLC) elevation and stronger association with disease activity in SLE than in rheumatoid arthritis. Furthermore, we reported the superiority of using tFLC over dsDNA antibody and immunoglobulin G levels as a biomarker of disease activity in 75 SLE patients. *Aims.* To measure tFLC in SLE patients and compare its utility with interleukin 6 (IL 6), interleukin 10 (IL10) and interferon α (IFN α) as a biomarker for disease activity. *Methods.* 211 SLE patients at a University Hospital were enrolled. Disease activity was assessed using physician global assessments (PGA) and SLEDAI. Adjusted SLEDAI (adjSLEDAI = SLEDAI - [C3C4 and ds-DNA antibody abnormality]) was obtained. serum FLC (kappa (κ), lambda (λ)) and total FLC [$\kappa + \lambda$] was measured using nephelometry. Additionally, serum dsDNA (antibody positivity and titers), complement (C3 & C4 abnormality and titers), IL 6, IL10 and IFN α levels were available. Spearman correlation was used to correlate tFLC and other biomarkers with PGA, SLEDAI and adjSLEDAI.

Table 1. Correlation of markers with disease activity.

	TFLC	DsDNA abnormality	DsDNA titer	Complement abnormality	C3 titer	C4 Titer	IL6	IL10	IFN α
N	211	182	171	171	109	109	121	117	120
PGA	0.39 ($p=0.0001$)	0.39 ($p=0.0001$)	0.34 ($p=0.0001$)	-0.14 ($p=0.07$)	-0.24 ($p=0.01$)	-0.14 ($p=0.16$)	0.02 ($p=0.88$)	0.18 ($p=0.20$)	0.13 ($p=0.40$)
SLEDAI	0.44 ($p=0.0001$)	0.49 ($p=0.0001$)	0.47 ($p=0.0001$)	-0.13 ($p=0.16$)	-0.41 ($p=0.0001$)	-0.23 ($p=0.03$)	0.23 ($p=0.04$)	0.21 ($p=0.05$)	0.20 ($p=0.09$)
Adj.	0.39 ($p=0.0001$)	0.23 ($p=0.03$)	0.211 ($p=0.05$)	-0.08 ($p=0.45$)	-0.15 ($p=0.22$)	0.12 ($p=0.31$)	0.16 ($p=0.24$)	0.32 ($p=0.02$)	0.03 ($p=0.83$)

Results. The mean age was 42 $\pm$ 13 yrs. African American 45%, Caucasian 30%, Hispanic 12%, Asian 10% and Others 4%. PGA and SLEDAI (mean $\pm$ SD, median) were 0.7 $\pm$ 0.8, 0.5 and 4.2 $\pm$ 4.3, 2, respectively. The values (mean $\pm$ SD, median) were: tFLC (62.5 $\pm$ 69.8, 44.3), IL 6 (6 $\pm$ 7, 4), IL10 (16 $\pm$ 20, 13) and IFN α (21 $\pm$ 39, 4). tFLC correlated with IFN α ($r=0.37$, $p=0.0001$) and IL 6 ($r=0.25$, $p=0.009$). Table 1 depicts the association of various biomarkers with disease activity. *Summary/Conclusion.* tFLC is correlated with disease activity. Its correlation with PGA and adjSLEDAI is stronger than other biomarkers tested. These data suggest that tFLC may serve as a potential biomarker for SLE activity.

E47 - SJÖGREN'S SYNDROME AND SERUM FREE LIGHT CHAIN ANALYSIS IN NEWLY DIAGNOSED PATIENTS

M. Maignan,¹ A. Driad,¹ C. Jacob,² N. Jay,³ B. Dousset,² J.-M. Vignaud,⁴ J.-D. De Korwin¹

¹Service de Médecine Interne H- HOPITAL CENTRAL, Nancy France; ²Laboratoire de Biochimie, CHU, Nancy France; ³Département d'information Médicale, CHU, Nancy France; ⁴Laboratoire d'anatomo-pathologie, CHU, Nancy, France

Background. Sjögren's syndrome (SS) is a chronic autoimmune epitheliitis characterised by lymphocytic infiltration of the lachrymal and salivary glands. B cells activation has been well demonstrated in SS. Fatigue and sicca syndrome are the main complaints. Outcome of systemic extraglandular manifestations and a long term risk for lymphoma development justify a regular follow-up. **Aims.** We examined the presence of the serum free light chain (sFLC) in all patients with SS newly diagnostic in an internal medicine department. We compared the sFLC with clinical and biological markers of disease activity. **Methods.** Diagnosis of SS was made on the criteria of the American-European Consensus group in 2002. We compared value of sFLC (κ , λ and the κ/λ ratio) with levels of β_2 -microglobulin (β_2 M), blood lymphocyte count, gammaglobulin, Immunoglobulin G, complement factors and presence of cryoglobulinaemia. Disease activity at presentation was also compared with the value of sFLC. Spearman correlation coefficients were tested against zero. **Results.** In 2009/2010, we identified 22 patients with a new diagnosis of SS (21 females and 1 male). The mean age was 55.7 years (range: 27-78). Twelve patients had primary SS and 10 secondary SS. Secondary SS was associated with; autoimmune thyroiditis (5 patients), limited scleroderma (3 patients), primary biliary cirrhosis (3 patients) and 1 dermatopolymyositis. Eight patients had an enlarged parotid gland at presentation. Main systemic manifestations were arthralgia and/or Raynaud's phenomenon. All patients had a Cockcroft-Gault clearance of creatinine above 50 ml/min. None had HIV, hepatitis C infection, sarcoidosis, myeloma, amyloidosis, lymphoma or monoclonal gammopathy of undetermined significance. 19/20 patients had a positive labial salivary gland biopsy. 13/22 patients had significant anti-SSA or anti-SSB antibodies. 12/21 had an increased β_2 M above 1.5 mg/l. Hypergammaglobulinemia was observed in 11/22 patients. 8/18 patients had a high Immunoglobulin G. Eleven patients had a high κ free light chain or λ free light chain or abnormal κ/λ ratio. 1/15 patients had a cryoglobulinemia. κ sFLC levels were correlated with serum level of gammaglobulin ($\rho=0.76$, $p<10^{-4}$), of IgG ($\rho=0.85$, $p<10^{-3}$), and with β_2 M ($\rho=0.75$, $p<10^{-4}$). There was a significant negative correlation between blood lymphocyte and κ sFLC levels ($\rho=-0.66$, $p<10^{-3}$). λ sFLC levels were correlated with serum level of gammaglobulin ($\rho=0.56$, $p<6.10^{-3}$), of IgG ($\rho=0.67$, $p=0.002$) and with β_2 M ($\rho=0.66$, $p<10^{-3}$). Correlation between blood lymphocyte count and lambda sFLC levels was not significant ($\rho=-0.41$, $p=0.056$). Median value of κ sFLC in patients with enlarged parotid glands was 25.05 mg/L, and 12.85 mg/L in

patients without enlarged parotid gland ($p=0.07$). We did not observe any correlation between κ/λ ratio and serum gammaglobulin, IgG nor β_2 M levels. **Conclusion.** In newly diagnosed SS patients, serum free light chain was significantly associated with lymphopenia, β_2 M and gammaglobulin. We continue the study and follow-up the patients in order to specify the role of sFLC measurement for diagnosis and/or prognosis of Sjögren's syndrome.

E48 - SERUM FREE LIGHT CHAINS AND IgA κ /IgA λ RATIOS IN PATIENTS WITH SYSTEMIC INFLAMMATORY RHEUMATIC DISEASES WITHOUT MONOCLONAL GAMMOPATHY

B. Schlüter, G. Bonsmann, H. Becker, P. Willeke

University Hospital Münster, Centre for Laboratory Medicine, Münster, Germany

Background. Novel nephelometric assays allow for the specific and sensitive quantification of serum κ and λ free light chains (FLC) as well as intact IgA κ and IgA λ immunoglobulins in the routine clinical laboratory setting. The determination of κ/λ FLC and IgA κ /IgA λ ratios as derived parameters is helpful for the sensitive detection and monitoring of monoclonal gammopathies since values outside the normal range are indicative of monoclonal proliferation of immunoglobulin producing cells. A characteristic feature of some systemic inflammatory rheumatic diseases is polyclonal B cell activation which may affect the production of serum FLC, IgA κ and IgA λ immunoglobulins and potentially the respective ratios. This may be of particular importance since inflammatory rheumatic diseases lead to an increased risk for the development of myeloma and monoclonal gammopathies of undetermined significance (MGUS). **Aims.** We studied the serum FLC and IgA κ /IgA λ ratios in patients with systemic rheumatic diseases without monoclonal gammopathy. **Methods.** In a cross-sectional retrospective study sera obtained from 120 patients (92 female, 28 male, mean age 46.9 ± 15.4 years) with an established diagnosis of either a collagenosis (group CD: 51 SLE, 7 systemic sclerosis, 6 CREST, 3 dermatomyositis, 11 Sjögren's syndrome, 3 MCTD, 2 overlap syndrome), rheumatoid arthritis (group RA: 17) or Wegener's granulomatosis (group WG: 20). There was no evidence of monoclonal gammopathy in these samples as demonstrated by serum electrophoresis, quantification of serum IgG, IgA and IgM levels, and in individual cases by serum immunofixation. All sera were tested by immunonephelometric assays (Binding Site, UK) on a BN II analyser (Siemens, Germany) for serum FLC and intact IgA κ and IgA λ concentrations. **Results.** Polyclonal gammopathy (PG) defined as serum levels of IgG, IgA and/or IgM above the respective upper reference limit was present in 52 % of CD, 41 % of RA, and 10 % of WG samples ($p=0.0032$). The serum concentrations of FLC and intact IgA κ and IgA λ were significantly increased in PG-positive versus PG-negative sera (median values in mg/l for κ : 25.7 vs. 16.5; for λ : 25.6 vs. 16.5; for IgA κ : 2075 vs. 1110; for IgA λ 1595 vs. 933; $p<0.0001$ for each comparison). In contrast, the FLC and IgA κ /IgA λ ratios did not differ significantly between PG-positive and negative sera

(median κ/λ FLC ratio 0.92 vs. 1.01; median IgA κ /IgA λ ratio 1.32 vs. 1.33). Ratios ranged from 0.47 to 2.19 for κ/λ and from 0.68 to 2.40 for IgA κ /IgA λ (2.5 to 97.5 percentiles). The glomerular filtration rate (GFR) was reduced in 10 % of patients. The concentration of κ FLC, but neither of λ FLC nor of intact IgA κ and IgA λ was significantly increased in patients with a reduced GFR ($p = 0.0079$). In addition, the FLC and IgA κ /IgA λ ratios did not differ according to GFR status. *Summary/Conclusion.* Our data demonstrate that patients with systemic inflammatory rheumatic diseases have increased serum levels of FLC, intact IgA κ and IgA λ due to polyclonal B cell activation. FLC and IgA κ /IgA λ ratios were comparable to previously reported values for healthy subjects.

