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Guest Editor
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ORAL COMMUNICATIONS

FREE LIGHT CHAINS IN MULTIPLE MYELOMA I

FREE LIGHT CHAINS FOR MYELOMA DIAGNOSIS AND STAGING

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Diagnosis

The first clinical evaluations of serum free light chain (sFLC) measurement were retrospective diagnostic studies and it was discovered that sFLC measurements were abnormal in approximately 70% of patients previously classified as having nonsecretory disease. While sFLC were found to be abnormal in 100% of patients with light chain only myeloma. Subsequent studies confirmed these initial reports and also found >90% of patients with intact immunoglobulin multiple myeloma and >80% patients with smouldering myeloma had abnormal sFLC results. FLCs are cleared from the serum via the kidneys and sFLC measurements in patients with reduced renal function revealed elevated concentrations of both κ and λ light chains. The presence of an abnormal κ/λ ratio was therefore, accepted as the appropriate indication of monoclonal FLC production, rather than elevated concentrations. It was also apparent that because of the proportion of patients whose tumours do not produce FLC, sFLC analysis must be regarded as a complement to serum electrophoretic analyses and not as a replacement. Several studies have been published investigating the benefit of adding sFLC analysis into primary screening protocols for lymphoproliferative disorders. Bakshi *et al.* reported 9/1003 extra patients identified while Hill *et al.* reported 8/923. In these 2 studies, the additional patients identified included some with light chain only myeloma, nonsecretory myeloma, IgA MGUS, B-cell chronic lymphocytic leukaemia and non-Hodgkin lymphoma. sFLC analysis is of particular benefit when urine samples are not provided for the initial investigation; Bakshi et al made no reference to urine results in their study while Hill et al commented that >60% of their sera did not have accompanying urine samples. Results from primary screening studies, have identified some subjects with marginally abnormal FLC ratios but no evidence of a monoclonal plasma cell disorder on follow-up. This is most frequently seen in patients with borderline κ elevations. Minor increases in the κ/λ ratio are frequently a result of reduced glomerular filtration, which results in a change in the balance of FLC clearance towards removal by the reticuloendothelial system and increased retention of κ FLC. In 802 patients with chronic renal disease and no evidence of lymphoproliferative disorders, Hutchison *et al.*(a) found κ/λ ratios ranged from 0.37-3.1. The impact of applying this modified reference range diagnostically, was investigated in an audit of FLC results for 142 patients who presented with dialysis-dependent acute renal failure (Hutchison b). Use of the previously published reference range (0.26-1.65) identified all myeloma patients (41/41) plus 2 with MGUS and 5 "false-positives". Applying the modified reference range still identified 100% of the myeloma patients but with only 1 "false-positive" so the diagnostic specificity was improved.

Staging

The current international staging system (ISS) for multiple myeloma defines 3 stages based upon the measured concentrations of serum β2-microglobulin (β2M) and albumin. While other factors are known to be powerful prognostic indicators, this system was selected because it is simple and assays for measuring β2M and albumin are widely available. In 2007, Kyrtsonis *et al.* reported a study of 94 MM patients indicating that baseline κ/λ (or λ/κ) ratios greater than the median were predictive of reduced 5-year disease-specific survival. In addition, it was noted that the prognostic value of the FLC ratio was independent of the ISS and could discriminate between patients within ISS stages. These findings have recently been supported by an analysis of 790 MM patients by Snozek *et al.* In this study, break-points for the κ/λ ratio of <0.03 or >32 were used. Again, this was found to be independent of the ISS categories and a combined risk stratification model with 4 stages was proposed. There are now published reports indicating that abnormal FLC production is an adverse prognostic factor in most plasma cell disorders including MGUS, smouldering myeloma, plasmacytoma and AL amyloidosis in addition to multiple myeloma. The reasons why this is so are not clear, although the elevation of the tumour light chain as well as the suppression of the alternate light chain might be surrogate markers of tumour burden. Also, myeloma patients with high FLC production are more likely to have renal involvement which is known to have an adverse prognosis. It is possible however, that abnormal FLC production may reflect an inherent biological property of the tumour cells and extreme FLC ratios have been reported to be associated with adverse IgH translocations (Kumar *et al.*).

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USING SERUM FREE LIGHT CHAIN CONCENTRATIONS AS RAPID RESPONSE MARKERS IN MULTIPLE MYELOMA

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Serum free light chains (FLC) have several properties that make them ideal as a tumour marker in multiple myeloma. These include a short serum half-life of only a few hours, high specificity for the tumour and the sensitivity and rapidity of the assay. These features mean that changes in FLC concentrations can be used to rapidly follow changes in tumour killing. In contrast the serum half-lives of intact immunoglobulin are 20-25 days for IgG (IgG3 is 8 days), 6 days for IgA, 3 days for IgD and 2 days for IgE and intact immunoglobulin paraproteins slowly respond to changes in tumour killing. The long survival of IgG is due to it binding specific recycling receptors (the "Brambell" or "neonatal" Fc receptor) through which it is recycled back into the circulation and protected from unrestricted lysosomal catabolism. Following chemotherapy, changes in serum FLC concentrations and intact immunoglobulins occur in parallel but FLC levels usually fall much more rapidly particularly with IgG myelomas given the longer half-life compared to IgA. We have looked at the kinetics of serum FLC in patients undergoing peripheral blood stem cell transplantation (PBSCT) showing that FLC assays provided a sensitive monitor of changes in tumour and non-tumour plasma cells after PBSCT with raised tumour FLC falling within 48 hours following high dose melphalan.[1] In patients with monoclonal intact immunoglobulin, the tumour FLC fell quicker than the monoclonal intact immunoglobulin with normal FLC recovery occurring just after or around the time of neutrophil engraftment. The potential benefits of rapid assessment of response in multiple myeloma include halting treatment earlier in patients who have reached a maximal response in order to avoid further stem cell damage and limiting toxicity for all patients. However some caution is required as it is unclear whether stopping treatment earlier is beneficial for clinical outcomes. A retrospective analysis of UK MRC trial patients illustrated a number of IgG myeloma patients, who achieved a serological plateau, where chemotherapy continued to be administered after the point at which FLC concentrations had stabilised within the normal range.[2] More importantly, the early identification of poorly responding patients using serum FLC is clearly relevant and such an approach is likely to become increasingly adopted in order to stop exposure to ineffective treatment and change to other agents. This is particularly relevant for patients in whom light chain is causing direct and potentially reversible renal failure and is illustrated by the extended daily haemodialysis study using a protein-leaking Gambro filter.[3] A similar approach, with frequent FLC monitoring and amended treatment for non-responders, might also be appropriate for patients relapsing with aggressive disease. The value of early identification of patients who are responding but with less than a PR is more controversial. Certainly attainment of complete response is associated with a better survival but randomised clinical trials need to address the issue of whether treatment should be altered in this setting in order maximise responses in order to reach CR and this is hopefully being addressed in the MRC Myeloma 11 trial. Some caution is required in monitoring serum FLC in patients on bortezomib as some poorly responding patients show a fluctuating pattern with a temporary reduction following each course followed by a recovery in tumour FLC levels whilst in responding patients FLC levels rapidly fall and stabilise within the normal range.[4] For the majority of myeloma patients with an intact monoclonal immunoglobulin, measuring FLC is unlikely to be of additional benefit as an earlier marker of disease relapse as the rate of FLC production parallels that of the intact monoclonal immunoglobulin and their concentrations should rise together. However, there are situations in which serum FLC can be an earlier marker of relapse. In early relapse or progression following initially chemosensitive disease without any plateau phase a rise in IgG may be obscured by the falling IgG following chemotherapy. Secondly in myeloma tumours there is a poor correlation between the concentrations of the tumour FLC and the tumour monoclonal intact paraprotein with an asynchrony in immunoglobulin molecule production and thus at relapse a rise in one marker may be detected before the other simply due to differences in the relative amounts of protein production by the tumour. Thirdly a minority of myeloma patients produce excess free light chains at relapse with little or no change in intact monoclonal immunoglobulin, called free light chain breakthrough.[5,6] In some of these patients there may be some intact immunoglobulin rise at relapse but the protein production of the tumour has clearly changed towards greater FLC production and this provides the clearer indication of relapse. With the advent of newer agents and reduced intensity allogeneic transplantation, novel manifestations of relapse with

extramedullary disease and free light chain breakthrough appear more frequent.[6] Using a double immunofluorescence staining method on myeloma bone marrows, a significant minority of myeloma patients (18%) have two populations of plasma cells, one population expressing intact immunoglobulin and the other light chain only.[7] During the course of their disease some patients showed a progression from having tumour cells expressing intact immunoglobulin to a population of tumour cells expressing light chain only and patients with a light chain only phenotype had a shorter survival.[7] This observation indicates a cellular basis for the "light chain escape" observed serologically.

A number of studies have identified earlier relapses using serum FLC in 1 of 7 patients[2] and 1 of 11 patients.[8] Following allogeneic transplantation the assessment of FLC may be potential useful in detecting early relapse with a view to donor lymphocyte infusions. Indeed a single study of 26 myeloma patients who had become immunofixation negative after reduced intensity allogeneic stem cell transplantation showed that changes in FLC preceded corresponding changes in immunofixation by at least 3 months in a number of patients.[9] Appropriate clinical studies are required to determine whether it is possible to gain clinical benefit from the earlier detection of treatment responses. Nevertheless using FLC to identify quickly patients with resistance to current regimens is likely to become increasingly incorporated into the current management of myeloma patients.

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SERUM FREE LIGHT CHAINS: IMPACT ON URINE TESTING

J.A. Katzmann

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Plasma cell proliferative disorders can usually be identified by the presence of an M-spike on serum protein electrophoresis (PEL). Some patients, however, do not have a large amount of serum monoclonal protein and their serum PEL may be normal. This is especially true for patients with monoclonal light chain diseases [amyloidosis (AL), light chain deposition disease (LCDD), light chain multiple myeloma (LCMM)], plasmacytoma, and non-secretory multiple myeloma (NSMM). The identification of a monoclonal gammopathy in these patients may require PEL as well as immunofixation electrophoresis (IFE) of both serum and urine. With the development of The Binding Site free light chain (FLC) assay for detection and quantitation of monoclonal serum FLC, the laboratory approaches to identifying and monitoring monoclonal gammopathies may no longer require sensitive electrophoretic assays for both serum and urine. Our recent studies have led us to conclude: 1) if serum PEL, IFE, and FLC are the components of a screening panel, then the diagnostic sensitivity is sufficient such that urine studies are not required; 2) unconcentrated urine is an appropriate sample for defining the protein distribution and M-spike concentration; and 3) when possible, serum and/or urine M-spike quantitation should be used for routine disease monitoring.

Screening panel for suspected monoclonal gammopathy

A number of retrospective studies of defined disease cohorts have concluded that if urinary monoclonal free light chains are detected by IFE, then the serum FLC assay will be abnormal. To test these conclusions, we have reviewed clinical laboratory results in 2 separate studies of routine clinical practice. The first evaluated all patients with serum FLC results that were reported by our laboratory in 2003.[1] The serum FLC κ/λ ratio was abnormal in 44% of patients with monoclonal gammopathy of undetermined significance (MGUS), 88% of smoldering multiple myeloma (SMM), and 91% of AL. The percentage of AL patients with a detectable serum monoclonal protein increased from 91% to 99% (109/110 AL patients) when both the serum FLC and IFE assays were evaluated. These sensitivities were consistent with other published data on defined disease cohorts, and reinforce the need to use the serum FLC assay as a complement to serum PEL and IFE. In addition, urine studies did not identify the single AL patient with normal serum results. To confirm these observations, we reviewed all newly diagnosed patients with both a urinary monoclonal protein and serum IFE and FLC studies.[2] Among 428 patients with a monoclonal protein in the urine, 426 were abnormal by serum IFE and/or serum FLC. The 2 patients with normal serum studies and abnormal urine were diagnosed with MGUS and had small amounts of urine total protein. The use of serum IFE in conjunction with quantitative serum FLC

therefore captured all the clinically significant patients identified by urine studies. This diagnostic testing panel identifies patients whose monoclonal gammopathy is evident by serum PEL and IFE, virtually all patients whose urine IFE is abnormal, additional patients who are only apparent by serum FLC, and eliminates the cost of urine screening assays.

Unconcentrated urine

Depending on the initial urine total protein concentration, it has been recommended to concentrate urines from 0- to 200-fold in order to increase PEL and IFE sensitivity. The larger concentration factors can take up to 1 hour with centrifugal concentrators and may require the transfer of specimen to a new tube during the process. More significantly, to achieve the higher concentrations, larger sample volumes are required and these volumes are not always available. If, however, the screening panel no longer includes urine studies, then the increased sensitivity is not needed and unconcentrated urine can be used for assessing the distribution of urine proteins. A study evaluating the Sebia urine electrophoresis protocol for unconcentrated urine specimens indicated similar performance for detection, characterization, and quantification of M-proteins when compared to our current procedure using concentrated urines.[3] Specimens with low protein concentration (≤25 mg/dL) may need to be concentrated 10-fold in order to achieve comparable sensitivity. The quantitation of M-spikes is equivalent in both protocols, and the ability to test unconcentrated urines means fewer sample rejections due to insufficient sample volume.

Disease monitoring

Serum FLC quantitation is a significant improvement for monitoring patients with oligosecretory plasma cell diseases such as NSMM, AL, and LCDD, and it has been recommended for monitoring MM patients without a serum M-spike >1 g/dL or urine M-spike >200 mg/24 hours. An ECOG MM trial whose accrual was completed in 1992 was used to compare urine M-spike values and serum FLC quantitation to response rate and survival.[4] The quantitation of involved FLC or the difference between involved and uninvolved FLC were preferred over the FLC κ/λ ratio for patient monitoring. A receiver operator curve analysis indicated that approximately a 50% reduction in these concentrations was the best cut-point for predicting overall response. The FLC response, however, did not predict for overall survival or progression-free survival. In addition, when a measurable M-spike was present, the correlation with changes in serum FLC was not exact and the FLC did not provide added information for routine disease monitoring.

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HOW AND WHEN I USE SERUM FREE LIGHT CHAIN TESTS

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The serum immunoglobulin free light chain (FLC) assay measures levels of free k and λ immunoglobulin light chains. The three major indications for the FLC assay in the evaluation and management of multiple myeloma and related plasma cell disorders (PCD) include: screening, prognosis, and measurement in cases of oligosecretory disease. The fourth use is documenting stringent response in multiple myeloma. In contrast, there are no data to support its use in myeloma patients with measurable disease by other means.

Serum flc assay in screening for plasma cell disorders

It is clear that having excess involved FLC or an abnormal rFLC is common in virtually all plasma cell disorders (Table 1).

Table 1. Rates of abnormal FLC ratio in different plasma cell disorders.[3]

Disease	*Abnormal FLC ratio, %*
Multiple myeloma (MM)	
Symptomatic MM	95-97
Non secretory MM	68
Light chain MM	100
Smoldering MM	88-90
Smoldering MM	90
MGUS	33-44
Amyloidosis	91-98
Light chain deposition disease	93

FLC, immunoglobulin free light chain; MGUS, monoclonal gammopathy of undetermined significance.

The most important screening study was done by Katzmann *et al*.[1] They asked whether the serum immunoglobulin FLC assay could replace urine IFE for screening patients suspected of having a monoclonal protein related disorder. Within the Mayo Clinic plasma cell disorder data base, 428 patients who had a positive urine IFE and who had serum PEL with IFE and serum FLC assay testing as a clinical assessment were identified. Serum PEL with IFE alone would have missed the

diagnosis in 28 patients (6.5%): MM (n=2); AL (n=19); plasmacytoma (n=3); smoldering MM (n=1); and MGUS (n=2). In contrast, serum FLC alone would have missed 14% of patients, but the combination of serum IFE and FLC identified 99.5% of patients with a positive urine. The two patients, who would have been missed had the urine IFE not been done, had low risk MGUS.

The FLC assay at diagnosis is especially relevant in patients with AL amyloidosis or any disease that has predominantly free light chains. Among 110 AL patients who had not been previously treated and who had a FLC assay performed within 120 days of diagnosis, the rFLC was positive in 91% compared with 69% for serum IFE and 83% for urine IFE. The combination of serum IFE and serum FLC assay detected an abnormal result in 99% (109 of 110) of patients with AL.[2]

The serum FLC assay in combination with serum PEL and serum IFE is sufficient to screen for pathological monoclonal plasmaproliferative disorders other than AL, the last of which still requires all the serum tests as well as the 24 hour urine IFE. If a diagnosis of a plasma cell disorder is made, a 24 hour urine for PEL and IFE is essential for all patients.

Prognostic value of the serum FLC assay

Baseline values of serum FLC can be used for prognostication (Table 2).[3]

Table 2. Uses of serum immunoglobulin free light chain assay.[3]

Screening in combination with immunofixation electrophoresis
Baseline values prognostic
Monoclonal gammopathy of undetermined significance
Smoldering myeloma
Symptomatic myeloma
Plasmacytoma
AL amyloidosis
Hematologic response
AL amyloidosis
"Non-secretory" myeloma*
Stringent complete response in multiple myeloma*
Light chain deposition disease (personal experience of authors)

**Not yet validated*

The pathogenic rationale for this linkage is not well understood, but one possibility is that higher levels of FLC may be associated with IgH translocations[4] as well as increasing tumor burden.[5,6] The serum FLC assay should be measured at diagnosis for all patients with MGUS, smoldering or active multiple myeloma, solitary plasmacytoma, and AL amyloidosis.

Role of the flc assay in response assessment

Although FLC response can be considered in 3 contexts--oligosecretory diseases, light chain myeloma, and measurable intact immunoglobulin disease–routine serial use of this assay can only be recommended for the first indication. To date there have been only a few studies that have validated the usefulness of serial FLC measurements,[5,7–9] although efforts for standardizing FLC response have been proposed.[10,11] For serial measurements, either the involved FLC or the difference between the involved and uninvolved (dFLC) should be used.[12] Aside from the time of diagnosis and in the context of documenting stringent complete response, the FLC ratio is not useful because of the not infrequently observed treatment related immunosuppression of the uninvolved (κ for monoclonal λ patients and λ for monoclonal κ patients) FLC during chemotherapy; the ratios generated when one of the FLC numbers is very low will be extreme, reflecting the degree of immunosuppression more than tumor burden.

Published FLC Response Criteria in Multiple Myeloma

In MM, the International Myeloma Working Group has recently published updated response criteria which incorporate the FLC assay. The criteria are shown in Table 4 as they pertain to FLC.[11] There have been no formal studies performed yet to date to validate these criteria.

Published FLC Response Criteria in AL amyloidosis

The consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis has defined FLC response in patients with AL amyloidosis as a FLC response in those individuals in involved FLC (iFLC) greater than 10 mg/dL as a 50% reduction in iFLC and progression as a 50% increase in iFLC.[10] The definition used for amyloid patients has been partially validated based on the work of Lachmann,[7] Sanchorawala,[9] and Palladini.[8]

Serial FLC ascertainment should be routinely performed in patients with AL amyloidosis and multiple myeloma patients with oligosecretory disease. It should also be done in all patients who have achieved a CR to determine whether they have attained a stringent CR. In routine clinical practic it has no role in response assessment in patients with monoclonal proteins measurable by protein electrophoresis of the serum or urine.

Caveats

Although the serum FLC is a valuable assay in patients with plasma cell disorders, there are technical limitations of the assay which make its uses as a serial measurement potentially problematic including: lot-to-lot variation; assay imprecision; and instances in which they do not dilute in a linear fashion. The most important area for future investigation includes defining the clinical relevance of early FLC "response" or "relapse" in patients with measurable intact serum immunoglobulins or measurable urinary M proteins. Apart from initial diagnosis and documentation of stringent complete response, its use is not advocated in these patients.

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FREE LIGHT CHAINS IN MULTIPLE MYELOMA II

RECENT ADVANCES IN MYELOMA TREATMENT

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Myeloma treatment has changed substantially with the introduction of thalidomide, bortezomib and lenalidomide. Patients are diagnosed with earlier disease and can expect better quality of life plus much longer survival. Although many treatment options are promising, only a few Phase III randomized trials provide efficacy and safety data to support evidence based decision making. For patients eligible for transplantation, there are 4 primary induction options: thalidomide/dexamethasone (Thal/ Dex); lenalidomide/dexamethasone (Rev/Dex); bortezomib/dexamethasone (Vel/Dex); and bortezomib/ thalidomide/dexamethasone (VTD). Overall response rates (ORR) range from 63-93% with CR + VGPR rates of 42-60% and 2 year overall survival rates of 70-87%. In the non-transplant setting there are 2 new options: MPT and VMP for which the treatment results are also excellent with ORR from 71-76% CR + VGPR 45-47% and 2 year overall survival of 83%. It is now particularly important to assess pretreatment prognostic factors and magnitude plus duration of response as accurately as possible. The serum immunoglobulin free light chain (FLC) assay has emerged as an important test for pretreatment assessment as well as for serial quantitative monitoring of response and subsequent disease activity, especially for patients with oligo secretory disease. In addition, genetically high risk disease has been defined as any or all of: t(4;14); t(14;16); t(14; 20); deletion 17p by FISH or deletion of chromosome 13 or hypodiploidy by conventional metaphase cytogenetics. This classification, which identifies 25% of patients, can be the basis for alternate treatment decisions. Initial studies indicate that bortezomib (Velcade) and/or lenalidomide (Revlimid) can overcome the negative impact of genetically high risk disease. In addition, very recent follow up shows longer survival for patients treated in the TT2 (total therapy-2) protocol with thalidomide if cytogenetic abnormalities were present (46% 8 year survival versus 27%). A major open question is the added role of the novel agents in the setting of single or double transplantation. The corollary is whether novel agents combined within conventional dose regimens can produce results equal to what is achievable with the high dose therapy combinations. Long term follow up will be necessary to adequately assess ultimate survival benefit. For the foreseeable future, we are fortunate to have a myriad of options in the clinic and in development. The challenge is to individually assess patients and customize therapy for maximum long term benefit.