E49 - POLYCLONALLY RAISED SERUM FREE LIGHT CHAINS IN LIVER DISEASE COMPARED TO MARKERS OF LIVER DAMAGE

L.K. Assi,¹ R.G. Hughes,¹ B. Gunson,² G. Webb,¹ D.H. Adams²

¹The Binding Site Group Ltd, Birmingham, UK; ²Liver Research Laboratories, University of Birmingham, Birmingham, UK

Background. Monoclonal immunoglobulin serum free light chains (sFLCs) are elevated in many B-cell dyscrasias, including multiple myeloma, typically producing an abnormal κ/λ FLC ratio. Increased concentrations of κ and λ sFLCs can result from reduced clearance by the kidneys or increased polyclonal production. Abnormal B-cell activation is also observed in autoimmune liver diseases (LD), shown by the emergence of autoantibodies and high levels of immunoglobulins detected in many chronic patients. sFLCs have recently been shown to be particularly elevated in alcohol-related LD (ALD), possibly due to reduced clearance or increased production. *Aims.* To determine whether there is any association between sFLCs and other biological markers of liver dysfunction in LD. *Methods.* Serum samples from 80 LD patients, including 22 ALD patients, were obtained (with ethical approval) from the University Hospital, Birmingham. Immunoglobulins, sFLCs and cystatin C were measured turbidimetrically. Free κ and λ values were summated to give total sFLC values and κ/λ ratios were calculated. Values were compared against established normal ranges (κ : 3.3-19.4mg/L, λ : 5.71-26.3mg/L, ratio: 0.26-1.65). sFLCs were also corrected for renal function using cystatin C. Platelet cell counts (normal range: 150-400 $\times 10^9$ /L) and serum transaminase levels, to assess liver damage (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), were collated and analysed in relation to sFLC results. The model for end stage liver disease (MELD) score (ranges between 6-40 indicating increasing disease severity) was compared with the sFLC results. *Results.* Individuals in several LD groups had particularly high sFLC concentrations. Highest levels were detected in the ALD group (median concentration: 59.9mg/L, range: 20.4-256.4mg/L), with one particularly elevated total sFLC result of 754mg/L in a patient with cryptogenic cirrhosis. ALT values were generally within normal ranges (5-41U/L), with a slight elevation for AST.

No correlation was observed between corrected sFLCs and ALT in ALD or the whole LD population. Furthermore, although no association was determined between corrected sFLCs and AST in the ALD group, a correlation was observed when analysing the whole LD population ($r=0.244$, $p=0.03$). Both the ALD and cryptogenic cirrhosis populations had reduced median platelet cell counts (median: 125 $\times 10^9$ /L and 92 $\times 10^9$ /L respectively). There was no correlation with corrected sFLCs in the ALD group ($r=0.12$, $p=0.63$), although a significant correlation was determined in all LD patients ($r=-0.307$, $p=0.006$). 11/22 ALD patients had a MELD score <20 , but conversely had abnormally elevated corrected sFLCs. *Summary/Conclusion.* sFLCs are polyclonally elevated in LD particularly ALD (N=22) and no association was observed between sFLCs and other markers of liver dysfunction in this group. ALD and cryptogenic cirrhosis disease groups had reduced platelet cell counts, indicative of portal hypertension and advanced LD. In ALD, there was no correlation between sFLCs and platelet counts or AST, although a positive correlation was determined when analysing the whole LD population. Most interestingly, 50% ALD patients with a relatively low MELD score had elevated sFLCs. Therefore, elevated sFLC levels may be an additional useful biological marker for the diagnosis and monitoring of ALD, but further studies are required to verify these preliminary results.

E50 - DIFFERENCES BETWEEN C-REACTIVE PROTEIN AND FREE LIGHT CHAIN PRODUCTION AFTER TRAUMA

P. Lukacik,¹ R.G. Hughes,¹ G. Pratt,² C.A. Hutchison,^{3,4} G.P. Mead^{1,4}

¹The Binding Site Group Ltd, Birmingham, UK; ²Heartlands Hospital, Heart of England NHS Trust, Birmingham, UK; ³Renal Unit, University Hospital Birmingham, Birmingham, UK; ⁴Division of Immunology & Infection, The Medical School, University of Birmingham, Birmingham, UK

Background. The measurement of serum free light chains (FLCs) is now widely used as an aid in the diagnosis and monitoring of a range of monoclonal gammopathies including multiple myeloma, AL amyloidosis and plasmacytoma. However, potential applications for the measurement of polyclonal FLC elevations have recently been reported (e.g. in renal disease or autoimmune conditions). *Aims.* The aim of this study was to therefore determine whether FLC levels correlate with those of acute phase proteins. *Methods.* The relationship between the production of free light chains (FLCs) and the acute phase protein CRP was examined in 11 patients in the intensive therapy unit (ITU). Clinical problems included surgery, sepsis and respiratory failure. Plasma samples were collected as clinically indicated during ITU admission (range 3 to 32 days), residual volumes of which were used for this study. Plasma concentrations of κ FLC, λ FLC, IgG, IgA, IgM, cystatin C and CRP were measured by established nephelometric immunoassays. FLC levels were corrected for variations in kidney function by using cystatin C values, since it is a sensitive indicator of renal clearance. The corrected values,

which should be a reflection of FLC production, were therefore calculated as $[\kappa\text{FLC} + \lambda\text{FLC}] / [\text{cystatin C}]$. Total immunoglobulin concentration was estimated by summing immunoglobulins IgG, IgA and IgM. **Results.** 10 out of 11 patients showed a substantial elevation of CRP (range 57-367 mg/L). In 7 of these 10 patients, the peak in CRP was followed by a delayed gradual rise in FLC production. In general, the increase in FLC concentration was approximately 4-fold but did not exceed the upper normal range value ($[\kappa + \lambda] \leq 50$ mg/L in 9 out of 11 patients). Furthermore, in 4 out of 11 patients the changes in corrected FLC levels were not correlated with changes in total immunoglobulin concentration. This latter observation is at odds with the current understanding that polyclonal FLC production is a "by-product" of immunoglobulin synthesis and calls for further study. **Summary/Conclusion.** These preliminary results clearly show that FLCs do not behave as acute phase proteins. Additional studies in patients from a well defined clinical background (e.g. elective surgery) and with a longer follow up may help to dissect these observations more fully.

E51 - ABNORMAL SERUM FREE LIGHT CHAIN (sFLC) RATIO IS A PREDICTOR OF AGGRESSIVE FORM OF CLL AND IS RELATED TO RESPONSE TO TREATMENT

F. Durrieu, J. Dubois, H. Eghbali, P. Correia, F. Demarthe, G. Etienne

Institut Bergonie, Bordeaux, France

Background. Although chronic lymphocytic leukaemia (CLL) seldom has a detectable serum monoclonal immunoglobulin peak, previously published data have emphasized the utility of serum free light chain (sFLC) assessment in newly diagnosed chronic lymphocytic leukaemia.^{1,2} **Aims.** The aim of our study was to confirm the utility of sFLC assessment at CLL diagnosis, but also evaluate the use of the test in patients' follow-up, particularly in relation to the response to treatment. **Methods.** We investigated the sFLC ratio in 58 patients newly diagnosed with CLL in our centre between August 2007 and May 2010. Prognostic factors such as Binet stage, CD38 and ZAP-70 expression, FISH analysis for P53, ATM, or RB deletion and chromosome 12 number, serum thymidine kinase and serum soluble CD23 were systematically evaluated. In addition, the sFLC ratio was assessed during patients' follow-up over a 36-month period, allowing 116 control measurements (one to six per patient), and was compared to minimal residual disease (MRD) measured by 5-colour flow cytometry, according to international recommendations.³ **Results.** Twenty-one patients (36%) had an abnormal sFLC ratio at diagnosis. The ratio of kappa/lambda was increased in 19 cases (90%). We confirmed that an abnormal sFCL ratio was associated with adverse prognostic factors such as advanced Binet stage (B and C, $p=0.0004$), CD38 positivity ($p=0.0003$), ZAP-70 positivity ($p<0.0001$), adverse cytogenetics (P53 deletion, ATM deletion or trisomy 12, $p=0.06$) and increased serum thymidine kinase ($p=0.0001$). Surprisingly, no relation was found with the presence of a serum monoclonal immunoglobulin peak, which was detected in 3

patients only. However a decrease in normal immunoglobulin serum level was evidenced in 20 of the 21 patients with abnormal sFLC ratio (95%), with a significant correlation ($p=0.002$). Analysis of treatment-free survival (TFS) duration between diagnosis and first line treatment showed a relation of the sFLC ratio. Patients with abnormal sFLC ratios had shorter TFS (HR=2.19, 95% CI=0.84-7.38). Abnormal sFLC ratios distinguished a group with shorter TFS even in low risk CLL patients (less than 2 adverse prognostic factors), confirming that the disease was more aggressive in this group (HR=3.95, 95% CI=0.93-58.15). When analysed during patients' follow-up after treatment, an abnormal sFLC ratio was associated with positive MRD ($p<0.0001$), with a specificity of 86% and a positive predictive value of 96%. **Conclusion.** sFLC ratio assessment could be a simple and cost-effective test to appreciate the aggressiveness of CLL at diagnosis, and could be implemented as a first-intention test to estimate the response to treatment, saving flow cytometry for when the ratio is, or becomes normal.

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E52 - ABNORMAL SERUM IMMUNOGLOBULIN FREE LIGHT CHAIN (sFLC) RATIO PREDICTS THE RAPHY REQUIREMENTS AND ALLOWS DEFINITION OF A NOVEL PROGNOSTIC SCORE SYSTEM FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

F. Morabito,¹ R. De Filippi,² S. Cavalcanti,³ G. Iaccarino,² M. Gentile,³ I. Attolico,⁴ G. Cutrona,⁵ A. Neri,⁶ M. Ferrarini,⁵ S. Molica,⁷ V. Gigliotti,³ A. Recchia,³ E. Morelli,² B. Amoroso,⁸ L. Laurenti,⁹ G. Del Poeta,¹⁰ A. Pinto²

¹U.O.C. Ematologia Azienda Ospedaliera viale della Repubblica, Cosenza, Italy; ²Hematology-Oncology and Transplantation Unit, Fondazione G. Pascale, Napoli, Italy; ³Hematology Unit, Cosenza, Italy; ⁴Hematology Unit Ospedale San Carlo, Potenza, Italy; ⁵Divisione di Oncologia Medica C, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy; ⁶Centro di Ricerca per lo Studio delle Leucemie, Fondazione IRCS, Milano, Italy; ⁷Dipartimento di Oncologia/Ematologia, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy; ⁸Clinical Research Direction, The Binding Site, Rome, Italy; ⁹Istituto di Ematologia, Università Cattolica del Sacro Cuore, Rome, Italy; ¹⁰Division of Haematology, Ospedale S. Eugenio, University Tor Vergata, Roma, Italy

Background. The most common CLL biological prognostic factors include expression of CD38, ZAP-70, whose detection techniques are associated with significant inter-laboratory variations, and IGHV mutation status which requires DNA sequencing. Since the serum free light chain (sFLC) assay is a sensitive, simple and highly reproducible serum test, it is particularly attractive as a

putative prognostic tool in CLL. *Aims.* This investigation examined the value of an abnormal sFLC κ/λ ratio as a predictor of treatment free survival (TFS) in comparison with current biological assays and further defined a hierarchy of these markers in predicting outcome. *Methods.* sFLC levels were determined using a particle enhanced, high-specificity, immuno-FLC assay (**Freelite**; The Binding Site Group Ltd, Birmingham, UK).

Figure 1.

Results. An abnormal sFLC κ/λ was found in 134 of 403 untreated CLL cases (116 κ , 18 λ). A significant correlation was observed for sFLC κ/λ , CD38 and ZAP-70 as well as for *IGHV* status. CLL cases were divided into three groups according to sFLC κ/λ abnormality. The 3.0 year probability of TFS for cases with abnormal sFLC κ/λ was significantly lower than cases with a normal sFLC κ/λ (64% vs 63% vs 83%, $p<0.0001$). Since abnormal κ and λ sFLC curves overlapped, combining cases with abnormal sFLC κ/λ (i.e. κ plus λ) revealed these group had a significantly shorter TFS ($p<0.0001$, median TFS 12.3 years) as compared to patients with normal sFLC κ/λ (median TFS 4.1 years). Univariate Cox analysis showed 76 of 380 CD38+ cases (H.R. 2.8, 95% C.I. 1.9-4.1, $p<0.0001$), 134 of 303 ZAP-70+ cases (H.R. 4.5, 95% C.I. 3.1-6.4, $p<0.0001$) 114 of 333 *IGHV* unmutated cases (H.R. 4.1, 95% C.I. 2.8-6.0, $p<0.0001$), and 103 of 361 Binet stage B and C cases (H.R. 2.4, 95% C.I. 1.7-3.4, $p<0.0001$) had a higher risk of starting treatment when compared to corresponding cases (H.R.=1). Abnormal sFLC κ/λ (HR=2.3, 95% C.I. 1.4-3.7, $p=0.001$) was independently associated with TFS even after CD38, ZAP-70, *IGHV* status and Binet stage adjustment. In this model, only ZAP-70 (HR=3.0, 95% C.I. 1.7-5.1, $p<0.0001$), *IGHV* status (HR=2.6, 95% C.I. 1.4-4.7, $p=0.002$) and Binet stage (HR=2.8, 95% C.I. 1.6-4.7, $p<0.0001$) remained statistically associated with TFS. Following multivariate analysis, a scoring system was designed whereby 1 point was assigned to each unfavourable prognostic marker and the final score was given by the sum of all unfavourable markers. The 3.0 year TFS probability of 91 cases scored 0 was significantly higher (95.2%) than 87.8%, 83.1%, 36.0% and 0% of cases respectively scored 1 (100 cases), 2 (49 cases), 3

(40 cases) or 4 (12 cases) ($p<0.0001$). Considering that cases scoring 3 and 4 were not statistically different, these were combined to generate groups with low (no risk factor), intermediate-low (1 risk factor), intermediate-high (2 risk factors) and high risk with (3-4 risk factors). Using such criteria, 31.2%, 34.2%, 16.8% and 17.8% of cases clustered in low (HR=1), intermediate-low (HR= 2.0, 95% C.I. 1.1-4.1, $p=0.05$), intermediate (HR= 4.6, 95% C.I. 2.1-10.1, $p<0.0001$) and high risk groups (HR= 17.9, 95% C.I. 8.9-36.2), respectively (Figure 1). *Summary/Conclusion.* sFLC κ/λ is independent of all other prognostic factors in predicting TFS and should be incorporated to define risk-adapted therapeutic trials in CLL.

HEAVY CHAIN/LIGHT CHAIN ANALYSIS AT DIAGNOSIS AND COMPARISON WITH ELECTROPHORESIS

F53 - ABNORMAL HEAVY/LIGHT CHAIN RATIOS IN PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS

A. Wechalekar,¹ S. Harding,² H. Lachmann,¹ J. Gillmore,¹ N. Wassef,¹ M. Thomas,¹ S.D.J. Gibbs,¹ P. Sattianayagam,¹ C.J. Whelan,¹ A.R. Bradwell,³ P. Hawkins¹

¹National Amyloidosis Centre, Centre for Amyloidosis & Acute Phase Proteins, Department of Medicine Royal Free and University College Medical School, London, UK; ²The Binding Site Group Ltd, Birmingham, UK; ³Division of Immunity and Infection, Medical School, University of Birmingham, UK

Background. Primary systemic amyloidosis is a disease characterised by the accumulation of misfolded monoclonal free light chains. The monoclonal disease is subtle and in 5-20% of patients undetectable in the serum by serum protein electrophoresis (SPE) and 6% have no detectable clonal marker by any standard method including serum free light chains (FLC). FLC measurements are fundamental in the diagnosis of these patients. The sensitivity of the assay relies on the specificity of the antisera and the production of a FLC kappa/lambda ratio. The FLC ratio means that it is possible to detect clonality even when there are low levels of monoclonal protein produced. In a similar manner, antibodies which recognise kappa or lambda bound to their heavy chain partner allow accurate and specific quantification of serum IgG κ and IgG λ , IgA κ and IgA λ , and IgM κ and IgM λ proteins and the calculation of their ratios [heavy-light chain or (HLCr) ratios]. Here we report on the utility of these antibodies in nephelometric assays for detection and quantification of monoclonal proteins in patients with systemic AL amyloidosis. **Materials and Methods.** In retrospective analysis of 155 patients attending the UK National Amyloidosis Centre (NAC), IgG κ and IgG λ , IgA κ and IgA λ , and IgM κ and IgM λ were measured on a Siemens Dade Behring BN II Nephelometer. HLCr were compared with previously produced SPE and immunofixation (IFE) results. **Results.** IgA HLCr correctly identified all IgA (10 IgA λ and 4 IgA κ) IFE positive patients, 7/14 of these patients had monoclonal proteins which were not quantifiable by SPE because of their co-migration with other proteins or because they were below the sensitivity of SPE. Similarly, IgM HLC identified all of the IgM (2 IgM and 6 IgM λ) IFE positive patients, all of whom were also positive by SPE. There was concordance between IFE and HLC in 55/58 IgG (48 IgG and 6 IgG λ) patients. 3 patients had abnormal IFE results with normal HLC ratios while monoclonal protein concentrations were below the limits of SPE sensitivity in all cases. In 46 patient sera that were negative by traditional serum and urine analysis HLC ratios identified clonality in 9 cases (2 IgA κ , 3 IgA λ and 4 IgG κ). **Conclusions.** Analysis of serum Ig κ and Ig λ and the production of Ig κ /Ig λ ratios identified low levels of paraproteins in patients with systemic AL amyloidosis. This was particularly useful in the case of IgA patients where the monoclonal protein co-migrated with other proteins. In an addition Ig κ /Ig λ ratios identified clonality in patients that were normal by all other standard serum and urine tests.

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F54 - EXPERIMENTAL AND PRACTICAL SENSITIVITIES OF Ig κ /Ig λ NEPHELOMETRIC IMMUNO-ASSAYS

J. Harper,¹ N. Fourrier,¹ K. Sharp,¹ L. Mirbahai,¹ J. Harris,¹ J. Bradwell,² S. Harding¹

¹The Binding Site Group Ltd, Birmingham, UK;

²Division of Immunity and Infection, Medical School, University of Birmingham, UK

Introduction. The sensitivity of the serum free light chain tests (FLC) for clones which are producing low concentrations of monoclonal proteins relies upon the use of numerical κ/λ ratios to define clonality. Separate measurement of κ and λ light chains bound to their heavy chain partners may also offer similar advantages. The production of Ig κ /Ig λ ratios may be particularly useful when monoclonal proteins are difficult to measure, for example when they co-migrate with other proteins on serum protein electrophoresis gels (SPE). Novel polyclonal antibodies have been produced which recognise junctional, conformational epitopes between the κ or λ light chains and their heavy chain partners (HLC). Here we report on the use of these antibodies in nephelometric assays for the detection and quantification of IgA and IgG monoclonal proteins. **Methods.** SPE and immunofixation (IFE) were performed using a SEBIA Hydrasys II electrophoresis system in accordance with manufacturer's instruction. Total IgG and IgA concentrations were measured using a Siemens BN II nephelometer. In addition IgG κ , IgG λ , IgA κ and IgA λ were also measured nephelometrically and the results used to produce IgG κ /IgG λ and IgA κ /IgA λ ratios. Experimental sensitivities were tested by spiking known concentrations of monoclonal proteins into well characterised, pooled normal human sera and comparison between SPE and HLC ratios was made. Presentation sera from 530 IgG (374 IgG κ and 156 IgG λ) and 210 IgA (145 IgA κ and 65 IgA λ) multiple myeloma (MM) patients were retrospectively analysed and results compared with normal ranges. **Results.** The limits of SPE sensitivity for detection of 3 monoclonal IgG proteins were 0.48, 0.85 and 0.43 g/L compared with IgG HLC ratio sensitivities of 0.95, 1.7 and 1.7 g/L. For 3 IgA monoclonal proteins the limits were comparable between SPE and IgA HLC. There was good agreement between IgG HLC and IFE in 526/530 samples (99.3%). In 4 samples (0.7%), IgG HLC was normal when IFE was positive, however in all cases the concentrations of monoclonal proteins were below the detection limit of SPE. There was concordance between IFE and IgA HLC for all samples measured. In 83/210 (40%) samples, accurate quantification by SPE densitometry was not possible due to the co-migration of the monoclonal protein with other serum proteins. **Conclusions.** The experimental sensitivity of IgG HLC is lower than SPE, although this is likely to be an experimental anomaly which was not reflected in patient sera due to the role of immunoparesis. Overall there was good agreement between IFE and HLC measurement at presentation. In conclusion Ig κ /Ig λ ratios provided a sensitive method for the identification and quantification of monoclonal proteins.

F55 - SERUM Ig κ /Ig λ (HEVYLITE) RATIOS CAN CONFIRM MONOCLONALITY IN SERA THAT ARE AMBIGUOUS BY IMMUNOFIXATION

S. Harding,¹ E. Lachmann,² A. Legg,¹ J. Reibe,³ M. Bruegel,⁴ S. Schauseil,⁵ A.R. Bradwell,⁶ G. Mead¹

¹The Binding Site Group Ltd, Birmingham, UK; ²National Amyloidosis Centre, Centre for Amyloidosis & Acute Phase Proteins, Department of Medicine Royal Free and University College Medical School, London, UK; ³MVZ Cornely, Reibe and Berndt, Aachen; ⁴Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig; ⁵Medizinische Laboratorien, Düsseldorf; ⁶Division of Immunity and Infection, Medical School, University of Birmingham

Introduction. Immunofixation electrophoresis (IFE) is used to identify monoclonal immunoglobulins (Ig) alongside abnormal serum protein electrophoresis (SPE) and/or serum free light chains (FLC). Whilst the majority of intact monoclonal Igs can be satisfactorily classified by IFE, it is frequently inadequate for the detection of clonality in nonsecretory multiple myeloma, light chain only myeloma and AL Amyloidosis. In addition, patients with low concentrations of monoclonal Igs can be difficult to characterise in the presence of normal or high concentrations of polyclonal Igs. Here we describe the use of serum **Hevylite** (HLC) measurements for identifying and quantifying difficult to characterise monoclonal Igs. **Materials and Methods.** SPE and IFE were performed using a SEBIA Hydrasys 2 in accordance with the manufacturer's instructions. FLCs, IgG κ , IgG λ , IgA κ and IgA λ concentrations were measured on a Siemens Healthcare Diagnostics BN II nephelometer and results compared with published normal ranges. **Results.** A patient with a low level IgG κ monoclonal protein relapsed with heavy chain disease confirmed by the presence of a monoclonal gamma band with no associated light chain. Retrospective analysis of the patient's IgG κ and IgG λ concentrations and calculation of IgG κ /IgG λ ratio of 4.9 (normal range 0.98-2.75) indicated a persistent IgG κ monoclonal protein which was below the sensitivity of IFE. Serum IgA κ and IgA λ levels were measured in 3 patients with ambiguous IFE results. The first sample had a monoclonal alpha band with no associated light chain by IFE. Serum HLC measurements indicated a clear IgA λ monoclonal protein (IgA κ =1.06, IgA λ =2.69, IgA κ /IgA λ =0.394: normal range; 0.8-2.04) and FLC measurements confirmed a lambda clone (κ FLC=12.1, λ FLC=103 κ FLC/ λ FLC=0.12). In a second sample with a low total serum IgA concentration (0.95g/L) an IgA κ band was suggested by SPE but not clearly visible by IFE. The IgA κ /IgA λ ratio value of 10 clearly identified an IgA κ monoclonal protein. Third, a patient with increased levels of IgA was investigated for possible monoclonal gammopathy. Identification of a monoclonal band by IFE was difficult due to high levels of polyclonal Ig staining. Both IgA κ and IgA λ values were high but the IgA κ /IgA λ ratio indicated no monoclonal Ig (IgA κ =9.1, IgA λ =9.5, IgA κ /IgA λ =0.96). **Conclusion.** Analysis of immunoglobulin ratios can identify monoclonal Igs and may be particularly useful for assessing subtle clones below the sensitivity limit of IFE.