FREE LIGHT CHAIN KIT PRODUCTION AND QUALITY CONTROL

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As the demand for free light chain (FLC) assays has increased The Binding Site has had to respond by finding ways to produce large numbers of tests from what was originally a research scale process. This has posed several challenges, from simply being able to produce the sheer quantity of antibody required, to quality control issues, such as how to ensure consistency between batches and instruments. The intended analytes for the Freelite assays are monoclonal FLC this inevitably means that the assays are subject to all the issues associated with the measurement of monoclonal proteins. These include issues such as antigen excess and non-linearity that are exhibited by some monoclonal FLC with the Freelite assays; these have had to be addressed to enable the practical use of the assays. The measures that have been taken to scale-up production to ensure a consistent supply into the future, improvements to quality control (QC) and batch to batch consistency, and strategies for the practical use of the Freelite assays will be discussed. An assessment of the success of this work will be made by looking at the trends in the types and numbers of customer enquiries.

The key to the successful production of serum FLC assays was the raising of high affinity polyclonal antibodies that were highly specific for FLC and would not cross react with light chains bound to the intact immunoglobulin molecules. The development of a procedure to induce immunological "knock out" sheep enabled this. This process has been further refined to allow greater yields of specific antibody, over the past 5 years, yields have increased two-fold from around 150 mg/L to >300 mg/L. The capacity of the antiserum production has been expanded from around 2500L per year to >5000L per year. All the affinity and adsorption columns have been increased from a research to a manufacturing scale. Not only has capacity been increased but reagent reactivity has been improved by refining the immunological "knock out" process and by increasing the number of antigens used in the immunisations. Consistency between batches has been improved through pooling large numbers of antisera from different sheep, each pool now consists of bleeds from more than 100 sheep and is >1000L in size. Increasing the antiserum pool size also provides greater security of supply for the future.

The basis of FLC kit QC is the comparison of results obtained for a large panel of samples comprising normal samples, samples containing polyclonal elevations of FLC and samples containing monoclonal FLC and intact immunoglobulin. These panels have been expanded to include a greater variety of monoclonal proteins and larger numbers of normal samples. This has lead to higher level of control over batch to batch consistency. Comparisons are made statistically using the Passing and Bablock regression model and Pearson's correlation coefficient, minimum acceptance criteria are slope of 0.85 to 1.15 and r>0.95. Currently, in the majority of cases, the between-batch correlation will result in a slope of 0.9-1.1 and r>0.97. Acceptance criteria for imprecision during calibrator and control assignment have been tightened and the overall CV throughout control assignment has to be <7.5% but is routinely <5%. Overall, these measures have led to a measurable improvement in batch to batch consistency over the last 4 years.

Strategies for handling antigen excess and non-linearity have been developed and resources have been devoted to the assistance of Freelite users. Use of defined dilution protocols developed for each of the instruments that run the FLC assays ensure consistent results when dealing with those samples that display non-linearity. Antigen excess has been found to occur at a low incidence, a study on the BNII reported a frequency of 0.12% out of 7538 samples for κ and 0% out of 7538 samples for λ[1]. However, antigen excess detection capabilities have been developed on the Roche Integra and the SPA Plus from the Binding Site.

The numbers of customer enquiries per kit sold are shown in the Figure 1. It is evident that technical improvements, response to customer feedback, greater experience in production and larger reagent batch sizes have combined to give an upward trend. There are more than ten-times fewer enquiries per kit than when the FLC assays were first introduced and the trend is still improving.

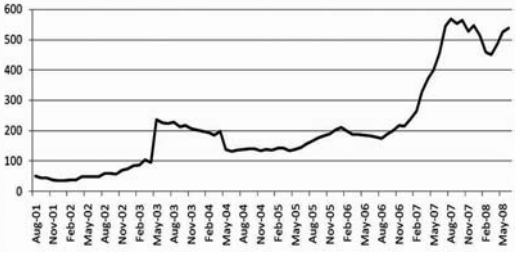

Figure 1. Chart showing the number of FLC kits sold per customer enquiry.

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HEAVY CHAIN/LIGHT CHAIN ANALYSIS IN MONOCLONAL GAMMOPATHIES

THEORETICAL BASIS OF HEAVY CHAIN/LIGHT CHAIN TESTS (HEVYLITE)

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Concept

Typical analytical tests for intact monoclonal immunoglobulins are SPE with scanning densitometry, IFE and nephelometry, all of which measure the total amounts of each class of immunoglobulin. In contrast, free light chains (FLCs) are measured separately, by immunoassays, as κ and λ molecules and κ/λ ratios derived. This provides: 1), a quantitative assessment of FLC clonality, 2), high analytical sensitivity, 3), wide clinical ranges due to immunosuppression of the non-tumour FLCs, and 4), ratios automatically compensate for variable renal metabolism and changes in blood volume.

Intact immunoglobulin molecules contain unique junctional epitopes between the heavy chain (CH1) and light chain (CL) constant regions. These are the target of "Hevylite" (HLC) antibodies. Such antibodies can separately identify the different light chain types of each immunoglobulin class, i.e., IgGκ, IgGλ, IgAκ, IgAλ, etc. When measured as pairs (e.g., IgGκ/IgGλ), ratios of monoclonal Ig/background Ig concentrations are produced in the same manner as serum FLC κ/λ ratios. Consequently, hevylite antibodies have similar analytical advantages to serum free light chain κ/λ ratios.

Production and clearance of immunoglobulins

Serum concentrations of FLCs and intact monoclonal immunoglobulins result from the balance between production and clearance rates. Production rates vary, not only between different patients but also in individual patients as their tumours progress or respond to treatment. It has been shown that for IgG myeloma, average synthetic rates per myeloma cell, per minute for IgG vary from 12,500 to 85,000 molecules between different patients. However, this is constant over time which means that changes in total synthentic rates reflect changes in tumour mass for an individual patient.

The clearance half-life of large molecular weight serum proteins (>65kDa - too large for renal filtration) is 2-3 days. Removal is by pinocytosis, a process that is active in all nucleated cells as they obtain their essential nutrients from plasma. However, for IgG the half-life is prolonged to 20-25 days by FcRn (neonatal) receptors. These membrane bound proteins have a structure similar to Class I MHC molecules with a heavy chain of 3 domains and a single domain light chain comprising β2-microglobulin. They are the same receptors that transport IgG from the pregnant mother to the developing foetus in the last trimester of pregnancy.

These heterodimeric FcRn molecules protect both 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. When there are no functioning FcRn receptors as in familial hypercatabolic hypoproteinaemia (a disease associated with a genetic deficiency of β2-microglobulin) the half-lives of IgG and albumin are only 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 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 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 is more than 21 days. 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).

Hence, in myeloma patients with monoclonal IgG production:

1. Concentrations do not accurately relate to tumour production rates (in addition to any variations in the efficiency of immunoglobulin production).
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 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. There is additional bone marrow suppression of IgG producing plasma cells in many patients.

Blood volume changes in monoclonal gammopathies

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 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 IgM, 50% of IgG and only 20% of FLCs are located in the vascular compartment. Hence, sFLCs are least affected because of their large extravascular distribution. Furthermore, sFLC ratios compensate for any changes in the individual FLCs or

immunoglobulin concentrations making them inherently more reliable for judging changes in tumour mass. Plasma 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 extravascular compartment, so again, molecular size is relevant. As the mass of monoclonal Igs 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, sFLC ratios are not 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 immunoglobulin measurements bear less relationship to tumour production rates than might be hoped. Hevylite Igκ/Igλ ratios are likely to be better than total IgG, IgA or IgM concentrations for predicting and monitoring responses to treatment in patients with monoclonal gammopathies.

CLINICAL STUDIES USING HEVYLITE TESTS

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Firstly, antibodies were produced which bound exclusively to conformational epitopes spanning the junction of the heavy and light chains in immunoglobulins. These antibodies were therefore specific to just one heavy chain/light chain combination. They were utilised to develop nephelometric assays for the measurement of their target immunoglobulin molecules and this allowed the determination of heavy/light chain (HLC) ratios for the different immunoglobulins, i.e. the IgGκ/IgGλ, IgAκ/IgAλ and IgMκ/IgMλ ratios. Normal ranges were established for the IgG, IgA and IgM HLC ratios using sera from healthy blood donors. Presentation sera from patients with multiple myeloma or Waldenstrom's macroglobulinaemia were analysed to determine HLC results and compare with the normal ranges. Serial samples, throughout the course of disease, were analysed for 9 IgG myeloma patients and 30 IgA myeloma patients. HLC measurements from these sera were compared with total immunoglobulin and monoclonal immunoglobulin measurements and the clinical assessments of disease.

The normal ranges are shown in the Table 1.

By comparison with the appropriate normal ranges, presentation sera from 19/19 IgG myeloma patients, 33/33 IgA myeloma and 7/7 Waldenstrom's macroglobulinaemia patients were all abnormal.

For the 9 IgG myeloma patients followed with serial samples, the change in the HLC ratio following treatment always showed a greater range of change than that of the monoclonal protein. This could be explained by changes in the degree of immunoparesis effecting the concentration of the non-tumour IgG because the non-tumour IgG is included as the denominator in the calculation of HLC ratios. Factors affecting the degree of immunoparesis would have included competition for plasma-cell niche-occupation in the bone marrow, TGFβ-mediated immune suppression and concentration-dependent changes in IgG catabolism.

Table 1.

	Number of subjects	*Concentration (g/L) Median (95 percentile range)*	*HLC ratio (Igκ/Igλ) Median 95 percentile range)*
IgG κ	N=103	6.98 (4.57-10.94)	1.77 (1.22-2.59)
IgG λ	N=103	3.95 (2.77-6.95)	
IgA κ	N=191	1.27 (0.44-2.36)	1.41 (0.58-2.52)
IgA λ	N=191	0.87 (0.34-1.85)	
IgM κ	N=119	0.77 (0.33-1.54)	1.64 (0.81-2.52)
IgM λ	N=119	0.50 (0.20-1.10)	

However, in 2/9 of the patients, salvage chemotherapy was followed by reductions in the concentration of monoclonal IgG and total IgG but no significant change in the HLC ratio. This would indicate that there was no tumour-selective killing with this chemotherapy but equal toxicity towards both the tumour and normal plasma cells. In addition, it has been observed that corticosteroids can reduce the expression of the IgG recycling receptor (FcRn) so it is possible that increased IgG catabolism could have been a contributory factor. For both patients, this (apparently non-selective) chemotherapy failed to induce a lasting remission.

Seven of the 30 IgA myeloma patients followed from serial samples, had IgA monoclonal proteins which were wholly or partially hidden in the β region of the serum protein electrophoresis gels. The monoclonal IgA could not, therefore, be measured directly at any time point while the HLC ratio could be determined throughout.

Of the 5 IgG and 10 IgA patients who became negative by serum protein electrophoresis after treatment, the HLC ratios remained abnormal at later sampling times or became abnormal earlier at relapse in 2/5 and 9/10 instances respectively. There were no serum samples in which protein electrophoresis revealed a monoclonal immunoglobulin while the HLC ratio was normal. Immunofixation was not performed on all samples but sensitivity of the HLC ratio for indicating monoclonality appeared similar to that of IFE.

In conclusion, these preliminary results indicate that the determination of HLC ratios gives a quantitative indication of monoclonal immunoglobulin production which is more sensitive than serum protein electrophoresis and similar in sensitivity to (qualitative) immunofixation electrophoresis assessment. Use of HLC ratios to monitor myeloma will intrinsically include a measure of immunoparesis irrespective of how that is induced. Further trials are needed to establish whether this will provide information which is of added value in patient management.

SERUM FREE LIGHT CHAINS IN OTHER MONOCLONAL GAMMOPATHIES

FREE LIGHT CHAINS IN AL AMYLOIDOSIS

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The amyloidoses are protein misfolding diseases, in which different soluble proteins aggregate as extracellular insoluble fibrils, causing organ dysfunction and death, unless this process is arrested by therapy.[1] In immunoglobulin light-chain (AL) amyloidosis, a usually small sized clone produces toxic light chains (LC) that cause multiple organ damage.[2] The introduction in the early 2000's of an assay that measures serum immunoglobulin free light chains (FLC) has revolutionized the care of AL amyloidosis. The FLC assay plays an essential role in the diagnosis and follow-up of this disease.

FLC assay in the diagnosis

The FLC assay is complementary to immunofixation electrophoresis in detecting the monoclonal protein in patients with AL amyloidosis. In the first two retrospective series by the United Kingdom National Amyloidosis Centre[3] and by the Mayo Clinic,[4] the quantitative FLC assay showed a greater sensitivity than the association of serum and urine immunofixation electrophoresis (98% vs. 79% and 91% vs. 81%, respectively). The Boston Amyloid Program, subsequently reported a lower diagnostic sensitivity of the FLC assay (75%) compared to immunofixation (sensitivity 96%),[5] and in a prospective study by the Mayo Clinic group,[6] the FLC assay and the combination of serum and urine immunofixation electrophoresis had a comparable diagnostic performance (sensitivity 91% *vs*. 95%, respectively). More recently, in a study from the Heidelberg group, including 11% of patients with multiple myeloma, the combination of serum and urine immunofixation had a slightly higher sensitivity than the FLC assay (92% *vs*. 87%), and the two tests proved complementary.[7] Our unpublished data obtained prospectively in 115 subsequent patients with AL amyloidosis at diagnosis showed a sensitivity of commercial IFE on serum and urine of 96% that reached 100% when associated with the FLC test. Our data also indicate that in patients with AL amyloidosis urine immunofixation should be performed to assure optimal diagnostic sensitivity. Indeed, not performing urine immunofixation led to missing 6% of the λ amyloidogenic clones in our study.

FLC and prognosis

In AL amyloidosis the structural characteristics of the pathogenic light chain is the main determinant of the disease, in particular, the conformational instability of the protein is considered at the basis of its aggregation and ensuing organ dysfunction.[1] Although the "quality" of the light chain is essential, the "quantity" of the misfolded protein may play a role in disease progression and prognostication. The Mayo Clinic group reported a significantly higher risk of death in patients with higher baseline FLC (hazard ratio 2.6, $p<0.04$). Baseline FLC correlated with serum cardiac troponin levels, and higher FLC levels were associated with more organs involved by amyloid, suggesting that high FLC levels may be associated with more advanced disease.[8]

FLC and assessment of response to chemotherapy

The introduction of the FLC assay has greatly improved the management of patients with AL amyloidosis and is now an essential tool in the care of this disease. The FLC response is included in the established consensus criteria for the definition of response to therapy.[9] Lachmann et al, were the first to relate changes of FLC with overall survival.[3] They demonstrated that those AL patients who achieved more than a 50% reduction in their involved serum FLC were more likely to live longer. Dispenzieri et al, reported, in a group of patients undergoing hematopoietic stem cell transplantation (HSCT), that normalization of FLC was the most important determinant to predict for hematologic response, organ response and overall survival.[8] Similar results were reported in 45 evaluable patients undergoing HSCT in whom normalization of FLC ratio at 3 months predicted for both progression free and overall survival.[10] Sanchorwala and colleagues, also demonstrated that the deeper the FLC response, the higher likelihood of both organ and hematologic complete response.[11] Furthermore, our group reported that FLC reductions correlate with reductions of NT-proBNP, a marker of cardiac function, and predict for overall survival.[12] This preliminary observation has been confirmed in a larger prospective population (unpublished results). Close monitoring using clonal (FLC) and cardiac (NT-proBNP) biomarkers allows the titration of therapy optimizing the toxicity/benefit ratio.

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THE ROLE OF SERUM FREE LIGHT CHAIN IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE, SOLITARY PLASMACYTOMA OF BONE, AND SMOLDERING MULTIPLE MYELOMA

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Monoclonal gammopathy of undetermined significance (MGUS) is characterized by the presence of a monoclonal immunoglobulin in the serum (M protein). The size of the serum M protein in MGUS is less than 30 g/L and the bone marrow contains fewer than 10% plasma cells. There is no evidence of end organ damage (CRAB – hypercalcaemia, renal insufficiency, anaemia, or lytic bone lesions). MGUS is found in approximately 3% of the general population 50 years of age or older and in 5% of those greater than 70 years of age (Kyle, *et al.,* 2006). The overall rate of progression is 1% per year (Kyle, *et al.*, 2002). This risk continues throughout the lifespan of the patient. Consequently, long-term follow-up is necessary in all persons with MGUS. Risk factors for progression include the size and type of M protein. Patients with a larger M protein or an IgA or an IgM MGUS progress at a higher rate. In a study of 1,148 patients with MGUS, an abnormal FLC ratio (κ/λ ratio <0.26 or >1.65) was found in 33% of patients. The risk of progression in patients with an abnormal FLC was significantly higher compared with patients with a normal ratio (hazard ratio, 3.5; 95% confidence internal 2.3-5.5; $p<0.001$). The FLC ratio was independent of the size and type of the serum M protein. Patients with an abnormal serum FLC ratio, IgA or IgM MGUS, and a serum M protein level ≥15 g/L had a risk of progression at 20 years of 58% (high-risk) versus 32% (high-intermediate risk) with any 2 of these risk factors, 21% (low-intermediate risk) with 1 risk factor, and 5% (low risk) when none of the risk factors were present (Rajkumar, *et al.,* 2005). Patients in the low-risk group can be followed less frequently than those in the intermediate or high-risk groups. The low-risk group accounts for almost 40% of MGUS patients.

Solitary plasmacytoma of bone

In a cohort of 116 patients with solitary plasmacytoma of bone, 43 progressed to multiple myeloma with a median time to progression of 1.8 years. The free light chain ratio was abnormal in 47% at the time of diagnosis. An abnormal FLC ratio is associated with a higher risk of progression to multiple myeloma ($p=0.039$). The risk of progression at 5 years was 44% in patients with an abnormal serum FLC ratio at diagnosis compared with 26% in those with a normal FLC ratio. Persistence of a serum M protein level of 5 g/L or greater 1 to 2 years following diagnosis was an additional risk factor for progression. The risk of progression to multiple myeloma in patients with a normal FLC ratio at baseline and an M protein <5 g/L at 1 to 2 years following diagnosis (N=31) was 13% at 5 years. Patients with either risk factor abnormal (N=26) had a progression in 26%, while those with both an abnormal FLC ratio and M protein level >5 g/L 1 to 2 years following diagnosis (N=18) had a risk of progression at 5 years of 62% (Dingli, *et al.,* 2006).

Smoldering (asymptomatic) multiple myeloma

Smoldering multiple myeloma (SMM) is an asymptomatic plasma cell proliferative disorder associated with a high risk of progression to multiple myeloma or AL amyloidosis. SMM is characterized by the presence of a serum M protein of 30 g/L or more and/or monoclonal bone marrow plasma cells of 10% or more but no evidence of end organ damage (CRAB). In a cohort of 276 patients with SMM, 59% developed multiple myeloma or AL amyloidosis. The overall risk of progression was 10% per year for the first 5 years, approximately 3% per year for the next 5 years, and 1-2% per year for the last 10 years with a cumulative probability of progression of 73% at 15 years. Risk factors for progression included the serum M protein level, type of M protein, presence of urinary light chains, extent and pattern of bone marrow involvement, and reduction in uninvolved immunoglobulins. The proportion of plasma cells in the bone marrow and the serum M protein level were combined to create a risk stratification model with three distinct prognostic groups (Group 1: Bone marrow plasma cells > 10% and M protein level > 30 g/L (N=106); Group 2: Plasma cells > 10% and M protein level <30 g/L (N=142); and Group 3: Plasma cells < 10% and M protein > 30 g/L (N = 27)(Kyle, *et al.,* 2007). The cumulative probability of progression

at 15 years was 87%, 70%, and 39%, respectively. The addition of the free light chain ratio (FLC) (<0.125 or >8.0) was an additional risk factor (hazard ration 2.3; 95% CI 1.6-3.2) and independent of the size of the M protein and number of bone marrow plasma cells(Dispenzieri, *et al.,* 2008). The free light chain ratio is a useful risk factor at diagnosis for predicting progression of MGUS, solitary plasmacytoma of bone, and smoldering multiple myeloma.

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USE OF SERUM IMMUNOGLOBULIN FREE LIGHT CHAIN IN WALDENSTRÖM MACROGLOBULINEMIA

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Background. Waldenström macroglobulinemia (WM) is a low-grade lymphoproliferative disorder of the elderly with an indolent course but a highly variable prognosis. The accurate diagnosis of WM is difficult in some cases, due to the ill-defined overlap between IgM-monoclonal gammopathy of undermined significance (IgM-MGUS), asymptomatic and symptomatic WM. The serum IgM level has been utilized as a marker of tumor progression and to assess response to therapy in WM. However, there are many limitations to the IgM protein level. The IgM level lacks sensitivity to early response of therapy and early relapse especially due to its prolonged half-life. Therefore, the serum IgM level does not reflect in a sensitive and accurate fashion the tumor burden or prognosis in WM, and there is a need to identify serum markers that reflects tumor burden in WM and correlate with the outcome of these patients. The prognostic significance of the IgM level at diagnosis remains controversial in WM. As a consequence, an international prognosis scoring system for WM (IPSS) was developed from the largest retrospective series of WM patients, based on independent factors of adverse prognosis (age >65 years, platelet count 100×10^9/L, β2M >3 mg/L, M-protein >7.0 g/dL, Hb 11.5 g/dL and albumin 3.5 g/dL). The Freelite assay is a new and sensitive immunoassay that measures serum immunoglobulin free light chains levels. The sFLC assay has shown significant clinical application in plasma cell dyscrasias, specifically in MM, primary systemic amyloidosis and MGUS. In MM, it is used to monitor response to therapy especially in patients with oligo- or non-secretory MM and is also now included in the new response criteria for MM based on its sensitivity to assess lower tumour burden compared to serum protein electrophoresis. The presence of an abnormal FLC ratio is a significant predictor of progression in MGUS patients, and also predicts early progression of disease compared to other tumour markers in MM. *Purpose.* Two published studies were conducted in order to evaluate the association between known tumour burden markers and prognostic factors with sFLC in patients with WM. *Results.* In the first study, Leleu *et al.* demonstrated in a large series of 98 WM patients along with 68 IgM-MGUS patients, that sFLC measurement accurately differentiated IgM-MGUS compared to WM reflecting a measurement of tumor burden. The median (IQ range) sFLC in IgM-MGUS was significantly lower than for WM patients (20 mg/L [16-33] *vs.* 36 mg/L [16-140], p=0.0003). In our series, the median (mg/L, IQ range) sFLCs values were 32 (16-76), 37 (16-124) and 95 (39-288), in patients with low (46%), intermediate (37%) and high (17%) risk WM-IPSS scoring system, respectively (p=0.05). Median sFLC levels were significantly higher ($p\leq0.05$) in patients with higher tumor burden (serum viscosity ≥1.8 cp, IgM level >40 g/L, and hemoglobin <10 g/dL or ≤11.5 g/dL) and with adverse prognosis markers (β2M >3 mg/L). In univariate and mutivariate analysis, median sFLC at the cut-off at 60 mg/L was higher for WM patients with low hemoglobin and high β2M, when authors applied the WM-IPSS cut-offs, but showed no association with IgM level. In the second study, Itzykson *et al.* studied 42 patients newly diagnosed with WM and confirmed in univariate analysis that the FLC level, expressed as a continuous variable, was higher in patients with β2M >3 mg/L (p=0.03) or with albumin <35 mg/L (p=0.04). Symptomatic patients also had significantly higher FLC values (p=0.037). In univariate analysis, the FLC level, expressed as a continuous variable, influenced the time to treatment (p=0.006) in the overall cohort (symptomatic and asymptomatic patients), as did the monoclonal component level (p<0.001), the albumin level (p=0.02), anemia value (p=0.02, HR=1.00), the monoclonal component level (p<0.001, HR=1.07), and the albumin level (p=0.03, HR=4.0). The FLC value correlates with the median time to treatment (TTT) in part because symptomatic patients, who receive treatment at diagnosis, have higher FLC values than asymptomatic patients (median FLC value 59.4 mg/L *vs.* 29.4 mg/L,

p=0.037). However, the correlation between TTT and FLC persisted in the subgroup of asymptomatic patients (*p*=0.047). Factors predictive of the time to treatment are of great value to clinicians. Treatment started a median of one year after diagnosis if the FLC value is over >80 mg/L. In multivariate analysis, a β2M level >3 mg/L (median sFLC value of 66.8 mg/L vs. 32.0 mg/L if β2M <3 mg/L, *p*=0.018) and a symptomatic disease both correlated with the sFLC value (median sFLC value of 59.4 mg/L *vs*. 32.0 mg/L if asymptomatic, *p*=0.046). *Conclusions*. Altogether, these results show that sFLC elevation is frequent in WM, and that sFLC is a new marker in WM disease. sFLC level measurement, either as a continuous variable or at cut offs determined at 60 mg/L and 80 mg/L, is not simply a surrogate of the M-component value and correlates with prognostic factors reflecting the tumor burden such as β2M level and albumin concentration, both of which have independent prognostic significance in WM. sFLC value, when expressed as a continuous variable, correlates negatively and independently with the time to treatment. Further analysis is required to prospectively study the role of sFLC in monitoring response to therapy and as a prognostic marker in WM patients. If confirmed in larger, prospective studies, FLC monitoring in WM patients will be warranted.

SERUM FREE LIGHT CHAINS IN NEPHROLOGY I

MONOCLONAL FREE LIGHT CHAINS AND RENAL DAMAGE

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Paraproteinemic renal diseases can be divided into those processes that affect primarily the glomerulus and those that promote tubulo-interstitial injury. Immunoglobulin free light chains (FLC) deposition is directly responsible for the pathology that produces tubulo-interstitial damage. Unlike most endogenous proteins, FLC have a propensity to produce tubular lesions. One reason for this association is related to the renal handling of these low molecular weight proteins. Metabolism of these proteins is directly related to glomerular filtration and reduction in glomerular filtration rate increases circulating concentrations of FLC. The approximate 500 mg of FLC produced by the normal lymphoid system daily is catabolized by the kidney in the proximal tubule, such that about 1-10 mg of polyclonal FLC normally appear in the urine daily. Once filtered, binding to a heteromeric receptor that consists of megalin and cubilin initiates reabsorption of the FLC into the proximal tubule. After endocytosis, the FLC are hydrolyzed and the amino acid residues are returned to the circulation. Although saturation of the multi-ligand endocytotic receptor complex of the proximal tubule permits FLC to appear in the tubule fluid of distal nephron segments and finally in the urine, overproduction of monoclonal FLC can result in significant concentration of these proteins in the proximal tubule.

Activation of the epithelium of the proximal tubule

Attention in recent years has turned to the possibility that the process of excessive protein reabsorption in pathological states by the proximal tubule results in chemokine and cytokine release, which accelerates interstitial fibrosis and progressive renal disease. Proximal tubular reabsorption of clinically nephrotoxic FLC promotes a unique oxidative stress with production of hydrogen peroxide, which initiates a signaling cascade that includes NF-kB and promotes the release of monocyte chemoattractant protein-1 (MCP-1). An important point is that the capability of FLC to activate the proximal tubule epithelium greatly exceeds the ability of other proteins, such as albumin, to activate these cells. Elaboration of pro-inflammatory chemokines and cytokines might facilitate the renal fibrosis that accompanies tubulo-interstitial renal injury. Understanding the process of tubular epithelial cell activation by FLC becomes important, not only in tubulo-interstitial injury in paraproteinemias, but also perhaps in chronic kidney disease (CKD) in general. As glomerular filtration rate falls, the serum concentrations of monoclonal (and polyclonal) FLC increase; thus the tubular concentration will increase in the remaining viable nephrons and proximal tubular epithelium. Although pro-

bably not all FLC produce oxidative stress, it is interesting to speculate that, regardless of the underlying cause, CKD *per se* increases the filtered load of FLC (polyclonal) presented to the remaining proximal tubules, resulting in persistent activation of the epithelium and perpetuation of a pro-inflammatory environment that accelerates renal disease progression. Perhaps the controversy regarding the role of proteinuria in accelerating progression of chronic kidney disease relates, in part, to study of the wrong protein, such as albumin.

Proximal tubule cytotoxicity

Endocytosis of FLC can produce severe injury to the proximal tubule epithelium, with associated clinical manifestations of renal failure. Proximal tubular epithelial damage was associated with endocytosis of the FLC into proximal tubule cells with subsequent distention of the endolysosomal system. Functional and morphologic alterations of these cells followed. The mechanism responsible for the more severe form of epithelial cell injury is unknown, but may relate to aggregation of FLC, as it is concentrated in the epithelial cell after endocytosis, but impairment of hydrolysis of the FLC permits retention and increases oxidative stress and cytotoxicity.

Cast nephropathy

Cast nephropathy, or myeloma kidney, represents the most common cause of renal failure in multiple myeloma. The initiating event in this process is intraluminal cast formation, which develops when a FLC binds to a specific 9-amino acid domain on Tamm-Horsfall glycoprotein (THP), which is synthesized exclusively by cells of the thick ascending limb of the loop of Henle. THP belongs to the class of glycophospholipid-anchored proteins, permitting extracellular localization on the apical surface of the cell. A soluble form of THP also appears in tubular fluid of the distal nephron and urine. Although constitutively expressed, an increase in dietary salt increases expression of THP in rats. Binding results in co-aggregation of the proteins, producing intraluminal cast formation and obstruction. Intravenous infusion of nephrotoxic human FLC in rats elevated proximal tubule pressure and simultaneously decreased single nephron glomerular filtration rate; intraluminal protein casts were identified in these kidneys. Additional studies using an *in vivo* isolated microperfusion model of cast nephropathy in rats demonstrated that casts formed exclusively in the distal portion of the nephron, beginning with the thick ascending limb of Henle's loop. Removal of THP prevented cast formation *in vivo* in this model. Interstitial inflammation commonly follows the development of intraluminal casts.

While cast formation is related directly to co-precipitation of the FLC with THP in the tubular lumen, the degree of interaction and functional significance is also dictated by numerous variables that include ionic composition of the tubule fluid, tubule fluid flow rates, concentration of THP and FLC, the strength of binding interaction between THP and FLC, and presence of furosemide. These observations have direct clinical relevance, since many of these factors, except the actual binding interaction between THP and FLC, can be modified with current treatment modalities.

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THEORETICAL ASPECTS OF FREE LIGHT CHAIN REMOVAL BY HEMODIALYSIS

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Background and objectives. When immunoglobulin free light chains (FLCs) are overproduced in patients with multiple myeloma, they can contribute to a spectrum of renal complications, such as cast nephropathy. Shortening the time which patients' kidneys are exposed to toxic levels of FLCs lowers the risk for permanent renal insufficiency. Therefore, a clinical strategy for reducing serum concentrations of FLCs is an urgent priority. The application of hemodialysis, already used for the treatment of the patients' renal failure, seems an obvious choice for reducing serum levels of FLCs. However, conventional high-flux dialysis membranes are unable to effectively remove substances with mole-

cular weights of greater than 20 kDa. FLCs which exist as monomers (22.5 kDa) and dimers (45 kDa), therefore have very low clearance rates. This poor efficiency of conventional dialysis membranes reflects primarily the molecular mass cut-off of the membranes, determined by pore sizes. Therefore, a dialysis membrane which would allow removal of FLCs must have a tailored pore size distribution to clear FLCs, while retaining larger essential proteins such as albumin. The aim of this study was to predict solute flux of FLCs and albumin as a function of membrane pore size and treatment modalities using a mathematical model. In addition, in vitro tests have been performed to investigate FLC elimination and trans-membrane albumin loss of a newly designed high cut-off (HCO) membrane with an altered pore spectrum. *Methods.* Urea, FLC and albumin clearances as a function of increasing membrane pore size were calculated using a mathematical membrane pore model, based on equations derived by Mason and by Michaels. For dialysers containing high-flux and HCO membranes, elimination profiles during hemodialysis were calculated as a function of molecular size. The high-flux membrane was assumed to be isoporous with a pore radius of 4 nm and the HCO membrane was assumed to be isoporous with a pore radius of 8 nm. The model was also used to calculate the influence of blood, dialysate and ultrafiltration flow rates on the elimination performance of a HCO membrane. The HCO membrane was assessed *in vitro*, for dialysis efficiency, using a closed dialysis circuit with a bovine plasma pool spiked with human FLCs. A Design of Experiments software tool (MODDE 7, Umetrics) was used to generate a design of 40 test runs, to determine optimal treatment conditions and the factors which significantly effected FLC and albumin clearance. The factors investigated were blood flow rate, dialysate flow rate, ultrafiltration flow rate and membrane surface area. The response factors FLC clearance and albumin clearance were fitted to a partial least squares model. *Result and Discussion.* The mathematical model of solute transport predicted that FLC removal is limited with conventional dialysis membranes, which typically have pore sizes of less than 5 nm. The calculated clearance for FLCs is almost one order of magnitude greater for hypothetical isoporous HCO membranes with a pore radius of 8 nm, than for membranes with a pore radius of 4 nm (Figure 1).

Analysing the impact of increasing convective flow on the solute clearance profile showed that convection permits increased solute transport across the HCO membrane, particularly for larger molecules. But, FLC clearance increased more slowly with increasing degrees of convection than albumin clearance. Pure hemodialysis, therefore, provides a higher ratio of FLC elimination to albumin loss than hemodiafiltration. The model also showed that in contrast to the clearance of small molecules, which largely depends on the blood and dialysate flow rates, the clearance of FLCs is less affected by changes in blood and dialysate flow rates. For a given dialysate flow of 500 ml/min, for example, FLC clearance increased rapidly with increasing blood flow until a plateau was reached at a blood flow rate of approximately 100 mL/min. Further increases in blood flow rates did not increase the clearance of FLCs significantly. The results of our in vitro hemodialysis experiments, with a newly developed HCO membrane dialyser conformed to the predictions of the mathematical model. The HCO hollow fiber membrane was prepared from a blend of hydrophobic and hydrophilic polymers. It is characterized by an asymmetric 3-layer structure with a selective layer of approximately 0.5 µm thickness; supported by a stabilization layer of 2 to 5 µm thickness, which again is supported by 45 µm finger type structure with a high void fraction. The selective layer which determines the overall mass transport has a narrow pore size distribution with the maximum number of pores in the range of 10 nm radius. Analysis of the test runs, using a full factorial design of experiment approach, yielded valid model equations for both response variables (FLC and albumin clearance). Both variables were heavily dependent on the membrane surface area and the ultrafiltration flow rate. Blood flow and dialysate flow rates did not show significant impact for the observed ranges of 150-300 mL/min and 300-700 mL/min, respectively. This indicates that transport through boundary layers at the blood-membrane and dialysate-membrane interface are not rate limiting for the overall mass transfer of FLCs within the applied ranges of blood and dialysate flow rates. Furthermore, the model allowed the determination of optimal conditions which provide a significant elimination of FLCs at modest albumin loss into the effluent. In summary, *in vitro* experiments and a mathematical model demonstrated that removal of FLCs by hemodialysis is dependent on the pore size of the dialysis membrane used. The novel HCO membrane studied allowed effective clearance of FLCs by hemodialysis. To enable effective clearance of FLCs with minimal loss of albumin, hemodialysis without convection should be used.

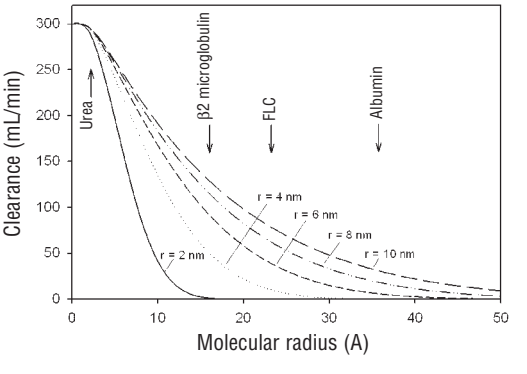

Figure 1. Calculated solute clearance profiles during hemodialysis when using dialysers containing isoporous membranes, with different pore radii r (pore radius = 2-10 nm, membrane surface area = 2.1 m^2, blood flow rate = 300 mL/min, dialysate flow rate =500 mL/min). The approximate molecular size of urea, β2 microglobulin, FLC and albumin are indicated.

REMOVAL OF IMMUNOGLOBULIN FREE LIGHT CHAINS BY HAEMODIALYSIS: A NOVEL MANAGEMENT STRATEGY FOR PATIENTS WITH MYELOMA KIDNEY

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Renal failure is a frequent complication of multiple myeloma and when severe it greatly increases patients' morbidity and mortality. Early intervention, however, can improve renal function which in turn improves the patients' survival.[1] Of the 10% of patients with multiple myeloma who develop dialysis dependent acute renal failure, 80-90% will require long term renal replacement therapy. The principal cause of this irreversible renal failure is cast nephropathy (myeloma kidney).[2–5] Myeloma kidney is a direct consequence of the very high serum concentrations of monoclonal free light chains produced by the clonal proliferation of plasma cells. The FLCs are filtered at the glomerulus and in these high concentrations overwhelm the re-absorptive capacity of the proximal tubules. In the distal tubules the FLCs co-precipitate with Tamm-Horsfall protein to form casts, obstructing the flow of urine. In turn, urine leaks into the interstitium and interstitial inflammation results.[6]

Treatment strategies have therefore focused on achieving early reductions in serum FLC concentrations. Use of plasma exchange (PE) in this setting may reduce serum FLC concentrations, but, randomised controlled trials have shown no evidence of renal recovery.[7] A model of FLC production, distribution and metabolism in myeloma patients indicated that plasma exchange might remove only 25% of the total amount over a three-week period. To address this failure of PE, we have assessed the utility of extended hemodialysis (HD), using protein permeable dialysers as an alternative approach to FLC removal.

In vitro studies indicated that a high cut-off (HCO) dialyser, the Gambro HCO 1100, was the most efficient of seven tested.[8] Model calculations suggested it might remove 90% of FLCs over three weeks. To optimise the removal of FLCs by this dialyser we studied the effect of dialysers in series, dialyser change and haemodiafiltration in 14 patients with multiple myeloma and renal failure. The clearance rates of both κ FLCs and λ FLCs were significantly increased on two dialysers from 19 (7.3-34) and 15.3 (9-28) mL/min to 47 (17-79) and 35.5 (20-57) mL/min, respectively. Clearance rates of both FLCs decreased over the course of the dialysis sessions (both $p<0.001$). Changing the dialyser during a HD session increased λ FLC clearance rates (22.5 (6-41) to 37.6 (9-52) mL/min; $p<0.001$) and decreased κ FLC clearance rates (39.6 (9-72) to 19 (8-59) ml/min; $p<0.003$). Ultra-filtration during HD increased the clearance rates of κ FLCs (R 0.52, $p<0.01$) but not λ FLCs (R -0.25; $p<0.076$). Haemodiafiltration increased the clearance rates of both κ (19 (SD 6.8) to 32 (SD 9.8) ml/min) and λ FLCs (15 (SD 7.8) to 20 (SD 7.7) mL/min).

The Gambro HCO 1100 dialyser has an effective molecular weight cut-off of 50 kDa, similar to that of albumin (65 kDa). We, therefore, assessed albumin loss in the cohort of patients studied and calculated albumin replacement requirements. Albumin loss increased when convection (filtration) was used in addition to dialysis and when two dialysers were used in series. Replacement requirements for 8 hours of HD were 12 g for a single dialyser and 45g for two dialysers in series. In addition to albumin replacement extended dialysis required regular replacement of magnesium and intermittent replacement of potassium and calcium.

Given the effectiveness of HCO-HD for removing serum FLCs, we assessed HCO-HD in combination with chemotherapy as a therapeutic strategy to rapidly reduce serum free light chain concentrations in patients with cast nephropathy and dialysis dependent acute renal failure. Dialysis independence and patient survival in 17 patients treated with HCO-HD were compared with a case matched historical control population (n=17). Standard chemotherapy regimens were used. HCO-HD resulted in sustained reductions in serum free light chain concentrations (median 86% [range 50-93]) in 12 of 17 patients. These 12 patients (71%) became dialysis independent at a median of 27 days (range 13-50). Five patients had chemotherapy stopped because of early infections and did not achieve sustained reductions; these patients did not recover renal function. Only two of 17 (12%) control patients became independent of dialysis ($p<0.0001$). In both groups, patients with cast nephropathy who recovered renal function had a significantly improved survival ($p<0.012$).

In conclusion, extended HCO-HD is highly effective for removing large quantities of FLCs in patients with multiple myeloma and renal failure. When used in combination with effective chemotherapy it resulted in sustained reductions in serum free light chain concentrations. Patients treated with this combination had high rates of renal recovery compared with a control population. Further evaluation of this treatment is now required to determine whether these results translate into improved patient outcome in a controlled setting.

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EUROPEAN TRIAL OF FREE LIGHT CHAIN REMOVAL BY EXTENDED HAEMODIALYSIS IN CAST NEPHROPATHY (EULITE)

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Dialysis-dependent acute renal failure is an important complication of multiple myeloma.[1,2] It is associated with a high morbidity and mortality: long-term patient outcomes are directly related to the severity of renal failure.[3] The commonest cause of renal failure is cast nephropathy (myeloma kidney);[4] a consequence of high serum concentrations of monoclonal free light chains (FLCs).