F56 - NEW QUANTITATIVE SERUM HEAVY CHAIN/LIGHT CHAIN ASSAY CONSISTENTLY IDENTIFIED IFE POSITIVE MYELOMA PATIENTS EVALUATED IN STUDY AT THE MYELOMA INSTITUTE

J. Bornhorst,¹ T. Kuus,² L. Traylor²

¹University of Arkansas for Medical Sciences, Little Rock, AR; ²The Binding Site, San Diego, CA, USA

Background. The current most sensitive method for detecting intact immunoglobulins in the serum is the non-quantitative immunofixation electrophoresis (IFE). The new serum heavy chain/light chain (HLC) immunoassay is a quantitative analysis of intact immunoglobulin heavy chain (IgH) and light chain kappa or lambda. The assay provides absolute concentrations of IgH kappa (κ) and IgH lambda (λ) as well as the clinically useful IgH κ /IgH λ ratio. **Aims.** The primary aim of this study was to evaluate the IgA HLC Assay on the Siemens BNII instrument using IgA myeloma patient and normal subject samples. **Methods.** The IgA κ/λ HLC reagent kits, provided by The Binding Site, Inc, were evaluated on 25 normal and 25 myeloma patient (20 IFE positive and 5 IFE negative) samples with known serum free light chain results using the Siemens BNII instrument. **Results.** The HLC assay identified all known myeloma patients and showed strong correlation with total IgA assay (see Figure 1). The IFE negative IgA myeloma patients also had normal IgA κ /IgA λ ratios. The serum free light chain ratios were abnormal in 18/20 (90%) IFE positive samples, consistent with previously reported percentages for intact Ig MM. **Summary/Conclusion.** In this study, quantitative HLC assay appears to offer similar analytical advantages as that of the serum free light chain assay including high sensitivity and quantitative assessment of clonality. Additional ways that HLC assays can be potentially utilized in the monitoring of myeloma patients are by providing numerical results for patients that are IFE positive but not readily followed by serum protein electrophoresis, and through calculation of the clinically beneficial IgA κ /IgA λ ratio. Biological variations that may impact electrophoretic evaluation of the immunoglobulin protein should not affect this ratio, for example changes in blood volume, hematocrit and metabolism. Further clinical studies are required to fully assess the clinical benefit of this assay in practice.

Figure1.

F57 - ANALYTICAL VALIDATION OF HEVYLITE IgA ASSAY FOR THE DIAGNOSIS OF MONOCLONAL GAMMOPATHIES

J. Eckold,¹ W. Poenisch,² J. Kratzsch,¹ J. Thiery,¹ M. Bruegel¹

¹Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany; ²Department of Internal Medicine, Haematology/Oncology, University Hospital Leipzig, Germany

Background. The analysis of monoclonal proteins in serum is an integral part of the diagnosis of monoclonal gammopathies. Currently used detection methods are mainly based on electrophoretic techniques, characterized by limited sensitivity and lack of quantification. **Aims.** The aim of our study was to analytically validate the Binding Site **Hevylite** IgA (HLC) assay, a newly developed immunoassay for the detection of monoclonal heavy chains type IgA κ and λ . **Methods.** Intra- and inter-assay precision were determined using patient sera (n=4) containing IgA monoclonal protein in a range between 0.5-31.0 g/L. Accuracy was estimated by testing linearity after dilution of patient sera with high monoclonal protein levels (n=2) and by spiking of assay calibrators into sera of healthy subjects (n=3). A potential interference with rheumatoid factors (RF) was investigated by dilution of sera containing monoclonal protein (n=2) with sera containing low or high amounts of RF IgM (34 and 974 U/ml). Sera containing monoclonal proteins in immunofixation electrophoresis (IFE) (n=22, 20 MM, 2 MGUS) were analyzed by the HLC assay. **Results.** Intra- and inter-assay precision ranged between 1.5 to 20.0% for the coefficient of variation. Testing accuracy of the analytical method resulted in a linear recovery from 0.9 to 13.0 g/L. RFs did not affect test results until a concentration of 974 U/ml. All 22 IFE positive samples could be confirmed by the HLC assay. **Summary/Conclusion.** Our test validation revealed high precision and accuracy of the analytical method and a high concordance between test results of IFE and the HLC assay. We are currently investigating the diagnostic value of the **Hevylite**-IgA assay in estimation of prognosis and disease activity.

F58 - SYSTEM HEVYLITE AND IgA MONOCLONAL GAMMOPATHY – FIRST EXPERIENCE

T. Pika,¹ Z. Hermanova,² P. Lochman,³ J. Zapletalova,⁴ J. Minarik,¹ V. Scudla¹

¹The Third Clinic of Internal Medicine, Medical Faculty of Palacky University and University Hospital, Olomouc, Czech Republic; ²Department of Immunology, Medical Faculty of Palacky University and University Hospital, Olomouc, Czech Republic; ³Department of Clinical Biochemistry and Immunogenetics, University Hospital, Olomouc, Czech Republic; ⁴Institute of Medical Biophysics, Medical Faculty of Palacky University, Olomouc, Czech Republic

Background. Typical features of monoclonal gammopathy (MG) include the presence of monoclonal immunoglobulin (MIG) molecules or their fragments in the serum and/or

urine. The latest test which extends the range of MIG examination possibilities is the **Hevylite** system, using principally specific antibodies against junctional epitopes between the domains of heavy and light immunoglobulin chains in the constant regions. **Aims.** The study aimed at implementing the **Hevylite** system and performing a pilot analysis in the group of IgA-type MG patients. **Methods.** The studied group consisted of 24 patients with multiple myeloma (MM) (4 patients in the stage of disease diagnosis, 6 patients in disease remission, and 14 in disease progression) and, in addition, 7 individuals with monoclonal gammopathy of undetermined significance (MGUS) (1× low-risk, 6× high-risk). The **Hevylite** system (The Binding Site Group Ltd, UK) was used to determine the serum levels of IgA κ , IgA λ on the BN II analyser (Siemens Healthcare Diagnostics). The ratio of IgA κ /IgA λ was determined by calculation. **Results.** The patients in the active disease stage revealed highly pathological levels of the dominant MIG, with suppressed levels of the alternative IgA immunoglobulin, and significant influence on the IgA κ /IgA λ ratio. 5 out of the patients who had achieved a complete remission (IFE negativity) showed a normal IgA κ /IgA λ ratio, 1 patient had suppression of both IgA pairs, evidently due to the short interval from their previous chemotherapy, with a modified IgA κ /IgA λ ratio. All individuals with MGUS revealed abnormal **Hevylite** levels as well as ratios of IgA κ /IgA λ , one patient with a low-risk disease type had the IgA κ /IgA λ ratio value slightly above the threshold. When comparing the results of M-protein determination by the method of **Hevylite** and membrane electrophoresis, Spearman's correlation analysis confirmed a very close correlation of M-protein levels regarding IgA κ (r=0.946, p=0.0001) as well as IgA λ (r=0.872, p=0.0001). The summations of the dominant as well as alternative IgA immunoglobulin levels substantially correlated with the general level of IgA immunoglobulin determined nephelometrically (r=0.994, p=0.0001). **Summary/Conclusion.** Preliminary observations confirm that the **Hevylite** system promisingly complements the set of examinations used routinely by MG monitoring, in particular for very low concentrations of MIG, or the mere positivity of the IFE. Further experience needs to be gained in order to fully assess the benefits of this examination for clinical practice.

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F59 - FIRST EXPERIENCES WITH MEASUREMENT OF IgA HEAVY/LIGHT CHAINS IN IgA MONOCLONAL GAMMOPATHIES

R. Assert, L. Eisele, V. Chapot, J. Dürig, A. Fleer, L. Volbracht, H. Nüchel

Department of Haematology, Essen, Germany

Background. Light chain restricted over-expression of complete or incomplete monoclonal proteins are a hallmark of most gammopathies which results in typically discordant serum heavy/light chain concentrations. Therefore, the separate monitoring of kappa and lambda bound heavy chains may be of value in the diagnosis and clinical assessment of gammopathies. **Aims.** We present our first experiences with a recently developed automated

immunoassay for the quantitative measurement of IgA heavy/light chains in patients with known IgA monoclonal gammopathies. **Methods.** We determined quantitative levels of IgA heavy/light chains (The Binding Site Group Ltd, UK) under routine laboratory conditions in serum samples from 15 patients with known Multiple Myeloma (MM) or POEMS with or without osteolysis, Monoclonal Gammopathies of Undetermined Significance, or Smouldering Multiple Myeloma. The monoclonal protein was determined by immunofixation electrophoresis in all cases. Concentrations of free light chains (FLC), IgA λ and IgA κ (The Binding Site Group Ltd, UK), serum β -2-microglobulin and total IgA (Siemens, Germany) were determined with commercially available kits on a BNII nephelometer with normal ranges provided by the manufacturers. Serum creatinine was measured according to a modified Jaffe method, serum protein concentration was estimated with Biuret endpoint (Advia 2400/1800, Siemens). **Results.** Cases were classified as IgA λ (n=7) or IgA κ (n=8) by immunofixation electrophoresis. Two patients had creatinine concentrations greater than 1.5 mg/dL, albumin was less than 3 mg/dL in one case. Concentrations of IgA kappa plus lambda correlated with total IgA ($r=0.933$, $p \leq 0.0001$), however, no correlation was found with beta-2-microglobulin or creatinine. The ratios of kappa to lambda bound IgA were abnormal in all studied cases, while the absolute concentrations of IgA kappa/lambda or free light chains were elevated in 94 % and 79 %, respectively. The free κ/λ light chain ratio was abnormal in 69 % of the cases. In 4 cases follow-up samples were available within an interval of 2 to 4 weeks. The concentration of IgA κ plus λ closely paralleled total IgA in 3 of 4 cases. In one MM case with proven clinical progression, both the IgA kappa/lambda ratio and the FLC ratio decreased, and the concentration of IgA lambda increased, whereas the concentration of β -2-microglobulin was unchanged. **Summary/Conclusion.** Taken together, our preliminary results might indicate a potential use of the IgA heavy/light chain antibodies in the diagnosis and monitoring of patients with monoclonal gammopathies. To better evaluate this novel assay higher patient numbers need to be tested.

F60 - EVALUATION OF NOVEL NEPHELOMETRIC ASSAYS FOR THE QUANTIFICATION OF SERUM IMMUNOGLOBULINS IN MONOCLONAL GAMMOPATHIES

P.S. Steele,¹ D.M. Hoefner,¹ S. Alvi,² S. Harding²

¹Marshfield Clinic Marshfield, WI; ²The Binding Site, San Diego, CA, USA

Introduction. Detection and quantification of IgA monoclonal proteins in serum can be difficult depending upon the concentration of the protein, co-migration with other proteins and the experience of the clinical scientist reviewing the gels. Antisera recognising quaternary epitopes between the kappa and lambda light chains and their associated intact heavy chains have been produced. The objective of our study was to evaluate the IgA κ and IgA λ reagents in our laboratory and to comment on their utility in routine laboratory investigations. **Material and Methods.** Electrophoresis

was performed using a SEBIA Hydrasys 2 in accordance with manufacturer's instructions. Total serum IgA (Siemens HealthCare Diagnostics) and IgA κ and IgA λ (The Binding Site, Inc.) concentrations were measured on a Siemens Healthcare Diagnostics BNII nephelometer. Sample analyses included 25 normal blood donor sera and 31 IgA (19 IgA κ and 12 IgA λ) myeloma patients. Monoclonal spikes were selected as too small to accurately quantitate due to polyclonal backgrounds in 23/31 IgA patients. **Results.** The median values for 25 normal blood donor serum (100 percentile ranges) were IgA κ 0.98 g/L (0.497-1.45 g/L), IgA λ 0.84 g/L (0.452-1.28 g/L) and IgA κ /IgA λ ratios 1.08 (0.88-1.78) and fell within the published normal ranges. All monoclonal proteins tested had abnormal IgA κ /IgA λ ratios. The median monoclonal protein concentration in IgA κ patients was 8.46 g/L (range 3.62-71.6 g/L), the median value for the uninvolved polyclonal IgA λ was 0.15 g/L (range 0.02-2.07 g/L) with a median IgA κ /IgA λ ratio of 38.87 (range 2.90-3.142). In IgA λ patients the median monoclonal protein concentration was 8.83 g/L (range 3.21-20 g/L), uninvolved polyclonal IgA κ median was 0.31 g/L (range 0.08-1.27 g/L) with a median ratio of 0.05 (range 0.001-0.27). In the 23 IgA patients whose monoclonal proteins were difficult to quantify by SPE 11/23 patients had uninvolved polyclonal immunoglobulin levels within the normal range.