There are three main areas of focus in the management of cast nephropathy in this setting. First, high quality supportive care; second, prompt commencement of chemotherapy; third, consideration of direct removal of serum FLCs. Although plasma exchange has been widely used to remove FLCs from the serum, the efficacy of this treatment has never been established;[5–7] *in vivo* studies and mathematical modelling indicate that the impact of plasma exchange on total FLC load is modest.

Recently we described the effective removal of large quantities of FLC from the serum of patients with myeloma and cast nephropathy by extended haemodialysis, using the protein permeable Gambro HCO 1100 dialyser.[8] An open label study showed renal recovery rates to dialysis independence in 70% of patients.[9] Recovery was seen in all patients treated with the dialyser who also responded chemotherapy.

These observations have led to the development of a randomised controlled European trial of free LIght chain removal by exTEnded haemodialysis in cast nephropathy (EuLITE). This study is designed to evaluate FLC removal haemodialysis against standard high flux haemodialysis in patients with dialysis dependent renal failure and multiple myeloma.

Design and Methods

The trial will address the hypothesis that FLC removal haemodialysis will increase the rate of renal recovery in patients with cast nephropathy, severe renal failure and de novo multiple myeloma. At enrolment patients are randomised into one of two treatment groups

Group A (intervention): this group will receive extended haemodialysis on Gambro HCO 1100 dialysers and trial chemotherapy as a modified PAD (bortezomib, doxorubicin and dexamethasone) regimen.

Group B (control): this group will receive standard high flux haemodialysis (using a Gambro Polyflux-S dialyser) at intervals determined on clinical grounds by the nephrologist supervising care (minimum 4 hours 3 times per week) and trial chemotherapy as a modified PAD regimen.

FLC removal haemodialysis (group A only)

Participants will receive haemodialysis on non bortezomib days: 6 hours on day 0; 8 hours on days 2, 3, 5-7, 9+10. After day 12 participants will receive 8 hours of haemodialysis on alternate days. Dialysis will be undertaken on two Gambro HCO 1100 dialysers in series at a blood flow of 250 mL/min and a dialysate flow of 500 mL/min, with adequate heparin (activated clotting time of 180-200 seconds).

The study chemotherapy comprises: bortezomib (1 mg/m^2 on days 1, 4, 8 and 11 of a 21 day cycle); doxorubicin (9 mg/m^2 on days 1 and 4 of the 21 day cycle) and dexamethasone (40 mg daily on days 1-4, 8-11 and 15-18 for 1st 21 day cycle and days 1-4 only on subsequent cycles). Patients who withdraw for any reason will be followed up for all outcome measures in an 'intention to treat analysis'.

Primary endpoint

The primary endpoint of the study is independence of dialysis at three months from enrolment. Independence of dialysis will be defined as an estimated GFR of >15 mls/min/1.73m^2, two weeks after the last dialysis session.

Secondary endpoints

(i) the efficiency of FLC removal HD versus a standard dialysis to result in sustained reductions in serum FLC concentrations; (ii) the duration of HD; (iii) the number of patients who subsequently receive a stem cell transplant; (iv) overall patient survival.

Chemotherapy response

After a maximum of four cycles of treatment, patients will be assessed for response to chemotherapy according to the International uniform response criteria for multiple myeloma. If there is no evidence of response, treatment will be switched to local centre practise. Patients with a partial response or very good partial response will be eligible to continue until a maximum of eight cycles. Patients who have achieved a stringent complete response or complete response will be eligible to stop chemotherapy and proceed to autologous peripheral blood stem cell collection if considered suitable for high dose therapy and stem cell rescue.

Sample size and statistical analysis

Data from previous studies suggest a recovery rate of 25% in the control group and 55% in the treatment group is a realistic expectation. Assuming an alpha error level of 5%, 41 patients are required on each treatment arm to detect this size of difference with 80%

power. The primary analysis will be on an intention-to-treat basis but to enable sufficient power for any per-protocol analysis. The final target is adjusted to allow for a 10% drop-out. The target recruitment is therefore 45 patients per arm, 90 patients in total.
The proportion of patients independent of dialysis at three months will be compared using relative risks and chi-square tests. Kaplan-Meier estimation and log-rank tests will be used to compare the groups. Serum FLC concentrations over time will be compared across the two groups using longitudinal analysis. Myeloma response at 6 and 12 months and the proportion to have undergone a peripheral blood stem cell transplant at 12 months will be compared across treatment groups. Mortality will be documented for a follow-up period of 24 months.
Final analysis of the primary outcome data will be carried out after all patients have been followed up for a minimum of 3 months and a further analysis of the survival outcome will be carried out after all patients have been followed-up for a minimum of 24 months.

Current status
The study opened in the UK in May 2008 and has started recruitment. The study centres will comprise 10 sites in the UK and 6 sites in Germany. German centres are scheduled to start recruitment later in 2008.

Summary
The EuLite trial will extend the evidence base for the management of myeloma and dialysis dependent acute renal failure by a rigorous assessment of the utility of serum FLC removal by the Gambro HCO 1100 dialyser.

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SERUM FREE LIGHT CHAINS IN NEPHROLOGY II

EXPERIENCE WITH EXTRACORPOREAL FREE LIGHT CHAIN REMOVAL FOR CAST NEPHROPATHY IN MULTIPLE MYELOMA IN 8 PATIENTS

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Renal involvement in myeloma affects up to 50% of patients with myeloma at initial presentation. The most common histological type of renal lesion is cast nephropathy in 30% of patients with myeloma and is rarely reversible. Cast nephropathy develops after glomerular filtration of free light chains, subsequent tubular precipitation of light chains together with other proteins and mechanical obstruction. Cast nephropathy is believed to be potentially reversible when circulating light chains are rapidly reduced. We report on 8 patients with multiple myeloma and acute renal failure treated with a bortezomib-based chemotherapy in addition to a newly developed high-cutoff polyflux hemofilter. Reduction in serum free light chain levels was rapidly achieved in most patients. Four patients recovered renal function. Characteristics of patients predicting recovery of renal function are analysed as well as side effects of therapy.

HAEMATOLOGY/RENAL CASE STUDIES

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A series of cases will be presented addressing common renal problems seen in patients with plasma cell dyscrasias, focussing on renal problems associated with myeloma. There are many causes for renal impairment in patients with myeloma and related disorders. These include sepsis, dehydration, hypercalcaemia and the toxic effects of drugs- particularly non-steroidal anti-inflammatory drugs given to patients for bone pain (usually prior to the diagnosis of myeloma).
The renal lesions seen include cast nephropathy, light chain deposition, heavy chain deposition and amyloid deposition as well as acute tubular necrosis. In cast nephropathy, effective treatment of the underlying myeloma must be achieved for reversal of renal damage to occur. Novel methods of light chain removal may increase the chances of renal recovery when combined with effective anti-myeloma therapy.
Newer anti-myeloma agents such as thalidomide and bortezomib are well-tolerated in renal impairment and raise the prospect of improving the previously inferior outcome that patients with renal failure faced. Response rates to drugs such as thalidomide and bortezomib in patients with renal failure are as good as

those in patients with normal renal function. Little or no dose adjustment is required. Lenalidomide has shown great promise in patients with myeloma. However, unlike thalidomide, significant dose adjustment is required in renal failure due to increased toxicity, particularly myelotoxicity.

For younger patients with myeloma, standard practice includes chemotherapy to best response followed by autologous stem cell transplant. Patients with renal impairment, including those on dialysis, face higher toxicity rates but can receive this treatment if thought appropriate. The newer approaches to the management of myeloma in patients with renal impairment raise the prospect of many patients having significantly improved renal function prior to autologous transplant with a significant reduction in both morbidity and mortality of the procedure. Data from Ludwig et al suggests that combinations of anti-myeloma therapy including bortezomib can lead to improvements in renal function. Early data from our group suggests that the removal of free light chains by novel filter methods both accelerate renal recovery and improve the chances of renal recovery. This is currently being tested in a randomised study. The cases presented will illustrate some of these exciting advances in the management of patients with myeloma and renal impairment.

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SERUM FREE LIGHT CHAINS IN CLINICAL PRACTICE

GUIDELINES FOR FREE LIGHT CHAIN MEASUREMENTS

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Guidelines for use of serum free light chain (FLC) analysis in multiple myeloma and related disorders have recently been summarized by Angela Dispenzieri and colleagues on behalf of the International Myeloma Working Group (IMWG).

Key elements in the guidelines

1. Establishing a focus upon elevation of kappa (κ) or lambda (λ) FLC in the presence of a significantly abnormal k/λ FLC ratio (rFLC) i.e. clonally selective production of FLC.
2. Identification of serum FLC levels required to produce overflow proteinuria: medians are 113 mg/L for κ and 278 mg/L for λ. This helps correlate with the more familiar 24hr urine measurements.
3. Serum FLC is recommended as part of screening for pathological monoclonal plasma proliferative disorders.
4. Serum FLC is recommended as a prognostic assay in MGUS, smoldering myeloma, solitary plasmacytomata, active myeloma and light chain amyloidosis. There are specific levels and cutoffs for each entity.
5. Serum FLC measurements are also recommended in the assessment of response. Oligo secretory disease is the major area for use. Rapid reduction in sFLC can be an early indicator of response. A major use of the rFLC is as the key element of stringent complete response (sCR) within the new international uniform response criteria for myeloma. Careful attention is required during ongoing therapy which can suppress the "normal" light chain levels and produce an abnormal rFLC.
6. The use of FLC measurements in many other settings is being actively studied. One potential utility is for identification of "Bence Jones escape" especially using novel therapy regimens following which "de-differentiated" relapse or extra-medullary oligo secretory progression occurs more frequently.
7. Particularly careful attention is essential for interpretation of FLC results in patients with renal insufficiency.

It is extremely important to be aware that although use of serum FLC analysis is a great advance there are caveats and cautions. One must be alert to practical day to day issues including measurements with possible "antigen excess", light chain polymerization, lot to lot kit variations and the potential for non-linear dilution problems. Many important correlative studies are currently ongoing. As physicians become more familiar with FLC assay, the potential broad utility becomes clear.

POSTERS

FREE LIGHT CHAIN INSTRUMENT COMPARISONS

A1. EVALUATION OF THE SPAPLUS FOR SERUM FREE LIGHT CHAIN MEASUREMENT

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The analysis of serum free light chain can improve the diagnosis and monitoring of multiple myeloma and AL amyloidosis. The SPAPLUS (The Binding Site Ltd.) automated analyzer was evaluated in comparison to the nephelometric method on BN II (Siemens Healthcare Diagnostics) analyzer. The correlation with the comparative method (nephelometric) was good, with Pearson correlation coefficients of 0,950 for serum free light chain κ in the range 3,6 to 120 mg/L and 0,976 for serum free light chain λ in the range of 6,6 to 120 mg/L. The within run precision showed an coefficient of variation between 2,5 and 7% for serum free light chain κ and between 2,2 and 2,3% for serum free light chain λ. The day-to-day precision showed coefficients of variation between 3,3 and 3,6% for serum free light chain κ and between 5,3 and 6,1% for serum free light chain λ.

A2. EVALUATION OF LATEX-ENHANCED TURBIDIMETRIC REAGENTS FOR MEASURING FREE IMMUNOGLOBULIN LIGHT CHAINS ON THE ROCHE COBAS C501

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Assays specific for serum immunoglobulin free light chains (FLCs) (Freelite) are now widely available for many laboratory nephelometric and turbidimetric analysers. Studies have shown that serum FLC measurements can aid in the diagnosis and monitoring of patients with AL amyloidosis, non-secretory myeloma and light chain myeloma, and may also give a more rapid indication of response to treatment than monitoring of intact monoclonal immunoglobulins. Here we describe development of serum FLC assays for use on the Roche cobas c501 and evaluate their performance. The main assay characteristics are summarised in the Table 1. Interference was within ±10% when either bilirubin (15 mg/dL), hemoglobin (500 mg/dL) or Intralipid (0.5%) were added to serum samples with known FLC concentrations. Linearity was assessed by assay of serially diluted serum samples and comparison of expected with measured results: κ free: y=0.99x+0.45 mg/L, R^2=0.99; λ free: y=1.01x-4.1, R^2=0.99. Comparison was made with The Binding Site FLC assays for the Roche Modular P. Serum samples from normal subjects and also from patients with systemic lupus erythematosus and multiple myeloma were assayed for FLC on both systems and were compared using Passing-Bablok analysis: κ free y=0.97x+1.24 mg/L (n=37); λ free y=0.89x+1.8 mg/L (n=39). We conclude that FLC assays for the Roche cobas c501 provide a rapid, precise method of measuring FLC in serum and show good agreement with the Freelite Roche Modular P assays.

Table 1. Table showing the main assay characteristics of the FLC assays for the Roche COBAS C501.

Assay	*Free κ*	*Free λ*
Range	3.8-56.3 mg/L	7.0-93.3 mg/L
Sample dilution	1/5	1/8
Min. sample dilution	Neat	Neat
Sensitivity	0.75 mg/L	0.88 mg/L
Assay time	7.3 mins	7.3 mins
Intra-assay precision	1.4% (5.6 mg/L)	4.1% (7.7 mg/L)
(n=20)	2.5% (18.5 mg/L)	5.5% (27.3 mg/L)
%CV (Mean)	1.7% (41.1 mg/L)	2.8% (60.3 mg/L)
Inter-assay precision	7.2% (5.8 mg/L)	5.6% (8.2 mg/L)
(n=10)	7.0% (19.8 mg/L)	3.6% (28.6 mg/L)
%CV (Mean)	2.9% (41.4 mg/L)	2.3% (63.3 mg/L)

A3. EVALUATION OF LATEX-ENHANCED TURBIDIMETRIC REAGENTS FOR MEASURING FREE IMMUNOGLOBULIN LIGHT-CHAINS ON THE ROCHE COBAS INTEGRA 800

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Assays specific for serum immunoglobulin free light chains (FLCs) (Freelite) are now widely available for many laboratory nephelometric and turbidimetric analysers. Studies have shown that serum FLC measurements can aid in the diagnosis and monitoring of patients with AL amyloidosis, non-secretory myeloma and light chain myeloma, and may also give a more rapid indication of response to treatment than monitoring of intact monoclonal immunoglobulins. Here we describe development of serum FLC assays for use on the Roche COBAS INTEGRA 800 and evaluate their performance. The main assay characteristics are summarised in the Table 1. Interference was within ±6% when either bilirubin (20 mg/dL), hemoglobin (500 mg/dL) or Intralipid (0.5%) were added to serum samples with known FLC concentrations. Linearity was assessed by assay of serially diluted serum samples and comparison of expected with measured

results:- κ free: y=1.01x-4.2 mg/L, R^2=0.99; λ free: y=1.0x-4.1, R^2=0.99. Comparison was made with The Binding Site FLC assays for the Roche cobas Integra 400. Serum samples from normal subjects and also from patients with systemic lupus erythematosus and multiple myeloma were assayed for FLC on both systems and were compared using Passing-Bablok analysis:- κ free y=1.09x – 1.8 mg/L (n=37); λ free y=0.9736x+0.3 mg/L (n=36). We conclude that the FLC assays for the Roche COBAS INTEGRA 800 provide a rapid, precise method of measuring FLC in serum and show good agreement with the FLC assays for the Roche COBAS INTEGRA 400.

Table 1. Table showing the main assay characteristics of the FLC assays for the Roche COBAS INTEGRA 800.

Assay	*Free κ*	*Free λ*
Range	2.9-127 mg/L	5.2-139 mg/L
Min. sample dilution	1/2	1/2
Sensitivity	0.6 mg/L	1.3 mg/L
Assay time	8 mins	8 mins
Intra-assay precision (n=20) %CV (Mean)	1.8% (5.7 mg/L) 2% (20.9 mg/L) 1.3% (82.5 mg/L)	4.5% (7.5 mg/L) 1.1% (28.6 mg/L) 1.0% (96.5 mg/L)
Inter-assay precision (n=10) %CV (Mean)	4.9% (5.8 mg/L) 2.3% (21.3 mg/L) 2.3% (82.9 mg/L)	2.9% (7.5 mg/L) 1.5% (29.3 mg/L) 1.9% (128.3 mg/L)
Antigen excess protection	An early reaction absorbance check flags samples likely to be in excess, prompting remeasurement at a higher dilution.	

A4. COMPARISON OF SERUM FREE IMMUNOGLOBULIN LIGHT CHAIN ASSAYS ON EIGHT NEPHELOMETRIC/TURBIDIMETRIC ANALYSERS

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Assays specific for serum immunoglobulin free light chains (Freelite) are available for the majority of nephelometric and turbidimetric analysers. The performance of these assays is determined to some degree by the analyser in question. We compared the performance of the Freelite assays on eight analysers in our production laboratory. Throughput was tested by measuring 20 normal serum samples (requiring no remeasurement) and 20 myeloma samples (requiring multiple remeasurements). These were run separately so that each instrument's ability to make automatic redilutions could be isolated from its basic throughput. The hands-on time, e.g. manual dilutions, was also recorded. Total precision (n=100) was assessed, and the availability of antigen excess protection was also documented. Instrument reliability was assessed by calculating the average number of breakdowns per unit, per year requiring engineer intervention. Finally, overall performance was assessed by awarding marks from 0-3 for each category, with the exception of antigen excess protection where only one mark was awarded. Basic throughput was similar, however the BNII and ProSpec were affected by longer assay times. Instruments offering multiple automatic redilutions might be expected to show better throughput where myelomas are concerned, however the Modular P and SPAPLUS showed the best processing speed, with only a few minutes hands-on required. Precision was good overall, although the best precision was seen with instruments using disposable cuvettes (***). Antigen excess protection was only available on the SPAPLUS and Integra 400, giving reassurance that very high myelomas will not be misreported. Where reliability was concerned, the Olympus and SPAPLUS performed best, with less than a single breakdown per unit per year. Overall performance showed the Integra, SPAPLUS and Olympus to be the most user-friendly instruments for running the Freelite assays.

Table 1. Table showing the comparative performance of the Freelite assays on eight nephelometric/turbidimetric analysers in the Binding Site production laboratory.

	Beckman Immage	*Binding Site SPA$_{PLUS}$*	*Roche COBAS Integra 400**	*Roche Modular p**	*Olympus AU 400*	*Siemens Advia 1650**	*Siemens BNII*	*Siemens BN ProSpec*
Sample redilution **	None	1	3	1	1	1	2	2
Normal samples	40 mins	37 mins	33 mins	29 mins	36 mins	18 mins	52 mins	52 mins
Myeloma samples	84 mins	68 mins	75 mins	51 mins	71 mins	106 mins	127 mins	172 mins
Hands-on time	21 mins	10 mins	5 mins	5 mins	15 mins	14 mins	0 mins	10 mins
κ precision (n=100)	2.7%	4.1%	1.8%***	6.3%	2.5%	4.5%	4.8%	1.6%***
λ precision (n=100)	2.7%	3.9%	1.2%***	5.4%	2.2%	3.1%	4.7%	1.3%***
Antigen excess	No	Yes	Yes	No	No	No	No	No
Breakdown unit/year	5	0.2	1	6	0	6	1.4	6
Overall performance	12 / 22	16 / 22	20 / 22	13 / 22	16 / 22	12 / 22	14 / 22	14 / 22

**Other compatible instrument models are available, but no data exists. **1 = Single auto-redilution. 2 = Multiple auto-redilutions. 3 = Multiple manually ordered redilutions.*

A5. STATISTICAL ANALYSIS OF FREE LIGHT CHAIN RESULTS BETWEEN EIGHT NEPHELOMETRIC AND TURBIDIMETRIC PLATFORMS

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Assays specific for immunoglobulin free light chains (Freelite) are now available on a number of analysers. Automated laboratory tests should produce comparable results between different platforms and this study compares results from the Freelite assay on eight different analysers within a single laboratory.

Comparison to the quoted normal reference range was assessed by measuring 100 sera (88 on the Advia 1650) from a normal population (negative by SPE) on all the analysers. Results were compared to the 95 percentile reference range (κ 3.3-19.4 mg/L, λ 5.71-26.3 mg/L) using Analyse-it for Microsoft Excel. 88 myeloma sera were identified by IFE and confirmed by bone marrow biopsy, all myeloma samples were run on the Siemens BNII, however, sample availability limited the numbers run on other analysers. The agreement to the BNII of the combined normal and myeloma sera results were calculated using the Passing & Bablok fit test on Analyse-it. It can be concluded that for both normal and myeloma samples the Freelite assay does produce comparable results on all these nephelometric and turbidimetric platforms.

Table 1. On all analysers very few normal sera gave results outside the reference range (≤6%). The Passing & Bablok test demonstrated good between-analyser agreement. There was no constant bias between the analyser results as shown by the low intercept values. There was very good agreement between results from different analysers as shown by the values for the slope. Receiver Operator Characteristic for each instrument was compared (Delong Delong Clark-Pearson), the area under the curves ranged from 0.96-1.00 and the differences between the platforms ranged from -0.03 to +0.02.