Table.

Assay	IgA κ	IgA λ
Regression Statistics	$y = 0.983x + 0.463$ $R^2 = 0.994$	$y = 1.030x + 0.100$ $R^2 = 0.989$
Intra-assay precision %CV (mean g/L n=10)	3.80 (1.74) 1.72 (7.82)	1.07 (1.59) 5.09 (5.90)
Inter-assay precision %CV (mean, g/L, n=3)	0.41 (1.72) 0.81 (7.88)	9.18 (1.54) 11.43 (5.51)
Median normal value, g/L, n=25 (range)	0.925 (0.497-1.45)	0.84 (0.452-1.28)
Median ratio, n=25 (range)	1.08 (0.877-1.78)	
Agreement with Standard Panel*	$y = 1.047x + 0.043$ $R^2 = 0.997$	$y = 1.122x - 0.151$ $R^2 = 0.999$

* 4 IgA κ , 4IgA λ , and 3 normal sera

Figure.

Conclusions. IgA monoclonal protein may be difficult to assess due to the co-migration with other serum proteins. Measurement of IgAk/IgAλ ratios provided a sensitive method for identifying monoclonal proteins and quantification was simple compared with SPE measurements in some anodal migrating samples.

F61 - NEPHELOMETRIC IMMUNOASSAYS IgAk AND IgAλ FOR ASSESSMENT AND MONITORING OF MULTIPLE MYELOMA PATIENTS

L. Mirbahai,¹ H. Avet Loiseau,² C. Mathiot,³ M. Attal,⁴ P. Moreau,⁵ J.L. Harousseau,² J. Bradwell,⁶ S. Harding¹

¹The Binding Site Group Ltd, Birmingham, UK;

²Laboratory of Hematology, Clinical Hematology Department, University Hospital, Nantes, France;

³Department of Hematology, Institut Curie, Paris, France;

⁴Centre Hospitalier Universitaire Toulouse, Toulouse, France; ⁵Centre Hospitalier Universitaire Nantes, France; ⁶Division of Immunity and Infection, Medical School, University of Birmingham, UK

Introduction. International guidelines recommend serum protein electrophoresis (SPE) and immunofixation (IFE) for the identification, quantification and characterisation of monoclonal proteins. Accurate quantification of IgA monoclonal proteins can be difficult particularly when they co-migrate with other serum proteins. IFE can identify these patients but it is a qualitative rather than quantitative test. Antibodies have been developed which recognise junctional, conformational epitopes between kappa or lambda light chains and the bound heavy chain partner (heavy/light chain - HLC). These antibodies have been used in nephelometric assays to quantify serum IgAk and IgAλ proteins. **Aims.** Comparison of IgAk/IgAλ ratios with SPE and IFE to identify and monitor IgA multiple myeloma (MM) patients. **Methods.** Serum of 46 IgA patients (28 IgAk and 18 IgAλ) from the IFM 2007 02 trial (comparing Velcade and Dexamethasone versus velcade, thalidomide and dexamethasone) were analysed at presentation, cycle 2, cycle 4 and after autologous stem cell transplant (ASCT) (mean=3, range 1-5 samples). IgAk and IgAλ were measured using a Siemens Dade-Behring BN II nephelometer. Results were compared with the 95 percentile normal ranges generated from 138 normal blood donor sera. Comparison with historic SPE, IFE and clinical assessments were made. All statistics were performed using SPSS version 18. **Results.** Abnormal IgAk/IgAλ ratios correctly identified all patients at presentation. By SPE, in 10/42 patients it was not possible to quantify the monoclonal protein accurately and in 2/42 patients there was no monoclonal protein visible. After 2 cycles of therapy there was monoclonal protein detectable in all patients by IFE and HLC, however in 8 patients the concentration of the protein was below SPE sensitivity. After 4 cycles of therapy there were concordant IFE and HLC results in 29 patient sera of which 21 had monoclonal protein levels which were measurable by SPE, in 3 patients IFE was positive when HLC ratio was normal and in 2 cases HLC was positive when IFE was normal. In the patients with an abnormal IFE and normal HLC ratio there had been significant

increases in the level of polyclonal IgA and in all instances the monoclonal proteins were below the sensitivity of SPE. After ASCT, 14/39 patients had abnormal IFE and HLC ratios while IFE was positive in 3 patients that had a normal HLC ratio. It is possible that the IFE bands were oligoclonal which occurs frequently after ASCT and is not indicative of the tumour clone. In these cases IgA levels were normal and the level of monoclonal protein production was below the sensitivity of SPE. **Discussion.** IgAk and IgAλ immunoassays provide a quantitative method for identifying and monitoring monoclonal proteins in MM patients. The IgAk/IgAλ ratio was frequently more sensitive than SPE and provided a quantitative measure of the protein concentration when SPE was negative. In a few instances there were disparate results between IFE and HLC that could be due to the presence of oligoclonal bands after therapy and ASCT.

F62 - QUANTIFICATION OF IgA-κ AND IgA-λ IMMUNOGLOBULINS IN 54 SERUM SAMPLES WITH DETECTABLE IgA MONOCLONAL COMPONENT

J.J. Rodríguez-Molina, J. Navarro, E. Fernández-Cruz
Hospital General Universitario Gregorio Marañón,
Madrid, Spain

Background. Quantitation of serum monoclonal component (MC) has been used as a criterion for the diagnosis, staging, risk stratification and response to therapy in multiple myeloma (MM) and other monoclonal gammopathies. The International Myeloma Working Group has stated that serum MC level should be quantified using densitometry on serum protein electrophoresis (SPE) except in cases where the SPE is felt to be unreliable (e.g., in some cases of IgA MC migrating in SPE beta region). The procedure for the quantification of IgAk and IgAλ immunoglobulin concentration in human serum samples is a new tool for monitoring monoclonal gammopathies. **Aims.** To evaluate the quantification of IgAk and IgAλ immunoglobulin concentrations in comparison with quantification of MC using high resolution capillary electrophoresis. **Methods.** 1.- Samples: 54 serum samples from patients with measurable IgA MC using a standard procedure: Capillary electrophoresis and densitometric evaluation of MC referred to albumin and subtraction of overlapping transferrin or C3 complement component. 2.- Typing MC by automated capillary electrophoresis and immune-displacement. 3.- Quantification of IgAk/IgAλ immunoglobulin concentration by an automated turbidimetric procedure. 4.- Comparison of the turbidimetric quantification of the involved IgAk or IgAλ immunoglobulin concentration versus standard quantification of serum MC. **Results.** a) Abnormal levels of IgAk or IgAλ in 45/54 IgA-MC serum samples. b) Abnormal levels of IgAk and IgAλ in 4/54 IgA-MC serum samples. c) Normal levels of IgAk and IgAλ in 5/54 IgA-MC serum samples. The figure shows correlation between quantification of MC and involved IgAk or IgAλ immunoglobulin. **Summary/Conclusion.** Quantification of IgAk/IgAλ immunoglobulin concentration is a useful tool for evaluating MC. Discrepancies between quantification of MC and the involved IgAk/IgAλ may be related to high reso-

lution procedure used for quantification of MC and/or to the overlap of monoclonal and polyclonal components in the involved IgA κ /IgA λ immunoglobulin quantification.

Figure.

F63 - QUANTIFICATION OF SERUM IgM κ AND IgM λ IN PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINAEMIA (WM): CLINICAL CORRELATIONS

E. Koulieres,¹ M.-C. Kyrtonis,¹ D. Maltezas,¹ T. Tzenou,¹ L. Mirbahai,² G. Mead,^{2,3} M. Dimou,¹ V. Bartzis,¹ P. Panayiotidis,¹ A.R. Bradwell,³ S. Harding²

¹First Dpt of Internal Medicine and Dpt of Hematology, Laikon University Hospital, Athens, Greece; ²The Binding Site Group Ltd, Birmingham, UK; ³Department of immunity and Infection, University of Birmingham, Medical School, Birmingham, UK

Introduction. Poor resolution of IgM monoclonal proteins (M-proteins) on serum protein electrophoresis (SPE) can make accurate quantification difficult. Specific polyclonal antibodies which recognise epitopes spanning the junction of the heavy and light chains (HLC) of the individual immunoglobulin isotypes have been produced. These have been used successfully in the identification and monitoring of IgA and IgG M-proteins in myeloma patients. Here we describe the use of the automated nephelometric assays to quantify serum concentrations of IgM κ and IgM λ and the use of IgM κ /IgM λ ratios to identify IgM M-proteins and assess patient outcome. **Patients and Methods.** Retrospective sera from 39 patients with IgM M-proteins (25 IgM κ , 13 IgM λ and 1 patient with IgM restriction but no identifiable light chain) diagnosed and followed during the last two decades were included in this study (31 WM, 4 IgM monoclonal gammopathy of undetermined significance (MGUS), 2 symptomatic MGUS (neuropathy) and 2 mantle cell lymphoma). Patients' median follow-up was 59 months. Analysis of IgM κ and IgM λ was performed on a Siemens Dade-Behring BNTMII nephelometer and results were compared with disease parameters (such as blood counts, beta-2-microglobulin (β_2 M), serum albumin, lactate dehydrogenase (LDH), lymphoplasmacytic bone mar-

row infiltration) and International Prognostic Scoring System (IPSS). **Results.** Abnormal IgM κ /IgM λ ratios were reported in 30/31 WM, 5/6 MGUS and 1/2 mantle cell lymphoma patient sera. There was a good correlation between summated IgM κ + IgM λ and total IgM ($r=0.832$ $p<0.0001$), although in 12 patients there was considerable disagreement between total IgM (range 12.9-80.4) and summated IgM κ + IgM λ (range 22.2-150.8, $p>0.01$). These 12 patients had polyclonal hypogammaglobulinemia with regard to their IgG and IgA levels. Median levels of the IgM κ /IgM λ ratios (expressed as IgM κ /IgM λ or IgM λ /IgM κ) were 90.9 (range 1.91-1000) in WM v 17.7 (range 1.08-34.57) in IgM MGUS ($p=0.06$). Median IgM κ /IgM λ ratio was higher in WM patients requiring treatment ($n=24$) at presentation than in patients not ($n=7$) requiring treatment (185.7 v 13.45 $p=0.023$). IgM κ /IgM λ ratios correlated with bone marrow infiltration ($p=0.029$), time to treatment ($p=0.003$) and IPSS ($p=0.001$). A simple model built on the presence of 0 or 1, 2 and 3 of the following: IgM κ /IgM λ ratio > median, β_2 M>5 and abnormal LDH, identified 3 prognostic arms with respect to survival ($p<0.0001$) (Figure). **Conclusions.** Analysis of HLC IgM seems useful in clinical evaluation of WM patients. IgM κ /IgM λ correlated with bone marrow infiltration, time to treatment need and outcome.

Figure.

F64 - SENSITIVITIES AND SPECIFICITIES FOR IMMUNOGLOBULIN HEAVY/LIGHT CHAIN (HEVY-LITE) ASSAYS

J. Harris,¹ A. Alvi,¹ N. Fourrier,¹ H. Carr-Smith,¹ G. Mead,^{1,2} A.R. Bradwell,² S. Harding,¹

¹The Binding Site Group Ltd, Birmingham, UK; ²Division of Immunity and Infection, Medical School, University of Birmingham, UK

Introduction. Specific polyclonal antibodies have been developed which recognise quaternary conformational epitopes spanning the junction between immunoglobulin light chains (kappa and lambda) and their heavy chain partners (IgG, IgA and IgM). Here we describe the development of

automated, nephelometric immunoassays on the Siemens BN II nephelometer; the main assay characteristic are summarised below. *Methods and Results.* Normal ranges were established by testing blood donor sera and comparisons made to the appropriate total immunoglobulin results are summarised in the table above. Interference was within $\pm 10\%$ for all antisera when either bilirubin (200mg/L), haemoglobin (4.56g/L) or Chyle (1000 FTU) were added to a characterized pool of normal serum. Susceptibility to interference and specificity was assessed using a panel of monoclonal serums: 22 IgG κ median 27.4g/L (range 7.8-70.8), 16 IgG λ median 24.6g/L (range 2.0-36.0), 14 IgA κ median 30.6g/L (range 6.3-43.0), 9 IgA λ median 22.2g/L (range 8.5-35.9), 15 IgM κ median 29.8g/L (range 3.6-39.1), 10 IgM λ median 45.8g/L (range 1.2-99.9), 2 FLC κ median 5460mg/L (range 4440-6480) and 6 FLC λ median 1797mg/L (range 605-8424). The data was expressed either as summated Ig' κ + Ig' λ or as ratios (i.e. Ig' κ / Ig' λ) and compared to total immunoglobulin and immunofixation results. For all assays median summated values were within 10% of the total immunoglobulin. Additionally abnormal ratio results correctly identified all monoclonal proteins in agreement with IFE. *Conclusion.* We conclude that serum assays provide a rapid and precise method for quantifying the isotype specific Ig' κ / Ig' λ in serum.