	κ			λ			κ	λ
	Passing & Bablok analysis			*Passing & Bablok analysis*			*Normal sera (n=100)*	*Normal sera (n=100)*
	Slope	*Intercept*	*Total no. of samples*	*Slope*	*Intercept*	*Total no. of samples*	*Outside reference range*	*Outside reference range*
Siemens BNII*	N/A	N/A	N/A	N/A	N/A	N/A	5.0%	3.1%
Beckman Coulter Immage**	0.97x	-1.28	174	0.99x	-1.36	134	1.0%	5.0%
Roche Modular P**	0.98x	-0.21	187	0.98x	-0.19	182	3.0%	1.0%
Olympus AU400**	0.92x	1.30	178	1.01x	1.32	158	5.0%	5.0%
The Binding Site SPA$_{PLUS}$**	1.02x	-1.76	135	1.00x	-1.01	183	4.0%	6.0%
Roche Cobas Integra 400**	1.02x	1.12	177	1.04x	-0.35	171	5.0%	2.0%
Siemens BN Prospec*	1.06x	0.33	188	1.04x	0.17	182	5.0%	6.0%
Siemens Advia 1650**	0.90x	-0.51	168	1.07x	-1.00	166	1.1%	2.3%

Key: * Nephelometric analysers. ** Turbidimetric analysers.

FREE LIGHT CHAIN ASSAY COMPARISONS

B6. SERUM FREE LIGHT CHAIN REFERENCE INTERVALS. A STUDY OF 133 BLOOD DONORS

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Introduction. Before the implementation of the free light chain (FLC) determination in our center, a control study with blood donors was undertaken. The serum references had been published by Katzmann and cols. who set 95% reference intervals and a diagnostic range for clonality. *Materials and methods.* From April to June 2007, 133 samples were collected in two lots and frozen, from anonymous blood donors, aged 18-65, who filled a health questionnaire. The second batch was screened with proteins studies, immunofixation and levels of immunoglobulins. Samples were defrozen and FLC quantified in a Dade-Behring BNII nephelometer, using antibodies Freelite from The Binding Site Ltd. *Results.* Results are given (Table 1) for the whole pool of blood donors, and for a subgroup of 50 controls with normal immune system, as assessed for the aforementioned studies.

Table 1.

	Mean FLC	*min*	*max*	*95% Interval*	*Reference Intervals*
Whole pool blood donors					
κ chains	11.2 mg/L	1.56	21.7	4.42-19.70	3.3- 19.4 mg/L
λ chains	12.5 mg/L	1.19	30.7	3.52-19.80	5.7- 26.3 mg/L
κ/λ ratio	1.0	0.19	6.18	0.47-2.19	0.26-1.65
Selected donors with normal immune system					
κ chains	13.2 mg/L	7.93	21.10	8.78-19.70	3.3-19.4 mg/L
λ chains	14.2 mg/L	8.85	30.70	9.19-19.80	5.7-26.3 mg/L
κ/λ n ratio	0.96	0.59	1.42	0.64-1.41	0.26-1.65

The 95% reference interval for the κ light chain was 4.42-19.70 mg/L and for the λ light chain was 3.52-19.80 mg/L, the latter down-displaced from the reference range. As shown in the histogram there is a cluster of low levels λ-FLC controls, who consequently disrupt the κ/λ ratio, giving rise to false positives for clonality. This contingency is solved applying the subgroup with a normal immune system in which the 95% reference interval are similar to the reported by Katzmann and the κ/λ ratio of 0.59- 1.42 falls entirely within the diagnostic range. *Conclusions.* We validated the diagnostic range of the serum free light chain (FLC) in a population of blood donors, with normal immune system and age somewhat younger than the one utilized to set the references, which better encompass the population with monoclonal gammopathies. Age, renal function and immune status seem to be the main features to be aware of when interpreting FLC results. We alert upon the need to demonstrate an elevated FLC when diagnosing the corresponding clonality, particularly for the κ chain.

B7. FREE LIGHT CHAINS IN URINE – AN ADDITIONAL DIAGNOSTIC ADVANTAGE?

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Background. The examination of free light chains (FLC) in serum is a very useful diagnostic tool in patients with monoclonal light chain production (e.g. multiple myeloma). About 30% of patients with multiple myeloma have renal involvement at the time of diagnosis and about 50% develop renal involvement during the course of disease. But different types of renal involvement have different prognostic value. Cast nephropathy usually progress very rapidly to end stage renal disease (ESRD), whereas nephrocalcinosis can disappear completely with bisphosphonate treatment. Recent data are controversial, whether a rapid remove of free light chains from serum is able to improve the renal outcome in patients with cast nephropathy. At the time the kind of renal involvement can be diagnosed correctly only by kidney biopsy, but problems with coagulation may delay the kidney biopsy in many patients with multiple myeloma. We investigated, whether the analysis of FLC in urine may be helpful to predict the kind of renal involvement. *Methods.* We examined the excretion of FLC in urine in patients with monoclonal light chain disease (e.g. AL amyloidosis, multiple myeloma) who underwent kidney biopsy because of unclear proteinuria or renal insufficiency. Patients were grouped according to their histological findings: 1: cast nephropathy (CN), 2: light chain deposit disease (LCDD), 3: AL amyloidosis (ALA), 4: other renal involvement (nephrocalcinosis, interstitial nephritis etc.) (ORI). Urine FLC were determined by electrophoresis and by nephelometry (Freelite, The Binding Site, Germany). *Results.* Kidney biopsies of 58 patients with multiple myeloma (MM) (n=30), immunocytoma (IM) (n=2), AL-amyloidosis (AL-A) (n=5) and monoclonal gammopathy of unclear significance (MGUS) (n=17) and B-NHL (n=1) were available. In 49 patients we had data about free light chains in serum. The findings in kidney biopsy were CN n=17(30%), LCDD n=4(7%), AL-A n=13(23%) and oRI n=23(40%). The mean light chain concentrations [mg/dL] were 357.7±390.5 (CN); 13.9±19 (LCDD); 15.7±17.9 (AL-A) and 45.3±103.8 (ORI) (p<0.01 CN *vs*. LCDD, ALA and ORD). If the critical FLC concentration was defined with >25 mg/dL the positive predictive value (PPV) was 0.64 for having a CN, sensitivity was 94%, specificity 78%. If the critical FLC concentration was defined with > 200 mg/dL PPV was 0.9, the sensitivity was 53% the specificity was 98%. *Discussion.* This data demonstrate that the examination of light chain concentration in urine may be helpful to find patients with cast nephropathy. Patients with LCDD or AL-A had significant lower light chain concentrations in urine and can be clearly discriminated from CN. Especially patients with a high excretion of λ light chains suffer from a cast nephropathy in most cases. Only patients with nephrocalcinosis had at times similar high FLC concentrations in urine. This might be important, because in patients with CN the renal function decreases often very rapidly and the exact diagnosis of renal involvement is important for treatment decisions. Further prospective studies are necessary to confirm this data.

B8. PITFALLS IN THE CALCULATION OF 24-HOUR URINE TEST RESULTS IN PATIENTS WITH MONOCLONAL GAMMOPATHIES: SERUM FREE LIGHT CHAIN ASSAYS PROVIDE A MORE ACCURATE ASSESSMENT IN THIS SETTING

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Background. 24-hour urine sampling is limited by imprecision, collection difficulties, noncompliance, and insensitivity. Evaluation of urine protein electrophoresis (UPEP) and 24-h urine protein for inaccuracy may be difficult at one time-point, but may become evident over time. For creatinine clearance (CrCl), normal ranges are defined, and results significantly above normal raise suspicion of error. A pattern of abnormally increased CrCl was observed from one large commercial US laboratory. *Aims*. To assess the scope of errors in CrCl, and correlate it with 24-h urinary M-protein, urine protein, and serum free light chain (sFLC) results. *Methods.* sFLC and 24-h CrCl, UPEP and urine protein sent to one commercial laboratory (because of insurance requirements) from January to November 2007 from patients with monoclonal gammopathies were reviewed. Normal values for CrCl were 97–137 mL/min (men) and 88-128 mL/min (women). *Results.* Among 624 urine samples from 208 patients, CrCl was abnormally increased (≥150 mL/min) in 119 samples from 59 patients (28% of all patients): 150–199 mL/min (60 samples); 200-299 mL/min (41 samples); and ≥300 mL/min (18 samples). For CrCl ≥ 300 mL/min, prior and subsequent CrCl were substantially lower on all evaluable patients: the median % increase from prior samples was 61%, and median % decrease with subsequent samples was 71%. Representative examples demonstrating corresponding errors on CrCL, M-protein and urine protein, but unaffected sFLC are shown (Table 1).

Table 1.

Pt Monthly time points	*A*	*B*	*C*	*D*
1				
Creatinine clearance, mL/min	63	75	207	70
M-protein, mg/24h	753	395	1107	291
24-h urine protein, mg/24 h	1285	665	2035	638
Free κ/λ mg/L	2/1590	<1/1300	<1/1130	1/1060
2				
Creatinine clearance, mL/min	57	37	185	94
M-protein, mg/24h	45	26	99	35
24-h urine protein, mg/24 h	136	119	969	248
Free κ/λ mg/L	49/9	37/6	21/10	25/12
3				
Creatinine clearance, mL/min	122	594	93	104
M-protein, mg/24h	141	737	89	106
24-h urine protein, mg/24 h	273	1992	173	261
Free κ/λ mg/L	98/12	95/14	107/16	113/13

Conclusions. Although laboratory error may occur with any test, results requiring calculations on 24-hour urine samples were highly susceptible to error as was observed in samples from one commercial laboratory. Clinicians relying on 24-h urine testing as a primary clinical parameter may be misled by these inaccuracies. sFLC testing was unaffected by these errors, and provided more reliable results to guide therapeutic decision-making.

B9. SERUM FREE LIGHT CHAIN TESTING IS A MORE SENSITIVE BASELINE MARKER THAN URINE PROTEIN ELECTROPHORESIS AMONG PATIENTS UNDERGOING PRIMARY AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Background. According to the International Uniform Response Criteria (IURC), serum free light chain (sFLC) assays are only to be used for monitoring patients without measurable baseline levels of M-protein in the serum or urine (Leukemia 2006; 20:1467-73). Thus, sFLC testing may be viewed as a default test rather than a primary monitoring tool. However, 24-hour urine protein electrophoresis (UPEP) is cumbersome, imprecise, and considered unpleasant by patients. *Aim.* To determine the sensitivity of sFLC testing and UPEP in assessing measurable disease at baseline. *Methods.* Among patients with multiple myeloma undergoing induction and primary autologous stem cell transplantation (ASCT) in 2005 and 2006, electronic laboratory records were accessed for results of sFLC testing, UPEP, and urine immunofixation at baseline.

Table 1.

Urine protein electrophroreis (UPEP)	*sFLC diagnostic n*	*sFLC not diagnostic n*
UPEP diagnostic: ≥200 mg/24h	13	0
Non-diagnostic: quantifiable but <200 mg/24h	13	4
Non-diagnostic: bands too faint to be quantified	2	4
Normal	*2*	*7*
Serum free light chain testing (sFLC)	*UPEP diagnostic n*	*UPEP not diagnostic n*
sFLC diagnostic: abnormal ratio, diff ≥50 mgL	13	47
Non-diagnostic: abnormal ratio and ↑ clone but difference <50 mgL	0	4
Non-diagnostic: abnormal ratio or ↑ clone	0	5
Normal	0	6

Measurable disease on UPEP was defined as ≥200 mg/24h as per the IURC. Measurable disease with sFLC was defined as an abnormal κ/λ ratio and ≥50 mg/L difference between involved and uninvolved clones (Dispenzieri et al. Blood. 2008;111:4908–15). *Results.* Among 177 patients undergoing ASCT, 45 patients had results available for baseline sFLC and UPEP (Table 1). All 13 patients with diagnostic UPEP were correctly classified as abnormal by sFLC testing, and 76% of patients with quantifiable but nondiagnostic M-protein (<200 mg/24h) on UPEP had unequivocally abnormal sFLC results. In contrast, 30 patients had measurable disease on baseline sFLC assays, but only 13 of them had diagnostic UPEP. UPEP did not provide any additional diagnostic information when the sFLC testing was normal or nondiagnostic. Urine immunofixation demonstrated a heavy chain in addition to a light chain in 25 of 36 abnormal UPEP, and light chain only in the remaining 11 UPEP. *Conclusions.* sFLC testing is a more sensitive marker than UPEP as a baseline marker prior to ASCT. UPEP provided no additional diagnostic information that was not already detected by sFLC.

B10. COMPARISON OF SERUM IMMUNOFIXATION ELECTROPHORESIS AND FREE LIGHT CHAIN ASSAYS IN DETECTION OF MONOCLONAL GAMMOPATHIES

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Our previous study on Light Chain Disease (LCD) consisting of patients with myeloma and AL amyloidosis indicated that 80% had detectable Bence Jones proteinemia (Stone and Frenkel, Am J Med 58:601-19, 1975). However, the free monoclonal light chains could not be accurately measured. The advent of serum free light chain (FLC) assays has made quantification possible. Between 11/06 and 11/07, results on routinely ordered serum immunofixation electrophoresis (IFE) were compared with independently conducted FLC assays. Monoclonal gammopathies were identified in 144 specimens by IFE; 73 (50.7%) had a normal κ/λ ratio in the FLC assay. Results for IgG and IgA M-proteins are shown in the Table 1. 44.6% of these specimens had a normal κ/λ ratio. Of 357 sera that showed no M-protein by IFE, 95.8% exhibited a normal κ/λ ratio. Nine of 11 patients with LCD had abnormal κ/λ ratios, whereas abnormal ratios were unusual in IgM or biclonal patients (6/32). A separate group of specimens ordered by clinicians for FLC only consisted of 946 samples of which 587 (62.1%) had abnormal κ/λ ratios. Of these 454 showed an increase and 133 a decrease in the κ/λ ratio. It is unclear why over 40% of patients with monoclonal gammopathies by IFE had normal κ/λ ratios. The magnitude of the M-spike by serum protein electrophoresis (SPE) was lower in these patients suggesting that some had monoclonal gammopathy of undetermined significance (MGUS). In the normal ratio subgroups, absolute values of both κ and λ light chains were elevated in 26 of 45 (57.8%) IgG and IgA specimens. Other patients, including some with MGUS, may have had balanced heavy-light chain synthesis without excess free light chains; this circumstance probably accounts for most of the discrepancies noted between IFE and FLC assays. *Conclusions.* Serum IFE should be carried out in addition to SPE and free light chain assays in patients with suspected monoclonal gammopathies.

Table 1.

	N Ratio	*Normal Ratio*	*% Normal*
G,κ	47	19	40.4
G,λ	29	14	48.3
A,κ	13	7	53.8
A,λ	12	5	41.7
Totals	101	45	44.6

B11. SERUM FREE LIGHT CHAIN MEASURMENTS BY ELECTROPHORETIC AND NEPHELOMETRIC METHODS MAY DIFFER DUE TO THE PRESENCE OF AGGREGATED LIGHT CHAINS

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Background. Overproduction of serum free light chains (sFLC) in multiple myeloma (MM) patients can be measured using nephelometric assays and densitometry following serum protein electrophoresis (SPE). *Aim.* To compare the concentrations of sFLC by SPE and a nephelometric sFLC assay in 5 different patients. *Methods.* SPE and immunofixation (IFE) was performed by Sebia hydrasys electrophoresis. sFLC was measured using a sFLC assay on Dade Behring BNII analysers. Western blot (WB) and size exclusion chromatography were run in accordance with manufacturers' protocols. *Results.* See Table 1. Using IFE 4/5 samples stained for monoclonal free λ proteins, 1 sample shows a faint κ restriction but no monoclonal free κ protein. In all cases sFLC measurements were greater than the reported SPE values. WB analysis of the patient sera was undertaken using HRP linked antibodies raised against total IgG, IgA, κ and λ. Visualisation of these blots with intact immunoglobulins confirmed bands between 116-180 kDa in size. WB visualised with anti-κ and anti-λ revealed bands from 25-250 kDa which indicated the presence of monomeric, dimeric and multimeric light chain aggregates. Samples from patients 1, 2 and 3 were fractionated using size exclusion chromatography. sFLC measurement and WB analysis of the fractions obtained verified the presence of aggregated free κ or free λ protein in these 3 samples. *Conclusions.* In all patient sera investigated, sFLC con-

centrations by nephelometry and by SPE densitometry differed. Results indicate that monoclonal sFLC can exist in several polymerisation states, as observed in all 5 patient samples. These polymerised paraproteins are likely to be the cause of any discrepancy between SPE and sFLC measurements.

Table 1. IFE, SPE and sFLC results.

Patient	*IFE result*	*PP Conc by SPE (g/L)*	*Free κ*	*sFLC Assay free λ*	*K/λ ratio*
			NR: 3.3-19.4	NR: 5.71-26.3	NR: 0.26-1.65
1	Free λ	0	8.03	4720	0.00170
2	negative	0	3060	4.63	660.907
3	Free λ	25100	1.75	79800	0.00002
4	Free λ	9100	7.69	67800	0.00011
5	Free λ	0	9.7	16300	0.00060

B12. COMPARISON OF SERUM VERSUS URINE ANALYSIS FOR THE IDENTIFICATION OF MONOCLONAL FREE LIGHT CHAIN PRODUCTION IN 219 PATIENTS WITH AL AMYLOIDOSIS

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Introduction. In a number of studies, the presence of an abnormal immunoglobulin free light chain (FLC) ratio in serum has been found to be a more sensitive indication of monoclonal plasma cell disease than immunofixation of urine samples. This would be expected because although FLC are predominantly cleared from the plasma by glomerular filtration, the renal tubules in a healthy kidney have the capacity to re-absorb and catabolise 10-30g/day of filtered protein before there is significant overflow into the urine. Nevertheless small amounts of monoclonal light chain have been identified in the urine of a number of patients who had normal serum FLC ratios. The aim of this study was to quantify and investigate this phenomenon in patients with AL amyloidosis. *Methods.* Paired serum and 24 hour urine samples, collected within 24 hours of each other, were obtained from patients with AL amyloidosis attending clinics at The Royal Free Hospital, London, UK. Serum FLC concentrations were measured by nephelometry (Binding Site). Both sera and urine were analysed by protein electrophoresis and immunofixation electrophoresis (Sebia). *Results and Discussion.* Among all the patients, 52/219 had abnormal serum FLC ratios but urine that appeared normal by immunofixation electrophoresis. Conversely, 16/219 patients had monoclonal bands visible in their urine by immunofixation but serum FLC ratios within the normal range. Only 2 of these 16 urine samples had FLC concentrations which were measurable by densitometry and these were both ~100mg/L. Furthermore, 12/16 of these patients had nephrotic-range range proteinuria (>3 g/day) so saturation of the protein reabsorption by albumin and other serum proteins could explain the increased passage of FLC into their urine; for the remaining 4/16 patients, some other mechanism must have been responsible. It was noticeable that the frequency of nephrotic-range proteinuria was higher in the subset of patients with abnormal urine/normal serum (12/16 =75%) than in those who had abnormal serum FLC results but normal urine (14/49=27%). Of the 4/16 patients with unexplained urine positivity, most had serum FLC ratios biased towards the tumour light chain (0.30, 0.34 & 0.49 for the λ patients and 1.61 for the κ patient). *Conclusions.* While serum FLC analysis is generally more sensitive than urine electrophoresis for indicating the presence of monoclonal FLC, this advantage is dependent upon efficient renal reabsorption of FLC. If the reabsorption mechanisms are impaired, monoclonal FLC can be detected preferentially in the urine. In the AL amyloidosis patients studied here, increased competition with albumin and other serum proteins could have accounted for the impaired FLC reabsorption in most but in some patients, other mechanism(s) must have been involved.