Table.

Assay	IgG κ	IgG λ	IgA κ	IgA λ	IgM κ	IgM λ
Range (g/L)	1.72-27.50	0.88-14.00	0.35-11.20	0.33-10.40	0.20-6.40	0.18-5.60
Sample dilution	1/100	1/100	1/100	1/100	1/100	1/100
Min sample dilution	1/5	1/5	1/5	1/5	1/5	1/5
Sensitivity (g/L)	0.086	0.044	0.018	0.016	0.010	0.009
Assay time (min)	12	12	12	12	12	12
Assay format	Antisera	Antisera	Antisera	Antisera	Latex	Latex
Total precision						
CV	5.6%	4.7%	5.1%	6.4%	5.7%	10.6%
(g/L)	(24.05)	(10.83)	(9.14)	(8.45)	(4.85)	(4.24)
CV	7.4%	4.6%	5.5%	3.3%	6.5%	8.7%
(g/L)	(11.25)	(5.90)	(2.48)	(1.93)	(1.16)	(0.77)
CV	6.5%	8.5%	3.6%	4.0%	7.7%	6.9%
(g/L)	(2.92)	(1.38)	(0.56)	(0.50)	(0.35)	(0.30)
Median normal	6.85	3.81	1.19	0.98	0.61	0.42
(95%ile) g/L	(4.03-9.78)	(1.97-5.71)	(0.48-2.82)	(0.36-1.98)	(0.17-1.98)	(0.18-1.20)
Ig' κ / Ig' λ median	1.87		1.27		1.57	
normal (95%ile)	(0.98-2.75)		(0.80-2.04)		(0.79-2.28)	
Ig' κ / Ig' λ median	y=0.94x +0.43		y=1.09x +0.05		y=1.00x -0.03	
Summation v						
Total Ig' (g/L)						

F65 - NEPHELOMETRIC IMMUNOASSAY MEASUREMENTS OF IGM κ AND IGM λ FOR THE ASSESSMENT OF IGM MONOCLONAL GAMMOPATHY PATIENTS

N. Fourrier,¹ L. Mirbahai,² G.P. Mead,¹ A.R. Bradwell,¹ S. Harding¹

¹The Binding Site Group, Ltd. Birmingham, UK;

²Division of Immunity and Infection, The Medical School, University of Birmingham, Birmingham, UK

Background. Serum protein electrophoresis (SPE) and immunofixation (IFE) are used to detect, quantify and type monoclonal immunoglobulins. Problems in the detection and measurement of the IgM monoclonal proteins using gel techniques can occur, particularly when the IgM monoclonal proteins aggregate thereby preventing migration into the gels. Specific polyclonal antibodies have been produced that target epitopes in the region spanning the junction of the heavy and light chain of the IgM molecule. Using these antisera, nephelometric IgM κ and IgM λ assays have been developed and optimised on the Dade Behring BNTMII nephelometer. Here we report the use of the IgM and IgM immunoassays for the detection and the quantification of IgM monoclonal protein in patients with Waldenstrom's Macroglobulinemia (WM) and monoclonal gammopathy of undetermined significance (MGUS). *Methods.* IgM κ and IgM λ assay characteristics are summarized in the table below. Normal ranges for IgM κ and IgM λ were derived from 89 normal (blood donor) sera and summated values compared with total IgM (Siemens Dade Behring BNII). IgM κ and IgM λ concentrations were measured in 19 IgM WM (14 IgM κ and 5 IgM λ) and 4 MGUS (4 IgM κ) patients. *Results.* Normal range results were; median IgM κ 0.611g/L (range 0.126-2.020g/L), median IgM λ 0.423g/L (range 0.168-1.290g/L), median IgM κ /IgM λ ratio of 1.566 (range 0.750-2.314). IgM κ +IgM λ summation correlated well with total IgM (Passing Bablok, Y=-0.03+1.01X). In 14 IgM κ WM and 4 IgM κ MGUS patients the median concentration of IgM κ was 17.9g/L (range 3.57-53.1g/L) the median ratio was 179.48 (range 1.08-695.12). In 5 IgM λ WM patients the median concentration of IgM was 20g/L (range 15.7-40.40g/L) the median ratio was 0.013 (range 0.003-0.037). In all WM and 3/4 MGUS patient samples the IgM isotype was identified correctly and matched the IFE results. One MGUS patient had high polyclonal levels of IgM and a normal IgM κ /IgM λ was reported. *Summary/Conclusion.* Normal ranges for IgM κ and IgM λ in patient serum were established and we demonstrated that IgM κ /IgM λ ratios can be a relevant indicator of IgM monoclonal protein production.

Table.

Assay	IgM κ	IgM λ
Range (mg/L)	200-6400	175-5600
Sample dilution	1/100	1/100
Min. sample dilution	1/5	1/5
Sensitivity (mg/L)	10	8.8
Assay time (mins)	12	12

HEAVY CHAIN/LIGHT CHAIN ANALYSIS FOR PATIENT MONITORING AND PROGNOSIS

G65 - HEVYLITE Ig κ /Ig λ RATIOS IN MULTIPLE MYELOMA CORRELATE WITH CLINICAL STATUS

S. Harding,¹ E. Koulieres,² M.C. Kyrtsolis,³ A. Levoguer,¹
L. Mirbahai,¹ M. Drayson,⁴ A.R. Bradwell,⁴ G. Mead,¹

¹The Binding Site Group Ltd, Birmingham, UK; ²First
Dpt of Internal Medicine and Dpt of Hematology,
Laikon University Hospital, Athens, Greece; ³Laikon
University Hospital, Athens, Greece; ⁴Division of
Immunity and Infection, University of Birmingham, UK

Introduction. Serum protein electrophoresis (SPE) and immunofixation (IFE) are the most commonly used laboratory tests for monitoring monoclonal immunoglobulin (Ig) production in multiple myeloma (MM). Levels of Ig in individual patients are commonly assumed to assess tumour burden and reductions relate to therapeutic responses. However, SPE measurements can be inaccurate if there is significant polyclonal immunoglobulin production and/or when large changes occur in haematocrit and plasma volume. Assays measuring the heavy/light chain (HLC) subsets, IgG κ , IgG λ , IgA κ and IgA λ , have been developed that quantify the concentrations of the monoclonal Ig as well as the uninvolved Ig's of the same isotype. Here we report three case studies where HLC Ig κ /Ig λ ratios closely matched clinical assessments but were at variance with SPE & IFE measurements. **Materials and Methods.** Sequential serum samples (median n=10, range 5-14) from 3 MM patients (1xIgG λ , 1xIgA κ and 1xIgA λ) were retrospectively analysed. The IgG κ , IgG λ , IgA κ and IgA λ concentrations were measured on a Siemens BN II nephelometer. Results were compared with clinical assessments, total Igs and monoclonal Igs by SPE and IFE. **Results.** The IgG λ MM patient responded well to cyclophosphamide therapy and achieved a complete response (CR) after 264 days (SPE and IFE negative). The CR lasted until 732 days when the patient relapsed with a low level IgG λ . Salvage therapy with cyclophosphamide, vincristine, adriamycin, melphalan and prednisone resulted in a monoclonal Ig reduction but a terminal relapse occurred by day 1,160. The patient's IgG λ /IgG κ ratio showed a similar response to the initial treatment but showed no change during the salvage therapy supporting the clinical response. The IgA κ MM patient responded well to the initial treatment of vincristine, adriamycin and dexamethasone (VAD) achieving a stringent Cr (sCR) by 342 days. The response lasted 220 days when the patient relapsed and was treated with velcade. A second response by SPE was recorded after 8 days but the patient continued to clinically decline and bone marrow aspirations showed considerable plasma cell infiltration. The IgA κ /IgA λ ratio normalised at sCR, became abnormal during relapse and increasingly abnormal during the second clinical relapse in contrast to the SPE results. The IgA λ patient showed a partial response to VAD therapy after 111 days by SPE. However, the patient deteriorated clinically and presented with sponta-

neous fractures at 351 days. Subsequent treatment with velcade and high dose melphalan (with stem cell rescue) achieved sCR. The IgA κ /IgA λ ratio became increasingly abnormal after presentation and did not show any responses to VAD treatment. The ratio normalised when the patient achieved sCR. **Conclusion.** Serum Ig κ /Ig λ ratios can be used to monitor MM patients through the course of their disease. In the 3 patients described, HLC ratios corresponded better with the clinical status than SPE and IFE and suggested alternative management strategies.

G66 - HEAVY/LIGHT CHAIN ANALYSIS AS AN AID IN MONITORING IgA AND IgG MULTIPLE MYELOMA PATIENTS

E. Koulieres,¹ L. Mirbahai,² D. Maltezas,³ M.C. Kyrtsolis,³ N. Kafasi,³ M. Dimou,³ V. Bartzis,³ A. Efthymiou,³ G. Georgiou,³ P. Panayiotidis,³ G. Mead,^{2,4}
A.R. Bradwell,⁴ S. Harding²

¹First Dpt of Internal Medicine and Dpt of Hematology,
Laikon University Hospital, Athens, Greece; ²The
Binding Site Group Ltd, Birmingham, UK; ³Laikon
University Hospital, Athens, Greece; ⁴Division of
Immunity and Infection, University of Birmingham, UK

Background. Monoclonal protein quantification by serum protein electrophoresis (SPE) and scanning densitometry is perhaps the most commonly performed laboratory test for assessing the response to therapy in multiple myeloma (MM) patients. The accuracy of the test depends upon the amount of protein, the position of the protein on the SPE gel (particularly in IgA MM) and may require skilled interpretation. Operator error can account for unexpected results, particularly at low concentrations. Assays for measuring specific heavy chain/light chain pairs (eg. IgG κ or IgA λ) have recently been developed and initial evaluations have reported prognostic value in MM patients at presentation. Here we report the use of these assays for monitoring IgA and IgG multiple myeloma patients. **Materials and Methods.** SPE and immunofixation (IFE) were performed on a SEBIA Hydrasys in accordance to manufacturers' instruction. A Siemens Dade Behring BNII nephelometer was used for all serum protein measurements (Total IgG, total IgA, IgG κ /IgG λ and IgA κ /IgA λ). Multiple serum samples from 53 patients were analysed retrospectively (median number of samples 4; range 1-15). The patients comprised: 1 AL amyloidosis, 2 free light chain MM, 5 IgA κ , 5 IgA λ , 27 IgG κ , 11 IgG λ , 1 plasmacytoma relapsing with MM and 1 smouldering IgG κ MM. All statistics were performed using SPSS version 18 and results were compared to the clinical assessments. **Results.** In the presentation sera, abnormal IgA κ /IgA λ ratios were identified for all 10 IgA MM patients; there was sufficient sample to analyse IgG κ /IgG λ ratios in 6/10 patients all of whom had a normal ratio. Similarly, abnormal IgG κ /IgG λ ratios were identified in all 38 IgG MM patients, there was sufficient sample to analyse IgA κ /IgA λ ratios in 20/38 patients all of whom had normal ratios. Normal IgG κ /IgG λ and IgA κ /IgA λ ratios were identified in the FLC MM patient sera, and in the presentation sera of the patient with plasmacytoma. An abnormal IgG λ result was reported for

the amyloidosis patient, identifying a previously unidentified monoclonal protein. In the 10 IgA patients followed through the course of their disease IgAk/IgAλ ratios matched clinical assessment; IgAk/IgAλ ratios normalised only in patients achieving a complete response (Cr) or stringent complete response (sCr). In one IgAλ patient, where SPE quantification was not possible and total immunoglobulin measurements were used, IgAk/IgAλ ratios were able to monitor the course of the patient's disease, even during periods of response when the serum concentration levels were within the normal range. In the 38 IgG patients followed through the course of their disease IgGκ/IgGλ ratios matched clinical assessment in the majority of cases. In all patients achieving a sCr IgGκ/IgGλ ratios were normal, in 4/8 patients achieving a near Cr (nCr) IgGκ/IgGλ ratios remained abnormal. In one patient presenting with a plasmacytoma, the IgGκ/IgGλ ratio was initially normal, however, when the patient relapsed with MM, abnormal ratios indicated the disease progression. **Conclusion.** Monitoring patient responses to treatment is important in ensuring effective therapy. Traditional methods can be inaccurate and dependent upon skilled personnel. Intact immunoglobulin ratios matched clinical assessments in these patients and may add additional information.

G67 - SERIAL SAMPLE ANALYSIS OF 5 MULTIPLE MYELOMA PATIENTS USING HEAVY/LIGHT CHAIN SPECIFIC IMMUNOGLOBULIN RATIOS

O. Decaux,¹ M.P. Beaumont,² S. Besnard,³ N. Collet,² M. Sebillot,³ B. Grosbois,³ L. Guenet²

¹Hôpital Sud, Rennes, France; ²Laboratory of Biochemistry, University Hospital, Rennes, France; ³Internal Medicine Department, University Hospital, Rennes, France

Background. The standard for measuring paraproteins in patients with multiple myeloma (MM) to assess tumour burden is the quantification of protein bands by serum protein electrophoresis (SPE). There are several limitations with this method. The development of antibodies which bind to conformational epitopes spanning the junctional regions between bound κ or λ light chains and their respective heavy chain partners has allowed development of nephelometric immunoassays for specific measurement of serum IgGκ, IgGλ, IgAκ and IgAλ concentrations. These assays have enabled the calculation of IgGκ/IgGλ and IgAκ/IgAλ ratios (heavy/light chain or HLC ratios) for individual patients. Measurement of the involved and uninvolved heavy/light chains in serum may provide an accurate and quantifiable measurement of tumor burden. **Aims.** The aim of this study was to perform retrospective analysis of tumour burden in 5 multiple myeloma patients using this novel nephelometric assay. **Methods.** We analysed 41 samples from 5 multiple myeloma patients (2 IgGλ and 3 IgAκ). Informed consents of patients were obtained. We collected presentation sera and monthly serial samples through the course of the disease. IgGκ, IgGλ, IgAκ and IgAλ concentrations were determined and HLC ratios calculated. **Results.** In all presentation sera, the involved immunoglobulin concentra-

tion and HLC ratio were abnormal, including one patient where the SPE did not allow quantification of monoclonal IgAλ. The sum of the IgGκ + IgGλ or IgAκ + IgAλ measurements correlated well with total IgG or IgA in all 41 sera samples ($R^2=0.95$). Moreover, the changes observed in HLC ratios during serial follow up reflected the clinical assessments and were correlated with changes in SPE or free light chain evaluation. All patients initially achieved at least a partial response. In one patient, relapse from partial response was indicated by an increase in HLC ratio 2 months before SPE. No patients achieved complete response and HLC ratio remained abnormal throughout, except in one patient who achieved very good partial response. Serial samples analysis of this patient showed normalisation of IgGλ concentration and HLC ratio after respectively 3 and 6 months of treatment, whereas a quantifiable protein band was still present in SPE and persist after 9 months of treatment. When immunoparesis was present with very low concentration of uninvolved immunoglobulin (2 patients), the use of HLC ratio was difficult because very small variations of uninvolved immunoglobulin concentration induced high modification of HLC ratio for which interpretation was ambiguous. **Summary/Conclusion.** These preliminary results indicate that it is possible to monitor response to treatment using HLC ratio. Analysis of HLC ratio in multiple myeloma seems to be very a sensitive marker of tumour burden which could result in a significantly earlier detection of relapse when compared with traditional methods. However, further studies are necessary to define more precisely the interest of this new assay among traditional methods.

G68 - USEFULNESS OF A HEVYLITE IMMUNOASSAY IN SERUM FOR THE DIAGNOSIS AND THE FOLLOW UP OF IgA MONOCLONAL GAMMOPATHY

D. Bengoufa,¹ B. Arnulf,² L. Bugnot,¹ D. Charron,¹ J.P. Fermand²

¹Laboratoire d'immunologie et histocompatibilité, hôpital Saint Louis, Paris, France; ²Service d'immuno-hématologie, Hôpital Saint Louis, Paris, France

Background. Protein electrophoresis (SPE) and immunofixation electrophoresis constitute the two conventional assays for the evaluation of monoclonal immunoglobulins as markers for monoclonal gammopathies and an indicator of the tumor mass. However, these assays do not always provide satisfactory results in terms of sensitivity and objectivity. This is particularly relevant for IgA because of its electrophoretic migration positions overlapping with other bands such as transferrin. Recently, a new automated immunoassay has been developed by Binding Site (**Hevylite**) for diagnosis and monitoring of monoclonal gammopathies. **Aims.** The aim of this study was to evaluate the usefulness of the **Hevylite** assay for diagnosis and monitoring of IgA gammopathies. **Methods-Patients.** In a retrospective study, we analysed sera from 81 patients with monoclonal gammopathies (50 myeloma and 31 MGUS). Sera from 38 healthy donors were included as control. Serum was taken at the time of

diagnosis for all patients. For 10 of the myeloma patients the disease was further monitored and serum taken at various time points during the course of the disease. *Methods.* All samples were subjected to SPE for the evaluation of the monoclonal spike, when the peak was measurable, and identification by immunofixation using Hydrasys Sebia. The determination of **Hevylite** levels was performed using a Siemens Dade Behring (BNII) nephelometer. Normal ranges: IgAk: 0.48-2.82 g/l, IgAλ: 0.36-1.98 g/l, Ratio IgAk/IgAλ: 0.80-2.04. *Results.* Results for healthy donors were in the normal range. For MGUS and myeloma patients, all the monoclonal Ig were confirmed by the ratio (>2.82 for IgAk and <0.80 for IgAλ). 45% of MGUS and 46% of myeloma did not have a measurable peak. A correlation was found between the levels of **Hevylite** and the monoclonal Ig peak when this was measurable. For the myeloma monitoring cohort, there was a correlation between the **Hevylite** levels and the therapeutic response. The ratio was an early indicator of the disease evolution as an out of range ratio could be detected prior the reappearance of the spike and relapse. *Summary/Conclusion.* The determination of **Hevylite** by nephelometry allowed a more precise and sensitive measurement of the monoclonal Ig. This method is of particular relevance in the follow up of monoclonal gammopathies when the monoclonal component is not measurable using the conventional methods. Moreover, the sensitivity of the method allowing a more accurate quantification of the serum **Hevylite** makes it suitable for diagnosis, follow up and treatment monitoring of IgA myeloma.

G69 - IgA HEAVY/LIGHT CHAIN ASSAY FOR DIAGNOSIS AND MONITORING OF MYELOMA PATIENTS

D. Lakomy,¹ T. Dejoie,² I. Lafon,³ S. Lemaire-Ewing,¹ B. Jean-Noel,³ B. Bonnotte,⁴ D. Caillot³

¹Hopital du Bocage-Biochemistry, Laboratory, Dijon, France; ²University Hospital-Biochemistry Laboratory, Nantes, France; ³Hopital du Bocage-Hematology Department, Dijon, France; ⁴Hopital du Bocage, Immunology and Internal Medicine Department, Dijon, France

Background. Multiple myeloma diagnosis and follow up are based on monoclonal protein measurements. The estimation of monoclonal immunoglobulin production requires serum protein electrophoresis (SPE), serum immunofixation (IFE) and serum free light chain assays. Recently, a new nephelometric assay has been developed allowing the measurement of IgA heavy/light chain ratio: IgAk/IgAλ (IgAk/IgAλ). *Aims.* The aim of this study was to determine the performance of the IgAk/IgAλ ratio measurement for the diagnosis and follow-up of myeloma patients. *Methods.* Forty four sera from 26 patients were assayed (10 non myeloma patients and 16 myeloma patients). Total IgA, IgAk, IgAλ concentrations, SPE and IFE were performed for all patients. For the myeloma patients, two evaluations were performed: at diagnosis and during follow-up. *Results.* The IgAk/IgAλ ratio was normal in the non-myeloma group with the exception of 2 patients (one patient hospitalized for renal transplanta-

tion follow-up and the second for urinary infection and colitis). In the myeloma group, all the patients had an abnormal IgAk/IgAλ ratio at diagnosis and monoclonal isotype values were often more elevated than the standard IgA measurements. During follow-up, 5 myeloma patients had normal SPE and IFE. Three of them had a normal IgAk/IgAλ ratio. The 2 remaining patients had an abnormal IgAk/IgAλ ratio due to the decreased non monoclonal isotype but with normalized monoclonal isotype. This could indicate a residual disease. Four myeloma patients had negative SPE but positive IFE during follow-up. Despite normal values of monoclonal isotype, all the IgAk/IgAλ ratios were abnormal, in accordance with IFE. Finally, 7 patients had still positive SPE and IFE during follow-up, with an abnormal IgAk/IgAλ ratio in all cases. When compared with standard IgA concentrations, the addition of IgAk and IgAλ showed a very good correlation for the concentrations below 10 g/l, but a weaker correlation for the values beyond 10 g/l. For the cases where the monoclonal immunoglobulin migrates in the gamma-globulin region on electrophoresis, we compared the values of monoclonal isotype IgAk or IgAλ with the densitometric estimation of the monoclonal spike. The IgAk or IgAλ values were greater than the monoclonal spike but they followed the spike evolution over the time. During disease monitoring, the correlation between the percentage of decrease of monoclonal component given by the M-spike and the IgAk or IgAλ concentration was 0.97. *Summary/Conclusion.* IgA heavy/light chain assay appears to be an interesting test, especially when the monoclonal immunoglobulin has an anodal migration on electrophoresis and co-migrates with other normal protein, making a reliable estimation impossible. In our study, the IgA heavy/light chain assay can be as sensitive as IFE. Further investigations are needed to assess its role in the determination of residual disease. Finally, our results suggest that for high monoclonal IgA concentrations, the values of IgAk or IgAλ can be used for monitoring. At the stage of complete or near complete remission, the IgAk/IgAλ ratio seems to be more sensitive.