B13. POSSIBLE ROLE OF MYELOMA BIOLOGICALLY IMPORTANT CYTOKINES IN THE INAPPROPRIATE LIGHT CHAIN SECRETION OF MALIGNANT PLASMA CELLS

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Background. We and others observed that baseline free light chain (FLC) values and their ratio (FLCR) are prognostic in multiple myeloma (MM). The underlying biologic mechanisms of FLC hypersecretion by malignant plasma cells, even in intact immunoglobulin (Ig) MM type, are unknown. Numerous cytokines secreted mostly by the bone marrow microenvironmental cells were shown to play a role in MM pathogeny; among them interleukin-6 (IL-6) and its soluble receptor (sIL-6R) are considered the major plasma cell growth factor complex, syndecan-1 and its cleaved soluble form (s-syndecan-1) contribute to myeloma cell adhesion and behavior, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) promote neoangiogenesis and B-cell–stimulating factor (BLyS) modulates normal B-cell development and differentiation. *Aims.* The purpose of the present study was to determine the baseline serum levels of the aforementioned cytokines, at the

same time FLC were measured, in order to seek a possible correlation with FLC levels. *Methods.* FLCs were determined with the "Freelite" immunoassay (The Binding Site, Birmingham, UK) in 152 MM patients at diagnosis. Patients' median age was 66 years (42-85) and 50% were males. 24.6% were staged I according to Durie and Salmon, 33.3% II and 42.1% III while 32.8% were staged I according to the ISS, 25.5% II and 41.7% III respectively. MM Ig type was IgG in 61%, IgA in 20% and LC in 19%. 15% of patients presented with renal failure, 32.4% had haemoglobin levels <10 g/dL and thrombocytopenia (PLT <100×10⁹/L) was observed in 3.9%. Serum LDH and CRP levels were abnormal in 14.7% and 48.2% of patients respectively. Serum s-syndecan-1, VEGF, IL-6, sIL-6R, and BLyS were determined by ELISA in 91, 72, 86, 89 and 74 available frozen aliquots respectively, all drawn at diagnosis. Commercially available ELISA kits were used (Diaclone Research for s-syndecan-1 and R&D Systems for all the others). Measurements were performed in duplicate, according to the manufacturer's instructions and were reproducible. sFLCR was calculated with the uninvolved light chain as denominator. *Results.* Commercially available ELISA kits were used (Diaclone Research for s-syndecan-1 and R&D Systems for all the others). Measurements were performed in duplicate, according to the manufacturer's instructions and were reproducible. sFLCR was calculated with the uninvolved light chain as denominator. Median sFLCR was 5.6 in κ-MM and 57 in λ- MM. Serum s-syndecan-1 ranged from 7 to 5070 ng/mL (median 72 ng/mL) while serum VEGF ranged from undetectable to 4000 pg/mL (median 302 pg/mL). IL-6 was not detectable in the majority of the patients and was not included in our study. sIL-6R ranged from undetectable to 43301 pg/mL (median 5949 pg/mL) and BlyS ranged from undetectable to 921 pg/ml (median 122 pg). sFLCR values correlated with serum s-syndecan-1 (p=0.012) but not with serum VEGF (p=0.27), IL-6 (p=0.59), sIL-6R (p=0.74) and BLyS (p=0.83). *Summary/Conclusions.* Concluding, the correlation between sFLCR and serum s-syndecan may suggest that the last could play a role in the inappropriate FLC secretion observed in myeloma, unless a common factor regulate both s-syndecan release and FLC production.

B14. COMPARISON OF NEW SCIENTIFIC COMPANY AND BINDING SITE KITS FOR FREE LIGHT CHAIN MEASUREMENTS IN SERUM

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The current study describes a direct comparison between The Binding Site Ltd (TBS) free light chain (FLC) assays on the Siemens BNII nephelometer and a kit manufactured by the New Scientific Company (NSC). NSC currently holds an Italian patent for nephelometric measurement of FLC in urine, but also market their kits as being suitable for measurement of FLC in serum. A cohort of 20 normal sera (negative by SPE) were analysed using both sets of assays. Ten myeloma sera (five κ & five λ) were identified by IFE and confirmed by bone marrow biopsy, all samples were run on both manufacturers assays (one sample did not provide a result on the TBS assay due to limited sample volume). When the myelomic sera values were compared to the normal sera values produced in this comparison, all myelomic sera reported above the 95th percentile measured normal κ or λ ranges for the TBS assay and gave abnormal ratio values. Three of the κ myelomic sera did not report above the 95th percentile measured normal range on the NSC assay. Three of the λ myelomic sera did not report above the 95th percentile measured normal range on the NSC assay. All myelomic samples gave ratios outside the measured 95th percentile range. Calculation of the regression coefficients for the normal and myelomic sera between the two manufacturers produced R2 values of 0.0657 for k-FLC and 0.005 for λ-FLC. On the TBS assay, the myelomic sera all gave results and ratios that were significantly different to any normal samples, providing an excellent aid in the diagnosis of multiple myeloma. On the NSC kits produced many of the myelomic sera gave results that fell within the range of values given by the normal sera, this means that the NSC kit does not produce results that would be of clinical utility.

Table 1.

	κ-FLC		*λ-FLC*		*Ratio*	
	NSC mg/L	*TBS mg/L*	*NSC mg/L*	*TBS mg/L*	*NSC*	*TBS*
2.5%tile	25.55	7.61	19.29	7.64	0.49	0.76
97.5%tile	321.73	18.04	242.78	18.60	1.72	1.76

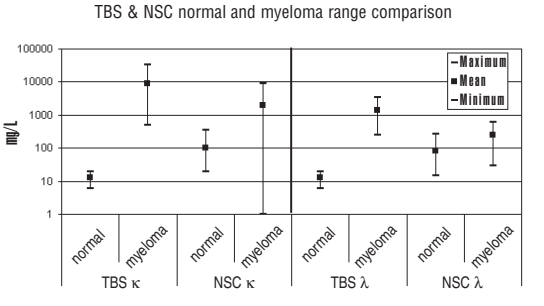

Figure 1.

B15. COMPARISON OF NORMAL AND MULTIPLE MYELOMA SAMPLES MEASURED BY AN ELISA FREE LIGHT CHAIN ASSAY (BIOVENDOR) AND THE BINDING SITE LTD NEPHELOMETRIC FREELITE ASSAYS

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The current study describes a direct comparison between The Binding Site Ltd (TBS) free light chain (FLC) assays on the Siemens BNII nephelometer and a research use only ELISA kit developed by Biovendor Laboratory Medicine Inc. 88 normal serum samples (negative by SPE) were analysed by both the Biovendor and TBS assays and the normal range data assessed for both κ and λ FLC for both manufacturers. The Biovendor κ and λ FLC ELISA assays generated normal results with a large number (21/88, 24%) of samples falling below the standard assay range of the κ ELISA assay (1.94 mg/L) and 5/88 (5.7%) falling below the standard measuring range of the λ assay (3.5mg/L). It was not possible to calculate the 95% range or the normal range for κ/λ ratio for the Biovendor kits. Serum from patients diagnosed with myeloma were analysed by both the Biovendor and TBS assays and compared to the normal ranges above. Of the 73 κ myeloma sera measured by the ELISA kit, only 37 (51%) reported abnormal results (gave values outside the normal range), whereas the TBS assays positively identified 71 samples (97%) with abnormally high κ FLC values (whilst the remaining 2 samples had abnormal κ/λ ratios, due to suppressed λ FLC levels). Of the 44 λ myeloma sera tested, the ELISA kits positively identified 40 (91%: with values outside the normal range), and the TBS λ FLC assay positively identified 43 (98%). Calculation of the regression coefficients for the normal sera between the two manufacturers produced R2 values of 0.18 for κ FLC and 0.08 for λ FLC. In summary, the TBS assays exhibit greater diagnostic sensitivity than those manufactured by Biovendor, positively identifying a greater number of myeloma sera. We postulate that the use of monoclonal antisera in the ELISA assays, as opposed to specific polyclonal antisera used in the TBS assays, restricts the number and spectrum of myeloma patients which can be diagnosed.

Table 1.

	k-FLC		*λ-FLC*		*k/λ ratio*	
	Biovendor mg/L	*TBS mg/L*	*Biovendor mg/L*	*TBS mg/L*	*Biovendor*	*TBS*
2.5%tile	-	6.95	-	9.18	-	0.53
97.5%tile	19.05	22.66	26.23	24.69	-	1.21

DIAGNOSTIC AND PROGNOSTIC USE OF FREE LIGHT CHAIN ASSAYS

C16. FREE LIGHT CHAINS BEHAVIOUR IN SERA FROM PATIENTS PRODUCING CRYOGLOBULINS

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Background. The diagnostic performance of the recently introduced assays for quantitative measurement of serum free light chains (FLCs),[1] has been studied in the recent years. The so-called Bence-Jones proteinuria and the increase of FLCs in serum have been associated with disease progression.[2] Immunofixation of urine or serum samples can detect free light chains at a maximum of 100-150 mg/L, while with the new FLCs assays, 3-4 mg/L are detectable. To quantify small amounts of FLCs, the test has been established as an latex-enhanced immunoassay suitable for different nephelometers. Given that the antibodies are specific for the hidden region of the immunoglobulin light chains, overestimation or underestimation can not be excluded. *Aims*. In this context we were interested to compare results obtained in the last six months from all samples processed in our laboratory for serum and urine fixation together with FLCs measurements. Out of this group, 25 samples were also characterised for the presence of cryoglobulins. The samples were collected from 208 patients recovered in our hospital with suspected plasma cell dyscrasia and processed in our laboratory for FLC measurements. *Methods*. The FLC assay (Freelite; The Binding Site Ltd.) was performed on a Delta (Radim) automated nephelometer. As normal reference ranges we considered 3.3-19.4mg/L for free κ chains, 5.7-26.3 mg/L for free λ chains (95% range) and 0.26-1.65 for the κ/λ ratio (100% range). All samples were simultaneously immunostained on a Hydrasis automated electrophoresis system (SEBIA) to evaluate the presence of monoclonal components. At the same time urine samples from all patients were processed on the Hydrasis system for the screening of Bence Jones proteinuria. The samples obtained from patients with suspected cryoglobulinemia were processed with the recommended SEBIA protocol, to isolate the cryoprecipitate and subsequently to characterize the cryoglobulins components. Before processing, cryo-samples were analysed for FLCs at 37°C, and after cryo-separation, the supernatants were also analysed for FLCs. *Results*. Our data show that some interaction with cryoglobulins can occur, but the Freelite assay still correlates with serum free light occurrence also in cryoglobulin positive patients.

C17. UTILITY OF SERUM FREE LIGHT CHAIN ANALYSIS IN THE DIAGNOSIS OF MONOCLONAL GAMMOPATHIES IN A COMMUNITY HOSPITAL

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Background. The serum free light chain (sFLC) assays in combination with serum protein electrophoresis (SPE) and serum immunofixation electrophoresis (IFE) provide a sensitive serum panel when screening for monoclonal gammopathies. The preponderance of supporting data has been observed at major reference laboratories. *Aim.* The goal of our study was to evaluate the performance of sFLC, SPE and IFE testing for the identification of monoclonal gammopathy in a community hospital setting. *Methods.* sFLC assays were performed on 356 serum samples using a SPAPLUS (The Binding Site) protein analyzer. SPE was performed on a Paragon Electrophoresis System with an Appraise Densitometer (Beckman-Coulter). IFE was performed on a Paragon Electrophoresis System. Samples were collected for routine analysis and stored at the Sharp Healthcare Laboratory from October 2006 to December 2007. 166 samples were evaluated by sFLC and SPE testing. 190 samples were evaluated by sFLC and IFE testing. *Results.* Clinical diagnoses with either SPE or IFE as well as sFLC results were available for 108 patients. 24 patients have been diagnosed with Multiple Myeloma. 20/24 (83%) of these patients had both abnormal SPE or IFE results and elevated free light chain ratios. 3 patients had elevated sFLC ratios with normal IFE or SPE values and one patient demonstrated an abnormal IFE result with a normal sFLC ratio. This patient had a polyclonal increase in sFLC and a diagnosis of MM with concomitant diabetes and renal insufficiency. 40 patients were diagnosed with renal disease. 24/40 (55%) exhibited normal SPE results with polyclonal elevations of sFLC and normal ratios. 7/40 (16%) exhibited polyclonal increases in SPE and polyclonal increases in sFLC levels with normal ratios. 5 patients had a normal SPE with a polyclonal increase in sFLC and an elevated FLC ratio (<3.0) consistent with published reports that patients with renal insufficiency can have FLC ratios as high as 3 without monoclonal gammopathy. 16 patients were diabetic. Of these, 11/16 (69%) had normal SPE or IFE, with polyclonal increases in sFLC and normal ratios. 2/16 had normal SPE or IFE with normal sFLC levels and slightly elevated FLC ratios (1.81, 2.16). In 3 patients all results were normal. 12 patients were diagnosed with an infection. 6/12 patients had polyclonal increases in sFLC levels and 6/12 had entirely normal laboratory findings. Patients diagnosed with a chronic inflammatory condition or cancer had variable results. 16 patients were diagnosed with a variety of other conditions. Of these, 14/16 had no evidence of any paraprotein anomalies and 2 had slightly elevated FLC ratios (1.87, 1.67). *Conclusions.* Many of our community hospital patients are over 50 years of age and present with non-specific symptoms potentially related to disorders associated with monoclonal gammopathies requiring laboratory evaluation. Older patients are also more likely to have renal impairment, hypertension and diabetes. Our preliminary data confirms that the sFLC assay provides additional laboratory information for the identification of patients with monoclonal gammopathies, renal insufficiency and diabetes.

C18. THE PRESENCE OF SERUM FREE-IMMUNOGLOBULIN LIGHT CHAINS AND ABNORMAL AND κ/λ RATIOS IS A FREQUENT FINDING IN PATIENTS WITH HODGKIN'S AND B-CELL NON-HODGKIN'S LYMPHOMA

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Introduction. The application of sensitive nephelometric immunoassays able to specifically identify free immunoglobulin light chains (FLC) has significantly improved the ability to detect and quantify monoclonal proteins (M-protein) in sera from patients (pts) with plasma cell disorders including monoclonal gammopathies and multiple myeloma (MM). Since demonstration of abnormal κ/λ ratios by serum (s) FLC testing supports the presence of an expanding population of monoclonal B-cells in lymphohemopoietic tissues, we have evaluated the potential value of sFLC as additional disease markers in pts with classic Hodgkin's (cHL) and B-cell non-Hodgkin's lymphomas (NHL). *Materials and Methods.* Frozen serum samples from 348 untreated pts with cHL (n=69) and NHL (DLBCL, n= 86; FCL, n= 55; MCL, n= 20; MZL, n= 29; CLL/SLL, n= 29 and Burkitt lymphoma -BL-, n= 5), were analyzed by the Freelite serum-free light-chain assay (The Binding Site, Ltd., Birmingham, UK) according to manufacturer's instruction. The FLC κ/λ ≥ratio was used to determine a positive or negative test and data was analyzed 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 positive. As internal controls, sera from 35 MM pts and 43 pts with solid tumors were concurrently analyzed. *Results.* Elevated sFLC concentrations with abnormal κ/λ ratio (positive test) were found in a consistent fraction of NHL with different histology. In detail, sera from 23% of DLBCL, 30.9% of FCL, 76.7% of MCL, 20.8% of MZL, 50% of BL and 70.5% of CLL/SLL pts, scored positive for sFCL testing. In most NHL cases (90%-100%) monotypic sFLC were of κ isotype. In control MM pts testing was positive in 80% of samples while none of the sera from solid tumor pts displayed an abnormal sFLC test. In positive NHL cases, the median sFLC κ chain value (mg/L) was: DLBCL, 23.93 (r 8.94-68.38); FCL, 26.29 (r 12.13-108.31); MCL, 54.88 (r 10.62-168.36); MZL, 41.22 (r 25.63-67.24); CLL/SLL, 55.77 (r 14.93-83.9), as compared to a median κ value of 120.545 mg/L (r 20.99-1823) of MM pts. Median abnormal λ values (mg/L) were of 113.63 (r 100-127), 75.33 (r 36.88-110.98) and 133.69 (r 74.56-192.82) for DLBCL, MCL and CLL/SLL, respectively. An intriguing finding

of our investigation was that 20% of cHL pts at diagnosis displayed a positive sFLC test, with a median κ value (mg/L) of 24.685 (r 5.93-37.06) and a single and λ positive case (79.77). *Conclusions.* Our results indicate that a unexpectedly high frequency of monoclonal serum FLC is present in sera from pts with different NHL histotypes representing various stages of B-cell differentiation. While a positive sFLC test in NHL may provide an additional tool for diagnosis and clinical monitoring of the disease, its biologic significance in cHL remains to be unveiled. This latter data, however, strongly supports and expands previous results that tissue bystander B lymphocytes may play a critical role for tumor cell growth in cHL.

C19. A NOVEL STAGING SYSTEM FOR LIGHT CHAIN AMYLOIDOSIS INCORPORATING FREE LIGHT CHAIN LEVELS

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Background. Primary systemic amyloidosis (AL) is characterized by multiple organ dysfunction secondary to deposition of light chain derived amyloid. Significant heterogeneity exists in outcome with in these groups, based on the degree of organ involvement as well as clonal characteristics. *Aims*. To develop a staging system that allows better prognostic stratification. *Methods.* We examined the baseline clinical and laboratory data from patients with AL who were seen at our institution with in 90 days of their diagnosis. Cox proportional hazards analysis was performed to estimate the prognostic values of different variables.

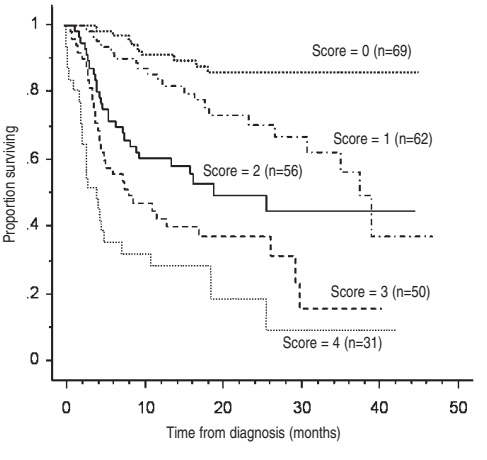

Figure 1.

Results. Variables related to the plasma cell characteristics (Difference between involved and uninvolved FLC, bone marrow plasma cell%, plasma cell labeling index, beta 2 microglobulin, circulating plasma cells) and organ involvement (troponin-T, BNP, NT-Pro-BNP, ejection fraction, septal thickness, 24 hour urine protein, creatinine, alkaline phosphatase) were all found to be prognostic for overall survival. In a multivariate analysis incorporating all variables that were significant on univariate testing, using a step wise selection, we arrived at four variables that had maximum impact on the outcome (FLC difference, troponin-T, BNP, and B2M). Patients were assigned a score of 1 for presence of each characteristic (FLC difference > 35 mg/dL, troponin-T > 0.035, BNP > 350, and B2M > 3.5) or 0 if the value was at or below the cutoff. The scores were added to obtain a composite prognostic score that grouped the 268 patients (who had all the variables available for analysis) into 5 groups with very different outcomes (Figure 1). *Conclusion.* Incorporation of plasma cell related measurements into the existing staging system using cardiac biomarkers allows better risk stratification of patients with AL amyloidosis. The system has the advantage of easily available laboratory values and can be widely adopted.

C20. ABNORMAL SERUM FREE LIGHT CHAIN RATIOS ARE ASSOCIATED WITH POOR SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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Serum free light chains (FLC) have prognostic significance in monoclonal gammopathy of undetermined significance, solitary plasmacytoma of bone, smouldering myeloma, multiple myeloma, Waldenstroms macroglobulinaemia and AL amyloidosis. The incidence of abnormal FLC in other lymphoid malignancies including chronic lymphocytic leukaemia (CLL) is unclear with only one published report which found 8/18 CLL patients with an abnormal FLC. There have been no studies correlating FLC with other biological variables and clinical outcomes in CLL or lymphoma. This is a retrospective study that analysed 259 (Stage: A, 209; B, 23; C, 21; Male: Female ratio 1.6:1, mean age 75: range 29-98) archived patient sera. Sera had been collected before (n=181) or during (n=79) treatment. The levels of FLC and B2M were assessed using nephelometric immunoassays (The Binding Site) on the Dade-Behring BNII analyser. Previously recorded measurements for biological and clinical markers (age, sex, CD38, ZAP70, and VH mutational status) were used in Kaplan Meier Survival Hazards and Cox Regression analysis. The population of CLL patients were analysed using Kaplan Meier Survival Hazards, abnormal FLC ratio was a significant indicator of poorer survival in the untreated (n=181, p=0.0001) and whole populations (n=255, p=0.006). Furthermore in both the untreated and whole populations a λ abnormal

ratio was associated with a poorer outcome (p=0.001). Using Cox Regression analysis (n=179, missing =63, with 17 cases excluded before the first event) we analysed age, stage, sex, CD38, ZAP70, mutational status and B2M and found 4 significant, independent, prognostic factors ZAP70 (p=0.001), mutational status (p=0.002), B2M (p=0.003) and abnormal ratio (p=0.006). Furthermore, analysis of patients with an abnormal λ ratio showed significant correlation (Spearman's rho 0.396 p=0.01) with VH3-21, 48 and 53 usage. Elevated levels of κ and λ FLC are evident in CLL patients. An abnormal FLC ratio associated significantly to worse outcome and is independent of the established markers ZAP70, CD38 and mutational status. VH3-21, 48 and 53 are known to be indicators worse outcome in mutated patients, here we provide the first indication that an abnormal λ FLC ratio may be indicative of the VH usage. The importance of abnormal FLC ratios in CLL requires further studies as does the biological rationale for their occurrence.