G70 - IMMUNOGLOBULIN HEAVY/LIGHT CHAIN RATIOS CAN BE USED TO EFFECTIVELY MONITOR IgA MULTIPLE MYELOMA PATIENTS

L. Ludwig,¹ L. Mirbahai,² N. Fourrier,² N. Zojer,³ A.R. Bradwell,⁴ S. Harding²

¹Department of Medicine I, Wilhelminenspital Vienna, Austria; ²The Binding Site Group Ltd, Birmingham, UK; ³Department of Medicine I, Wilhelminenspital Vienna, Vienna, Austria; ⁴Division of Immunity and Infection, Medical School, University of Birmingham, UK

Background. Accurate monitoring of monoclonal protein levels in multiple myeloma patients is important in determining treatment times and for prognostication. Guidelines recommend the use of serum protein electrophoresis (SPE) and immunofixation (IFE) in the identification, quantification and typing of monoclonal proteins. However, SPE may be inaccurate especially when the monoclonal protein co-migrates with other serum protein or at low concentrations. Total immunoglobulin assays may be used in this

instance however they may be clinically insensitive. Novel nephelometric assays have been produced which quantify the serum IgA κ and IgA λ . Quantification of these proteins enables the calculation of an IgA κ /IgA λ (HLC) ratio which has been suggested as having a greater sensitivity than IFE. Here, we report the evaluation of these reagents in monitoring a cohort of IgA patients through the course of their disease and comment on its usefulness compared to SPE, IFE and free light chain (FLC) measurements. **Methods.** SPE and IFE were performed using the SEBIA Hydrasys according to manufacturer's instructions. FLC and heavy/light chain analysis was performed on a Siemens Dade Behring BN II analyser. Longitudinal samples from 53 IgA (29 IgA κ /24 IgA λ) multiple myeloma patients were analysed (mean =4, range 1-12). **Results.** IgA HLC ratios correctly identified monoclonal protein in 29/29 baseline samples and matched IFE. In 14 of the 53 IgA patients the monoclonal protein was obscured by other proteins and accurate quantification with SPE densitometry was not possible. In these cases the HLC ratio could be used to monitor the patients through the course of their disease. One patient presented with 60g/L IgA κ which co-migrated with other serum proteins making accurate quantification impossible. The patient responded well to VAD and became IFE and HLC negative after 58 days. The patient relapsed after 86 days which was identifiable by SPE and HLC ratios. The patient underwent autologous stem cell transplant after 235 days. SPE normalised and remained normal through the course of the patient's disease whilst HLC ratio became increasingly abnormal reflecting a biochemical unidentified relapse. In 5/53 patients HLC ratios became abnormal before any other test, in 3/5 cases the abnormal ratio correctly identified early disease progression (75-282d) before other tests. In 2/5 patients disease progression occurred >1000d after the ratio had become abnormal. **Conclusions.** Abnormal HLC ratios correctly identified monoclonal proteins at presentation. Furthermore, HLC ratios quantified monoclonal proteins, even when they were obscured by other proteins on SPE. HLC ratios predicted a patient's relapse which was missed by standard laboratory tests.

G71 - IgA HEVYLITE PILOT STUDY IN CLINICAL PRACTICE

K. Arrowsmith,¹ M. Knapp,¹ S. Simpson,²

¹Biochemistry BNHFT, Basingstoke, Hants, UK;

²Haematology BNHFT, Basingstoke, Hants, UK

Background. Biochemical identification of monoclonal gammopathies is currently performed at Basingstoke and North Hampshire NHS Foundation Trust (BNHFT) by serum protein electrophoresis (SPE) followed by scanning densitometry, immunofixation, serum free light chain (FLT) and total immunoglobulin analysis. SPE coupled with densitometry is known to be insensitive for quantitation at low paraprotein concentrations and can lead to overestimation due to co-migration with other serum proteins. **Hevylite** utilises high avidity, specific antibodies to unique junctional epitopes between the heavy and light chain constant regions of intact immunoglobulin molecules enabling the nephelometric quantita-

tion of different light chain types of each immunoglobulin class. Measurement of serum **Hevylite** pairs (e.g. IgA κ /IgA λ) produces ratios of monoclonal immunoglobulin to background analogous to the serum FLC ratios and could provide a much more sensitive, specific and less labour intensive method of identifying and quantifying monoclonal immunoglobulins than current techniques. In this study three cases were followed over a one year period (2009-2010). **Aims.** 1) To evaluate the **Hevylite** quantification against SPE and scanning densitometry using co-migrating and non-co-migrating paraproteins 2) To investigate the use of the **Hevylite** assay in the following clinical settings: i) Response to therapy, ii) Disease relapse, iii) Light chain escape. **Methods.** **Hevylite** and FLC automated immunoassay using the SPAPLUS analyser. SPE, immunofixation and densitometry using the Sebia Phoresis system. Total immunoglobulin turbidimetry assays on the Siemens Advia 2400 multianalyser. **Results.** Patient A was followed from disease presentation through induction treatment prior to autologous stem cell transplant (SCT). The paraprotein co-migrated in the β -region on SPE. Patient B was post autologous SCT and started with an undetectable band on SPE. This patient was followed through relapse and the paraprotein co-migrated within the β -region. Patient C demonstrated the features of MGUS with a stable non-co-migrating paraprotein but demonstrated the light chain escape phenomenon. The **Hevylite** analysis demonstrated the following: In patient A, **Hevylite** analysis showed a greater reduction in paraprotein concentration than SPE densitometry due to masking of the paraprotein reduction by the presence of normal serum proteins that co-migrated within the same area on SPE. In this patient the IgA kappa concentration normalised when the SPE densitometry was 4g/L. In patient B, throughout disease progression increased IgA κ , **Hevylite** ratio and SPE densitometry concentrations were observed. The concordance between the IgA kappa and SPE densitometry concentrations decreased as the paraprotein level increased. This may be due to a loss of SPE densitometry linearity at very high paraprotein concentrations. In patient C, **Hevylite** results were in keeping with the diagnosis of MGUS and offered no additional information in relation to the patients light chain escape. **Conclusions.** 1) **Hevylite** analysis showed improved sensitivity at low serum concentrations when compared to SPE densitometry due to SPE interference with co-migrating proteins. 2) **Hevylite** gave higher results at high serum paraprotein concentrations when compared to SPE densitometry due to the possible loss of linearity of densitometry at high concentrations. 3) Further study is warranted on the clinical applications of **Hevylite**.

G72 - A NEW STAGING SYSTEM FOR MULTIPLE MYELOMA

H. Avet-Loiseau,¹ L. Mirbahai,² G. Mead,^{2,3} A.R. Bradwell,³ S. Harding,⁴

¹Centre Hospitalier Universitaire Nantes, France; ²The Binding Site Group Ltd, Birmingham, UK; ³Department of Immunity and Infection, Medical School, University of Birmingham, UK

Background. The current international staging system (ISS) for multiple myeloma (MM) relies upon the measurement of Beta 2 microglobulin (β_2M) and albumin. β_2M is thought to be a measure of tumour bulk in patients and correlates well with the Durie and Salmon staging system. The role of albumin is less well understood; hypoalbuminemia is a common occurrence in MM. The level of albumin is not influenced by the degree of proteinuria although it has been correlated to the concentration of monoclonal immunoglobulin. Somewhat counter-intuitively measurement of immunoglobulin production is not prognostic, but is the most commonly used laboratory method for monitoring patients' responses to therapy. Newly developed nephelometric assays enable the accurate quantification of serum heavy/light chain (IgA κ /IgA λ and IgG κ /IgG λ) proteins; the measurement of which gives a closer estimate of tumour-derived immunoglobulin than total immunoglobulin assays and indicates the level of isotype-specific immunoglobulin suppression. **Aims.** To determine the prognostic utility of serum immunoglobulin heavy/light chain (HLC) ratios and to compare their use with the current ISS in predicting outcome in MM patients. **Methods.** Presentation sera from 339 patients enrolled on the IFM 2005-01 trial were assayed retrospectively. Free light chains (FLC), β_2M and albumin were measured in all sera. IgG κ & IgG λ concentrations were measured in sera from the 245 IgG MM patients (166 IgG κ , 79 IgG λ). IgA κ and IgA λ concentrations were measured in the sera from the 94 IgA MM patients (60 IgA κ , 34 IgA λ). Association with progression free survival (PFS) was determined using univariate and multivariate analysis (SPSS Version 18). **Results.** Abnormal HLC ratios were recorded for all 339 patients tested, matching IFE sensitivity. 33% (31/94) of IgA patients had monoclonal immunoglobulins which could not be accurately quantified by SPE because of low concentrations or their co-migration with other serum proteins and all had abnormal HLC ratios. There was a weak, negative correlation between the HLC ratios and albumin concentrations ($r=-0.195$ $p<0.0001$), but there was no correlation with other serum markers including β_2M and FLC. Cox regression analysis identified abnormal the HLC ratio as being associated with reduced progression free survival (PFS, $p>0.001$). Separation of the HLC ratio into production and suppression clearly showed that polyclonal suppression had a greater influence on outcome than the level of production ($p=0.002$), but was not as significant as the ratio ($p=0.0002$). The degree of suppression varied greatly between individual patients although suppression was generally greater with IgA-producing tumours compared with IgG tumours. However, the correlation between suppression and production was greater in IgG patients (IgG κ $r=-0.456$; $p=8.7\times 10^{-10}$, IgG λ patients $r=-0.310$; $p=0.005$) than in IgA patients (IgA κ $r=-0.28$; $p=0.031$, IgA λ $r=-0.33$; $p=0.05$). It is possible that the correlation was greater for IgG patients than IgA patients due to saturation of FcRn receptors. Thus, greater concentrations of monoclonal IgG directly result in more rapid catabolism of any polyclonal IgG. Combining β_2M and the HLC ratio in a risk stratification model separated the population with 0, 1 or 2 risk factors and had greater statistical strength than the current ISS ($p=0.00000013$ v $p=0.023$). **Conclusion.** Levels of monoclonal protein have

only been weakly associated with outcome in MM and are not used in the current ISS. Heavy/light chain ratios are significantly associated with outcome and can be used to replace albumin in a risk stratification model. Heavy/light chain ratios correct for variations in plasma volume and haematocrit and in the case of IgG (the most frequent monoclonal protein) corrects for variable catabolism due to FcRn receptor saturation.

G73 - STABILITY OF FREE LIGHT CHAINS AND HEAVY/LIGHT CHAINS IN SERUM SAMPLES AFTER AN INITIAL SHIPPING PERIOD AT 22°C

D. Matters

The Binding Site Group Ltd, Birmingham, UK

Background. Serum stabilities of three weeks at 4°C for serum free light chains (FLC) and heavy/light chains (HLC) (IgA κ & λ and IgG κ & λ) are referenced in the assay manufacturer's instructions. Studies conducted to verify this stability data did not take into account time spent at ambient temperature during the shipping of the samples prior to storage. **Aims.** To determine the stability of FLC and HLC at 4°C and -20°C following storage at 22°C for 48h to mimic the effect of a two day shipping period. **Methods.** Venous blood samples ($n=10$) were collected from healthy donors and the serum separated by centrifugation. The serum FLC & HLC values were measured and the samples incubated at 22°C for two days, the analyte levels were re-measured and aliquots transferred to storage at 4°C and -22°C. The FLC & HLC levels were re-measured either weekly (4°C) or after 1 month (-20°C). **Results.** Following storage of sera at 22°C for 2 days the average levels of all analytes investigated were within $\pm 10\%$ of day 0 values. Following subsequent storage at 4°C for 4 weeks, average analyte levels were within $\pm 20\%$ for FLC and $\pm 15\%$ for HLC. The table shows the percentage difference of FLC and HLC levels in 10 normal serum samples following storage for 2 days at 22°C and subsequent storage at +4°C and -20°C. **Summary/Conclusion.** Storage of samples for FLC or HLC analysis at 22°C for 2 days does not have a detrimental effect on subsequent storage at 4°C or -20°C.

Table.

	Mean on day of collection (n=10)	Percentage difference from day of collection mean					
		Post 2 days 22°C storage	Day 9 at 4°C	Day 16 at 4°C	Day 23 at 4°C	Day 31 at 4°C	Day 31 at -22°C
FLC kappa	11.95mg/L	-0.2%	2.0%	-3.1%	-7.1%	-14.9%	2.2%
FLC lambda	14.64mg/L	-8.6%	-9.2%	-10.8%	-13.7%	-18.3%	-12.2%
H/LC IgA kappa	1.311g/L	3.6%	Not run	Not run	7.6%	4.7%	4.0%
H/LC IgA lambda	1.212g/L	1.3%	Not run	Not run	2.5%	1.6%	2.0%
H/LC IgG kappa	8.400g/L	0.7%	Not run	Not run	3.6%	2.6%	0.4%
H/LC IgG lambda	3.643g/L	0.9%	Not run	Not run	3.1%	-2.8%	-2.7%

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