C21. THE SERUM FREE LIGHT CHAIN ASSAY: AN ALTERNATIVE TO BENCE JONES PROTEIN ANALYSIS IN SCREENING FOR MONOCLONAL GAMMOPATHIES

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Background. The serum free light chain (FLC) assay is a sensitive technique for quantifying FLC. Abnormal serum FLC ratios strongly suggest monoclonal FLC production. Abnormal absolute serum FLC levels with a normal ratio are indicative of polyclonal increases in FLCs as a result of infection, inflammation or renal impairment. Recent publications demonstrate that the serum FLC assay can replace Bence Jones protein (BJP) analysis in screening for monoclonal gammopathies (MG) and other lymphoproliferative disorders (LPD). *Aim.* This prospective analysis is to confirm that the serum FLC assay is an appropriate alternative to urinary testing in screening our clinical population. *Methods.* 220 urine samples from patients evaluated for MG were subject to immunofixation (IFE). Equivalent serum samples were analyzed by electrophoresis, IFE if appropriate, and the serum FLC assay. *Results.* 17/181 BJP-ve patients had abnormal serum FLC ratios, including 12 patients who were subsequently diagnosed with MG. Of note, 2 patients diagnosed with multiple myeloma had highly abnormal ratios (#1 59.4, κ FLC - 522 mg/L, #2 14.07, κ FLC - 122 mg/L) despite their BJP-ve result. Of the remaining 5 patients, one (1) who is still under investigation, had an elevated ratio (4.89) and abnormal elevated serum FLC levels (κ FLC-640 mg/L and λ FLC - 131 mg/L), and 4 patients had a borderline ratio (>1.65/≤2.0) with no significant diagnosis. Of the 39 BJP+ve patients, 27 have confirmed diagnosis of MG/LPD, including 23 patients with abnormal serum FLC ratios. 4 patients had normal serum FLC ratios but all were diagnosed with monoclonal gammopathy of undetermined significance (MGUS) after being identified with a monoclonal protein by SPE and IFE. Finally, 48 patients had abnormal absolute levels of both κ and λ FLCs with a normal ratio, only 3 (1 BJP-ve and 2 BJP+ve) of them were subsequently diagnosed with MGUS. *Conclusions.* 12 BJP-ve patients with abnormal serum FLC ratios were diagnosed with MG demonstrating the potential for BJP analysis to miss monoclonal FLC production. Combination of the serum FLC assay and SPE identified monoclonal FLC in all BJP+ve patients diagnosed with MG/LPD. This study is clearly in agreement with recent reports supporting the replacement of BJP analysis with the serum FLC assay in diagnostic evaluation of patients with suspected MG or other LPDs. The results also highlight the importance of being aware that absolute concentrations of serum FLCs outside the normal range are abnormal results and further investigation may be appropriate after consideration of other clinical parameters.

C22. AN ABNORMAL SERUM FREE LIGHT CHAIN RATIO IS ASSOCIATED WITH HIGHER MONOCLONAL CONCENTRATION AND BONE MARROW PLASMOCYTOSIS IN A FRENCH COHORT OF PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background. In one retrospective study published by Rajkumar (Rajkumar *et al.*, Blood 2005), serum free light chains (FLC) ratio was abnormal in 33% of monoclonal gammopathy of undetermined significance (MGUS). Moreover, serum FLC ratio was identified as an independent risk factor for progression of MGUS. *Aims.* To describe the frequency of abnormal serum free light chains measurement in a French cohort of patients with MGUS and to correlate the results with the classic biological characteristics. *Methods.* We analyzed data from 246 patients with MGUS. FLC were measured during follow up consultation between January 2006 and January 2008. Demographic and laboratory data were compared using chi square tests for nominal variables and Kruskal-Wallis test for ordinal variables. *Results.* Patients were 107 men and 139 women. Median age was 66.1 years (32 – 92). Isotype was: IgG in 147 patients (59.8%), IgM in 50 patients (20.3%), IgA in 16 (6.5%) and biclonal IgG/IgA in 25 (10.2%). Median level of monoclonal protein was 12.6 g/L (4.4-28.2). A bone marrow aspiration was performed in 86 patients with IgG or IgA. Median bone marrow plasmocytosis was 1.7% (0-7.4). Free-κ light chain values ranged from 1.8 mg/L to 2300 mg/L (median, 15.85 mg/L), and free-λ light chain ranged from 2.2 mg/L to 824 mg/L (14.75 mg/L). One hundred and twenty seven patients (51.6%) had elevated levels of κ or λ FLC. The FLC ratio ranged from 0.0140 to 561 (1.04). An abnormal FLC ratio was detected in 82 (33.3%) patients. The FLC was of the same isotype as the serum monoclonal protein in all patients. We compared medical

data of patients according to FLC ratio. Patients with an abnormal ratio had higher monoclonal component concentration (median, 13.65 g/L) than patients with normal FLC ratio (12.20 g/L) (p=0.011). Similarly, the bone marrow plasmocytosis was more important in patients with abnormal FLC ratio (median, 2.50% vs 1.40%. p=0.011). There were no differences in the two groups about sex, age, hemogram, creatininemia, calcemia. According to the three factors risk models defined by Rajkumar (FLC ratio, size and type of monoclonal protein), 90 patients (36.6%) were classified in low risk group, 88 patients (35.7%) in low intermediate risk group, 59 (23.9%) in high intermediate risk group and 9 (3.8%) in high risk group. Summary/*Conclusions.* Our data confirm in a French independent cohort that approximately one third of patients with MGUS have an abnormal serum FLC ratio and that at least 70% of patients could be classified as low or low intermediate risk. These results are in accordance with those reported by Rajkumar. Moreover, we observed that abnormal κλ ratio was associated with higher monoclonal component concentration and bone marrow plasmocytosis. Further prospective studies are necessary to confirm a link between serum FLC measurement and bone marrow plasmocytosis and the predictive value of serum FLC ratio.

C23. SUGGESTED RISK-STRATIFICATION MODELS INCLUDING SERUM FREE LIGHT CHAIN RATIO FOR IMPROVED PROGNOSTICATION IN MULTIPLE MYELOMA

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Background. Serum free light chain ratio (sFLCR) was shown to provide independent prognostic information in patients with multiple myeloma (MM) and that its incorporation into the ISS improve prognostication. However, neither the best way to insert sFLCR into prognostic models nor its optimal cut-off to separate patients into different groups, have been fully validated. *Aims*. We attempted to assess the prognostic impact of different risk-stratification models incorporating sFLCR. *Methods.* 245 newly diagnosed MM patients were analyzed. sFLCs were measured by immunoassay (Freelite, The Binding Site, Birmingham, UK). The median age of the patients was 68 years, 53% were males, 31%, 30%, and 39% were in ISS stage 1, 2, and 3 respectively. The paraprotein type was IgG in 62%, IgA in 22%, LC in 14%; 59% presented κ LC monoclonality. Haemoglobin was <10 g/dL in 32%, creatinine >2 mg/dL in 16%, serum albumin <3.5 g/dL in 31%, LDH was elevated in 20%; CRP ≥4 mg/L in 51%, beta2-microglobulin (β2M) ≥5.5 mg/L in 39%, bone marrow infiltration ≥50% in 52% and 54% of patients presented extensive bone lesions. *Results.* Patients with "high" sFLCR (≥median) calculated as κ/λ or λ/κ according to light chain restriction had significantly inferior survival than the others. Disease parameters with prognostic importance were age, sFLCR, LDH, albumin, creatinine, β2M, CRP, haemoglobin, platelet counts, marrow infiltration. In multivariate analysis, only sFLCR, β2M, LDH and platelets kept their significance. Based on the former, two models were built, model A based on the presence of 0, 1, 2 or 3 of the following: β2M ≥3.5 mg/L, sFLCR ≥median, LDH above normal and model B based on the same with the addition of platelets <140×10^9/L (Table 1). *Summary/Conclusion.* We suggest that, along with sFLCR, the inclusion of LDH and platelets into prognostic models for MM patients, further contribute in the discrimination of a subgroup with very poor outcome. The optimal sFLCR cutoff for prognostication remains to be validated.

Table 1.

	Pts (%)	*3-yr DSS (%)*	*5-yrDSS (%)*	*p*
Model A				
0	24	93		<0.0001
1	34	73	64	
2	30	64	40	
3	12	0 at 28 months		
Model B				
0	22	93		<0.0001
1-2	61	75	57	
3-4	17	15		

DSS, disease specific survival

C24. FREE LIGHT CHAIN RATIO IN THE SERUM OF PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA AT DIAGNOSIS

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Background. Serum free light chains (sFLC) and their ratio constitute a useful parameter for the diagnosis, evaluation of response to treatment and prognosis of plasma cell dyscrasias, such as multiple myeloma and amyloidosis, as well as for the prediction of possible disease evolution in patients with monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. *Aims.* To evaluate the significance of FLCR in Waldenström macroglobulinemia (WM). *Methods.* sFLC were determined nephelometrically (Freelite serum free light chain assay, The Binding Site, Ltd, Birmingham, UK) in the serum of 35 patients

at diagnosis and 29 healthy individuals. FLCR was calculated either as κ to λ or as λ to κ according to the monoclonal free light chain restriction of each patient. Data were analyzed in order to detect possible correlations of sFLCR and clinical features and laboratory findings. *Results.* In healthy individuals κ sFLC ranged from 1.9 to 12.7 mg/dL (median 8.9 mg/dL) and λ from 12.7 to 37.1 mg/dL (median 20.4 mg/dL). The resulting normal median κ/λ sFLCR was 0.96 and λ/κ 7.55. 25 of the 35 patients were presenting κ light chain restriction and in them sFLCR ranged from 1 to 46.31. There was a strong positive correlation of sFLCR with the amount of the monoclonal protein as well as with bone marrow infiltration (p:0.038 and p:0.008 respectively) and an inverse one with hemoglobin (p:0.007). No correlation was observed between sFLCR and platelet count, β2 microglobulin and LDH, nor with lymphathenopathy or splenomegaly. In addition, it was observed that patients with sFLCR >5 needed immediate treatment. *Summary/Conclusions.* It seems that in WM the FLC secretion is more predictable than in multiple myeloma and correlates with the amount of the monoclonal protein and bone marrow infiltration, possibly because of the more indolent nature of the disease. In addition, sFLCR levels inversely correlate with hemoglobin. Patients with very increased sFLCR tend to need treatment sooner than the rest.

C25. SERUM FREE LIGHT CHAIN IMMUNOASSAY (FREELITE) AS AN ADJUNCT TO SPE AND IFE IN THE DETECTION OF MULTIPLE MYELOMA AND OTHER B-CELL MALIGNANCIES

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Background. Protein electrophoresis and immunofixation electrophoresis (IFE) of serum and urine are established as diagnostic aids for identifying monoclonal gammopathies. Many patient sera sent to laboratories however, are not accompanied by urine samples and recent reports suggest that the use of serum free light chain (sFLC) analysis in combination with serum protein electrophoresis (SPE) and serum IFE could eliminate the need for urinalysis. Additionally, the increased sensitivity of the serum FLC assay for detecting low levels of sFLC production may improve primary screening protocols. Thus the use of sFLC assays in combination with SPE in the initial screening for B-cell malignancies may provide an alternative to urine collection. Aim. To assess the utility of sFLC measurement in addition to SPE in the identification of patients with B-cell malignancies. *Methods.* 952 serum samples were analysed by SPE and those with abnormal bands were analysed by IFE. sFLC were measured in a retrospective manner by automated assay (Freelite, The Binding Site) using a Modular P analyser (Roche). *Results.* Of the 952 sera samples, SPE analysis identified 111 (11.6%) with abnormal patterns, indicative of a potential B-cell malignancy. IFE analysis confirmed the presence of monoclonal bands in 83 of 111 samples, and in 75 of 111, abnormal FLC ratios were detected. The remaining 841 (88.3% of all sera) samples had normal SPE patterns but among these, 58 (6.9%) were shown to have abnormal FLC ratios and IFE confirmed the presence of monoclonal bands in 24 of 58 samples. Thus, 24 samples shown to have monoclonal bands were indicated by the measurement of sFLC but not by SPE analysis. Examination of clinical records revealed that 11 of the 24 patients had a relevant clinical diagnosis (myeloma, lymphoma, leukaemia, MGUS or AL amyloid). Interestingly, 12 of 34 patients, those with normal IFE results but abnormal FLC ratios, also had relevant clinical diagnoses (myeloma, leukaemia or lymphoma). *Summary and Conclusions.* In this study, we have shown that by using conventional SPE and subsequent IFE analysis of patient sera, we detected monoclonal gammopathies in 83 of 952 sera. In addition 58 sera with abnormal sFLC ratios were identified. Of the 58 sera, 24 were found to have monoclonal bands by IFE and 11 of these had a relevant diagnosis. Thirty four of the 58 sera had normal IFE results, but 12 of the 34 also had a positive diagnosis for a lymphoproliferative disorder. Thus, a total of 23 extra diagnoses would have been made at the time of the initial presentation if sFLC analysis had been included in the screening protocol.

FREE LIGHT CHAINS AND DISEASE MONITORING

D26. CASE REPORT. SHIFT IN SECRETION FROM INTACT IMMUNOGLOBULIN TO FREE LIGHT CHAINS IN MULTIPLE MYELOMA AT RELAPSE: EARLY DETECTION BY FREE LIGHT CHAIN ASSAY

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Introduction. Multiple myeloma (MM) is a malignant disorder of the B-type lymphocytes. It is characterized by monoclonal plasma cells in the bone marrow. These monoclonal cells produce intact immunoglobulins and / or free light chains (FLC) in most cases. The quantitative measurement of FLC is a valuable tool for assessing the course of the disease, the response to the specific treatment, and the prognosis. *Materials and Methods.* The differentiation of monoclonal proteins was performed by serum electrophoresis, serum immunofixation (IF), (Sebia, France); quantitative measurement of FLC in serum by 'Freelite Human kappa/Lambda' (κ/λ) (The Binding Site, UK) and IgA using 'Immunoglobulin A Reagent' for IMMAGE (Beckman Coulter, Ireland). *Case and Results.* A 65 year old male presented himself in 2007 suffering from severe back pain. Initial laboratory revealed: anemia (8.5 g/dL), hypercalciemia (4.2 mmol/L, hyperviscosity (3.5 mPas), renal insufficiency, M-gradient, specified as IgAλ (IF), κ/λ ratio 0.14. MM Stage IIIB was verified by the recommended methods. IgA decreased in the course of three months under therapy with idarubicin/dexamethasone (ID), bendamustine/bortezomib/dexamethasone (BBD), and revlimid/doxorubicin/dexamethasone (RAD) from 70.1 to 0.29 g/L. The FLCλ concentration initially decreased from 109 to <4 mg/L, then increased again several months later to 78000 mg/L (Figure 1).

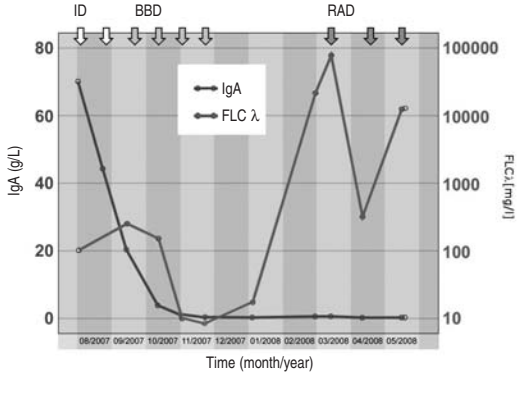

Figure 1.

Discussion. The switch in secretion from intact immunoglobulin to FLC in MM could be caused by mutations in MM-cells after cytostatic therapy. The exclusive raise of FLCκ during relapse may be explained by this mechanism. Most remarkably, the relapse was diagnosed by the routinely performed FLC quantification in the course of the disease management. It would never have been found by solely measuring IgA. This puts an emphasis on the importance of the serum FLC assay when monitoring MM-patients, especially following therapy with immunomodulators.

D27. FREE LIGHT CHAIN ANALYSIS: IS IT USEFUL TO MONITOR PATIENTS UNDERGOING HEMATOLOGICAL STEM CELL THERAPY FOR MULTIPLE MYELOMA? AN ANALYSIS OF "REAL LIFE" DATA

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Rationale. Free light chain analysis (FLCA) is relatively new, highly specific and sensitive laboratory parameter to be used for monitoring MM patients, even in the setting of "non-secretory" disease. Recently, it became the basis of a broadened new definition of remission depth (called stringent CR [sCR]) characterized by a normal serum free light chain ratio. We retrospectively analyzed FLCA results in our transplanted MM patients with regard to early diagnosis of relapse, assessment of treatment efficiency and its impact on clinical decision making, compared to standard M-protein measurements (MPM). Because of rapidly growing therapeutic alternatives in MM treatment the questions concerning estimation of remission depth and of early relapse diagnosis are becoming ever more important. So FLCA might open the way to better tailor subsequent therapies post transplant. *Patient characteristics.* Between March 2005 (when FLCA became available at our institution) and November 2007, 42 patients were either alive after, or undergoing hematological stem cell therapy (HSCT) for MM. This subgroup was identified out of 69 patients which were treated by either autologous (92 procedures) or allogeneic (19 procedures) HSCT since 1989. For 38 (14 females, 24 males) of this patients at least two consecutive FLCA were documented and they were thus considered to be evaluable. Paraprotein subtypes were IgG (15 patients), IgA (11 patients), Bence Jones (8 patients), IgD (2 patients), non-secretory (1 patient) and unknown (1 patient). Light chain subtypes were κ (22 patients), λ (15 patients) and unknown (1 patient). Treatments applied were autologous HSCT (33 patients), allogeneic HSCT (2 patients) or both (3 patients). Seven patients were pretreated with "novel agents" (thalidomide, bortezomib), while in 31 classical chemotherapeutic induction therapies were applied. Median time from first diagnosis to last HSCT was 11 months (range, 4-108 months). HSCT was integrated in MM first line (22 patients), 2nd line (14 patients), or 3rd line treatments (2 patients). *Results.* A total of 210 FLCA were performed with a median number of 8 analyses per patient (2-14). With the help of FLCA κ could be identified

as a tumor marker in one patient with a nonsecretory MM by MPM. sCR was achieved in 13 patients on 25 distinct occasions, but was not durable in any of these cases. Biochemical relapse (BR) was diagnosed in 13 patients by MPM, 17 patients by serum FLCA and 14 patients by urine FLCA. While the median time lag to BR was 686 days by classical MPM (178-1897), FLCA proofed to be much more sensitive with a median time to BR of 191 days (0-925) for serum measurements and 230 days for (0-1749) urine analysis. This eludes to a median advancement of relapse diagnosis of 495 days post HSCT, thus possibly opening the way to early implementation of modern disease modifying treatment approaches. An update of data, as well as pitfalls of FLCA and an outlook for prospective strategies will be discussed.

D28. IGD MULTIPLE MYELOMA: CASE REPORT

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Background. IgD multiple myeloma (MM) represents 1-2% of all MM cases having often an aggressive course. Predominates in men, affecting younger people (55-60) and more often features renal failure, hypercalcemia, amyloidosis, extramedullary plasmacytomas and lymphadenopathy. *Case report.* We report the case of a 62 year-old woman who was admitted for low back pain, weight loss and fever. The MRI revealed metastasic lesions in the hole vertebral spine. She developed chronic renal failure, which precised hemodialysis 3 times a week. A clavicle biopsy revealed plasmacytoma. A bone marrow biopsy showed 2% of plasma cells, but atypical cells were no found. Serum electrophoresis revealed an IgD λ monoclonal protein. Hypogammaglobulinaemia was present as well as serum free λ chains (10200 mg/dL) and total IgD level of 670.34 mg/dL. Bence-Jones protein was positive. The diagnosis of IgD λ MM was made, stage IIIB by Salmon and Durie criteria. Our patient received polychemotherapy with vincristine/ adriamicine/dexamethasone (VAD) regimen. In re-evaluation after the 3 cycles, the total IgD level reduced to 285 mg/dL, serum free light chain λ level to 4720 mg/dL and IgD was still detectable by immunofixation We decided to change treatment to bortezomib/dexamethasone, with a good response, IgD M protein was not detectable by immunofixation and reduction of the free light chain to 46.10 after 3 cycles of treatment. *Conclusions.* The follow up of our patient is being made by monitoring of free light chain levels. This test is useful for the evaluation of response to chemotherapy and the early detection of relapses in this kind of myelomas.

D29. PATIENT WITH MULTIPLE MYELOMA, IN EXTRAMEDULAR RELAPSE ASSOCIATED TO LIGHT CHAINS EXCRETION AND DISAPPEARING OF INTACT IMMUNOGLOBULIN "LIGHT CHAIN ESCAPE"

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This case study presents a 64 year old man with multiple myeloma with light chain escape at disease relapse. At diagnosis the patient had an IgAλ monoclonal protein (4,67 g/dL), Bence Jones proteinuria (1,59 g/day) and abnormal FISH analysis (13q deletion). Following treatment with chemotherapy (VBMCP/VBAD: vincristine, BCNU, cyclophosphamide, melphalan, prednisone/vincristine, BCNU, doxorubicin, dexamethasone) the patient demonstrated an initial response followed by subsequent progression at the 6th cycle associated with leukaemic plasma cells. At this time point the IgAλ M-protein had decreased, (0,4 g/dL), Bence Jones proteinuria had increased (4,12 g/day) and cytogenetics revealed a complex karyotype with multiple numeric and structural chromosomal alterations in 75% of metaphases. Subsequently the patient commenced treatment on bortezomib, liposomal doxorubicin and dexamethasone (2 cycles) and achieved a VGPR with normal cytogenetics and FISH.

Ten weeks following high dose melphalan and peripheral blood stem cell transplant (PBSC), disease progression was associated with the development of cutaneous plasmacytomas and leukaemic plasma cells. At this time point the serum M-protein was absent, the serum free light chain (sFLC) ratio was highly abnormal with detectable Bence Jones proteinuria (3.39 g/day). The patient commenced treatment with VDD (3 cycles) followed by lenalidomide, dexamethasone, doxil and bortezomib (3 cycles). Six months later, disease relapse occurred rapidly and was associated with a highly abnormal sFLC ratio that remained persistently abnormal and an undetectable serum M-protein. No urine was supplied for Bence-Jones analysis during this time.

This case study illustrates a patient with intact immunoglobulin multiple myeloma with disease relapse associated with light chain escape.

Light chain escape is characterized by rising free light chain concentrations at relapse with no associated increase in intact immunoglobulin. Light chain escape may be associated with extramedullary disease, plasmablastic morphology, complex karyotypes and poor prognosis. Periodic monitoring patients with intact immunoglobulin multiple myeloma with the serum free light chain assay is recommended for detection of light chain escape.

Table 1.

Patients details

Stage at diagnosis	IIIA
Serum M-protein	IgAλ, 4,67 g/dL
Urine M-protein	λ Bence Jones protein, 1,59 g/day
LDH	Normal
β2M	10 mg/L
Cytogenetics	46XY. Del 13q FISH
Treatment 1	VBMCP/VBAD (Vincristine, BCNU, Cyclophosphamide, Melphalan, Prednisone)/(Vincristine, BCNU, Doxorubicin, Dexamethasone)x6
Response	Progression with a serum M-protein 0.48 g/dL, urinay Bence Jones protein 4.12 g/day, and complex karyotype
Treatment 2	VDD (Velcade, Doxil and Dexamethasone)x2
Response	Very good partial response
Treatment 3	PBSC (Peripheral blood stem cell transplantation)
Response	Progression at 2.5 months with cutaneous plasmacytoma, leukaemic plasma cells, undetectable serum M-protein by immunofixation, urinary Bence Jones protein 3.4 g/day. Lambda serum free light chains (FLC) >1000 mg/L at disease progression, with further increases following treatment.
Treatment 4	VDD (Velcade, Doxil and Dexamethasone)x3 LblipDD (lenalidomide bortezomib, doxil, dexamethasone: Ruckser *et al*., 2007)x3
Survival	6 months after PBSC

D30. SERUM IMMUNOGLOBULIN FREE LIGHT CHAINS FOR MONITORING PATIENTS WITH OLIGOSECRETORY MYELOMA. A CASE STUDY

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This case study presents a 76-year old woman with oligoscretory myeloma. The patient presented with unexplained tiredness. Physical examination was unremarkable but laboratory tests revealed she was anaemic (Hb = 7,1 g/dL) and had renal impairment (Cr = 2,4 mg/dL). After further investigations (Table 1) the patient was diagnosed with oligosecretory λ light chain only myeloma in April 2003. The patient underwent conventional therapy with melphalan - prednisone (10 cycles) between April 2004 and June 2004 achieving a complete response (CR).
In May 2006 the patient remained in CR. However, serum free light chain (sFLC) analysis revealed a highly abnormal κ/λ ratio (κFLC = 67,9 mg/L, λ =3,4 mg/L, κ/λ = 19,9). The patient became symptomatic in January 2007 with incapacitating lower back pain and nausea. Bone marrow examination revealed a 50% infiltration of plasma cells and radiography identified vertebral lytic lesions (D12 and L1). Laboratory investigations revealed hypogammaglobulinaemia and an abnormal sFLC κ/λ ratio (κFLC = 102 mg/L, λFLC =32 mg/L, κ/λ ratio = 3,18). Subsequently the patient commenced treatment with melphalan, prednisone and bortezomib (8 cycles) with monthly cyclophosphamide. By December 2007 she had achieved a stringent complete response (sFLC κ/λ = 1,12, no detectable M-protein by immunofixation and bone marrow plasma cells = 1%). However, in January 2008, in spite of the CR, an abnormal κ/λ ratio was detected (κ/λ= 4,09), which became increasingly abnormal over the following 6 months. In June 2008 the sFLC κ/λ = 103,3 with no detectable M-protein by immunofixation. This case study illustrates that monitoring light chain only multiple myeloma with the serum free light chain assay identifies relapse of disease earlier than electrophoretic techniques. Although detection of biochemical relapse does not generally require immediate treatment, patient management can be modified to increase the frequency of monitoring and appropriate future treatment to be planned.

Table 1.

Patients details

Presentation

Diagnosis	Oligosecretory λ light chain only multiple myeloma (almost non-secretory)
Immunoglobulins	IgG = 264 mg/dL; IgA = 11 mg/dL; IgM = 5 mg/dL.
Serum protein electrophoresis	Hypogammaglobulinaemia. No visible M-protein.
Urine protein electrophoresis	No visible M-protein by UPE, λ BJP detectable by IFE
Biochemistry	Albumin = 3,7 mg/dL; creatinine = 2,4 mg/dL; calcium = 12,5 mg/dL
Lytic lesions	None detected
Bone marrow plasma cells	80%
Cytogenetics	t(11;14) FISH
Treatment 1	Melphalan, prednisone (10 cycles)
Response	Complete response
Treatment 2	Melphalan, prednisone, bortezomib (8 cycles) and monthly cyclophosphamide
Response	Stringent complete response
Stage today	Patient in CR (negative s/uIFE < 5% bone marrow plasma cells, no soft tissue plasmacytomas). From January 2008 to June 2008 the sFLC κ/λ ratio has progressively increased (from 4,09 to 103,3)

D31. INCIDENCE OF LIGHT CHAIN ESCAPE IN UK MRC MYELOMA VII TRIAL

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Introduction. For some intact immunoglobulin multiple myeloma (MM) patients in remission, relapse is accompanied by a marked rise in monoclonal free light chains (FLCs) with no associated increase in intact immunoglobulin concentrations - a phenomenon termed "light chain escape (LCE)". Recent case reports have suggested LCE might be more prevalent with modern chemotherapy and detected earlier by serum FLC analyses. *Objectives.* To quantify the frequency of LCE in MM patients recruited to the UK MRC Myeloma VII trial using urine or serum FLC analysis. To compare the incidence of LCE in patients with IgG vs IgA paraproteins and on intensive vs non-intensive chemotherapy regimens. *Method.* Stored sera from the first 58 IgG and 60 IgA patients recruited to the trial were utilised. There were sufficient frozen sera and complete clinical data for 36/60 IgA and 30/58 IgG patients. sFLC analysis (Freelite, The Binding Site) was performed using a BNII nephelometer on sera from presentation, maximum response and relapse time points and compared with recorded urine Bence-Jones protein (UBJP: light chain/creatinine ratio) and serum intact immunoglobulin measurements. Results were classified as "true" LCE (rising sFLC concentrations with stable/falling intact immunoglobulin concentrations) or "partial" LCE (the increase in involved serum FLC concentration was at least 40% greater than the increase in monoclonal intact immunoglobulin concentration). *Results.* Comparison of serum FLCs with UBJP: urine samples were not provided for all time points for 7/66 (11%) patients and 1 of these 7 was excluded due to lack of samples. In 18/65 (28%) patients, UBJP and sFLC results correlated. For 47/65 (72%) patients, UBJP was negative at one or more time points and serum FLCs provided additional monitoring information in 43/47 (91%) patients, including 4 with undetectable UBJP at all time points. Comparison of serum FLCs with monoclonal intact immunoglobulin: for 48/66 (73%) patients, changes in the involved serum FLC concentration correlated with changes in the monoclonal intact immunoglobulin concentration. For 7/66 patients, serum FLCs provided no useful monitoring information, but the ratio was abnormal at presentation in 5/7. LCE was detected in 11/66 (17%) patients. Incidence of light chain escape: IgA patients showed 8% (3/36) with true LCE and 11% (4/36) with partial LCE. For IgG patients the figures were 3% (1/30) and 10% (3/30) respectively. Of patients showing true LCE, 4/4 had received non-intensive treatment and of patients with partial LCE, 3/7 had been treated non-intensively. For all 11 patients showing some form of LCE, this was corroborated by the urine results in 5/11. For 6/11 the amounts of FLC in the urine were insufficient for consistent analysis. *Conclusions.* The results from this study support the use of the serum FLC assay for monitoring intact immunoglobulin myeloma patients to detect LCE. These preliminary findings do not indicate any greater frequency of LCE with intensive chemotherapy but suggest that it might be more apparent with serum FLC analysis compared with UBJP analysis. True LCE was seen in 3/36 IgA MM patients and 1/30 IgG MM patients.

D32. LIGHT CHAIN ESCAPE FROM PARAPROTEINAEMIA – MYTH OR REALITY?

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Background. There is well established evidence that myeloma patients treated aggressively with chemotherapy can eventually relapse with a more lymphomatous process that fails to secrete immunoglobulin. Recently this has occasionally been observed in patients treated with some of the newer forms of therapy, such as thalidomide or bortezomib.[1] Whole paraprotein estimation as a means of monitoring disease can thus become misleading, whereas in many cases light chain secretion continues. *Aims.* We aimed to identify a number of patients who demonstrated light chain escape from paraproteinaemia (LEPP) by looking at serum free light chain analysis and correlating this with clinical outcomes and paraprotein estimation in a cohort of myeloma patients seen in our institution over a two year period. *Methods.* Laboratory records over a two year period were used to identify myeloma patients and clinical records were then identified to establish responses and relapses. *Results.* A total of 806 SFLC analyses were performed at our institution over a two year period from April 2006 to April 2008 on 321 myeloma patients. The number of tests on an individual patient ranged from 1 to 19 (median 4). The median age was 66 years and there were 185 males and 136 females. In general κ:λ ratios (maximum observed was over 13,000) were higher than λ:κ ratios (maximum observed was just under 900), although the patients were almost exactly equally divided between κ and λ expressing myelomas. Of those patients (n=66, 20%) who had clinically useful SFLC follow up as opposed to just a single test, 44% achieved at some time at least a partial remission (>50% reduction in SFLC ratio) at some time. However 16% of these showed evidence of relapse at a later date (>50% increase in SFLC ratio) suggesting relapsed disease during follow up. 4 patients who presented with a measurable paraprotein and who were not being monitored by SFLC, but only paraprotein estimation had overt clinical relapses. All of these patients had been treated for an extended time with thalidomide and/or bortezomib. In each case, SFLC ratios were elevated at the time of relapse. *Summary/Conclusions.* Patients with myeloma may relapse with the loss of heavy chain immunoglobulin expression, presumably due to de-differentiation of the tumour cells. This has been thought to be more common in patients treated with very high dose chemotherapy regimens but now it appears that patients treated with thalidomide or bortezomib may also exhibit this phenomenon. More data is required to establish the frequency of this occurrence which may have significant impact on the way patients are monitored in plateau phase.

D33. EARLY PREDICTIVE VALUE OF SERUM FREE LIGHT CHAINS PROFILE IN RELAPSED OF MULTIPLE MYELOMA AFTER AUTOLOGOUS TRANSPLANT

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Background. The introduction of serum free light chain assays Freelite, a quantitative assay which can measure serum concentration of them, could be a biomarker to predict the early relapse in multiple myeloma (MM) patients in apparently complete response. Free light chains (FLC) are small proteins that pass easily through the pores in the glomeruli, entering the proximal tubule, where they are reabsorbed and metabolised. In patients with a low tumour burden, the amount of FLC produced can remain below this reabsorption capacity and so have no detectable urinary FLC. However, serum levels of FLC continue to rise and to become a good biomarker to detect an early progression. *Aims of study.* To determine the predictive value of serum FLC concentration in detect early relapsed in MM patients in complete response after autologous stem cell transplant. *Patients and methods.* Observational, analytical study performed in 20 MM patients included in an autologous stem cell transplant program in 2004-2006 in one center. All patients receiving induction therapy with alternate VBCMP/VBAD (x4), mobilization and collection of $CD34^+$ cells was performed after the second VBAD therapy and conditioning with high doses of Melphalan. The infusion of $CD34^+$ cells was performed with a mean of cells In all cases were recollected serum samples in days 0,+4, +7, +21, +30, +60 y +90 and stored frozen at -80ºC. We have analyzed serum FLC in a nephelometer system following the manufacturer instructions. We have compare the increase of FLC according M-component subtype, number of $CD34^+$ cells administered, time to immune reconstitution, administration of coadyuvant AM3-glycopeptical therapy, infections in early postrasplant period, maintenance therapy and time free relapsed survival. Descriptive statistical analysis, ANOVA comparative test and Kaplan-Meier survival study was performed. *Results.* 20 patients, 47.0 % females, mean age 63.2 y (38-72), IgG (46.1%), IgA (15.4%), light chains (23.0%), non secretory (7.7%), mean $CD34^+$ cells infused 5.39×10^6/kg (2.24-18.8), time to immune reconstitution 15-17 days, ratio of κ to λ at baseline 57.0 (CI95% 0.8,20.3), after 4 days: 37.3(CI95% 0.1,3.2), +7: 1.85(CI95% 1,3), +21 2.37 (CI95% 0.4,2.0), +30 0.87 (CI95% 0.2,0.9), +60 0.4 (0.2, 2.1) and +90 1.26 (CI95% 0.3, 2.9). Mean relapsed free survival 27.6 m (CI95% 9,55), OS 59.0 m (CI95% 27,76), 6 patients have dead for progression. At day +21 correlation κ/λ ratio and RFS was observed. *Comments.* The early determination of the ratio free serum κ/λ chains could be a good biomarker to predict RFS and OS in patients with MM in complete response after therapy.

FREE LIGHT CHAINS AND RENAL ISSUES

E34. IMMUNOGLOBULIN FREE LIGHT CHAINS IN SITU IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Background. Immunoglobulin free light chains (FLC) are primarily cleared from glomerular ultrafiltrate by kidney proximal tubule epithelial cells (PTEC). Monoclonal FLC have a profound pro-inflammatory effect on PTEC as well as co-precipitating with Tamm-Horsfall protein (THP) in distal tubules as casts. Polyclonal FLC have not been studied in the kidney in situ, but are potent activators of some non-renal cells. Recent studies have shown that serum and urine polyclonal FLC levels progressively increase with worsening CKD. *Aims.* To assess the presence of FLC in the kidney in CKD. *Methods.* We analysed renal biopsy specimens from 9 patients with established CKD secondary to ischaemic nephropathy. Simultaneous multi-colour immunofluorescence staining was performed using directly conjugated antibodies against κ-FLC, λ-FLC and Tamm-Horsfall protein. Images were acquired and examined using a confocal laser scanning microscope. We then examined periodic acid methenamine-silver (PAMS) stained sections from these 9 patients and 23 others with ischaemic nephropathy. Using image analysis software, the chronic damage index (CDI) for each biopsy was calculated as the ratio of total scarred area to total cortical area. Cast numbers were counted using the same software. A cast index was then calculated as the ratio of tubules containing casts in each biopsy to the total number of tubules visible. *Results.* Both κ and λ FLC were detected within tubular cells of all 9 patients. Casts stained positive for κ and λ FLC as well as Tamm-Horsfall protein (THP). There were no casts in tubules showing intracellular FLC, indicating proximal tubular uptake. In tubules containing casts, there was no intracellular FLC, indicating they were distal tubules. Staining for intact immunoglobulins was negative in all 9 biopsies. Cast numbers negatively correlated with CD34 expression, indicating that cast precipitation is associated with an ischaemic micro-environment. There were non-statistically significant relationships with ACR, FLC positive tubules and macrophage infiltration. *Conclusions.* We propose that as CKD progresses and nephron mass declines remaining nephrons are exposed to increasing concentrations of FLC. However, the potential for PTEC uptake of FLC may decrease as renal injury progresses. The increased delivery of FLC to the distal nephron may then promote cast formation. Further work is in progress to increase the size of the study and better dissect the in situ relationships.

E35. THE DIFFERENTIAL EVOLUTION OF RENAL SCARRING IN MYELOMA KIDNEY DESPITE EARLY REDUCTIONS IN FREE LIGHT CHAINS

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Background. Acute renal failure (ARF) in multiple myeloma (MM) is most frequently caused by cast nephropathy. Most patients do not recover renal function in this setting. However the *in situ* processes that lead to these poor outcomes are not known. Recent studies have indicated that chemotherapy combined with high cut-off haemodialysis (HCO-HD) may improve renal recovery rates. *Aims*. We report 4 cases of ARF treated with chemotherapy and FLC removal by HCO-HD. Whilst significant reductions in serum FLC levels were achieved, all 4 remained dialysis dependent at 6 weeks. They were then assessed by further renal biopsy. *Methods.* All patients had a confirmed diagnosis of MM and cast nephropathy before chemotherapy and HCO-HD were commenced. Dialysis was performed using a dialyser with a molecular weight cut-off of up to 60 kD. Two dialysers were used in series to increase both effective surface area and solute clearance by ultrafiltration. Dialysis was performed for 6-8 hours per session, up to 5 times during the first week, alternate days in the second week and then thrice weekly, until the serum FLC concentration was maintained below 500 mg/L. *Results.* Patient characteristics and outcomes are shown in the Table 1.

Table 1.

Patient	*1*	*2*	*3*	*4*
FLC κ/λ	κ	λ	κ	κ
FLC at presentation (mg/L)	15,700	9,918	5,870	7,675
Chemotherapy	TD	TD	CTD	TD, then B
Average FLC reduction per dialysis	74%	73%	63%	80%
Time to FLC <500 mg/L (days)	33	47	61	96
Biopsy 1: intestinal fibrosis	moderate	none	moderate	mild
Biopsy 2: casts present (Y/N) and intestinal fibrosis	No, moderate	Yes, severe	Yes, moderate	Yes, moderate
eGFR at dialysis independence (mL/min/1.73 m^2)	8	20	N/A	N/A

The biopsies showed differential progression of chronic damage from the first biopsy to the second, across the group: patient 1 showed moderate scarring on the biopsy with no progression of scarring by biopsy; patient 2 sustained a progression of scarring from 0% to 50% despite a rapid and sustained fall in FLC to <10% of starting level at 6 weeks; patients 3 and 4 showed intermediate levels of progression. *Conclusions.* Although chemotherapy combined with high molecular weight cut-off haemodialysis effectively reduces the FLC burden on the kidneys, the differential renal toxicity of light chain clones can promote rapid renal scarring. This identifies the importance of rapid reductions of FLC in myeloma and acute renal failure.

E36. ACUTE RENAL FAILURE BECAUSE OF LIGHT CHAIN DEPOSITION DISEASE AND ITS SUCCESSFUL COMBINED TREATMENT WITH CHEMOTHERAPY AND DIALYSIS WITH HIGH FLUX MEMBRANE

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Background. A 45-year-old man presented with terminal renal failure with no history of prior renal disease. High monoclonal IgG (38 g/L) λ (1050 mg/L) was detected by means of serum protein electrophoresis. Renal biopsy showed light chain deposition disease. Bone marrow biopsy revealed multiple myeloma (80% plasma cell infiltration). *Aims.* With beginning of chemotherapy the patient was treated with daily dialysis with high flux membrane. Serum levels of IgG λ fell immediately and renal parameters stabilized at levels of serum creatinine 1.7 mg/dL. *Methods.* Because of uremic syndrome dialysis had to be started after position of a dual-lumen cuffed tunnelled catheter. With beginning of chemotherapy -Z-Dex (idarubicin 10 mg/m^2/d for 4d and dexamethasone 40 mg/d for the same 4 d; each cycle was repeated at day 21) the patient was treated with daily dialysis for 4.5h with high flux membrane for 2 weeks without Sundays. The CT scan described bone lesions in the vertebral column and the pelvis, so we gave ibandronic acid 2 mg. *Results.* Hemodialysis could be stopped after 2,5 month of treatment including 18 times with high flux membrane. In the follow-up the patient received five cycles of Z-Dex with hematopoietic growth factors. We achieved a partial response and harvested stem cells for further high dose therapy. The re-biopsy of the bone marrow showed plasma cell infiltration <5%, IgG was 13 g/L, λ 4,30 mg/L. *Summary/Conclusions.* Daily dialysis was well tolerated, as side effect we had to substitute albumin 50% 200 mg for five times. The treatment was complicated by *E.coli* sepsis because of an infection of the tunnel catheter and one episode of chickenpox infection. Because of psychiatric problems the patient was not suited for high dose therapy. We started bortezomib/dexamethasone (2nd line therapy) for 4 cycles. After 2 month pause the FLC increased and we decided to start with lenalidomide/dexamethasone.

E37. QUANTITATIVE ASSESSMENT OF SERUM AND URINARY POLYCLONAL FREE LIGHT CHAINS IN PATIENTS WITH CHRONIC KIDNEY DISEASE.

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Background and objectives. Monoclonal free light chains (FLCs) frequently cause kidney disease in patients with plasma cell dyscrasias. Polyclonal FLCs, however, have not been assessed in patients with chronic kidney disease (CKD) yet could potentially play an important pathological role. This study describes for the first time polyclonal FLCs in patients with CKD. *Design, setting, participants, and measurements.* A sensitive, quantitative immunoassay was used to analyse serum and urinary polyclonal FLCs in 688 patients with CKD of various aetiologies. *Results.* Serum kappa (κ) and lambda (λ) FLC concentrations increased progressively with CKD stage (both: $p<0.001$) and strongly correlated with markers of renal function, including cystatin-C (κ; R=0.8 ($p<0.01$) and λ; R=0.79 ($p<0.01$)). Urinary FLC concentrations varied significantly between disease groups (κ, $p<0.001$; λ, $p<0.005$) and also rose significantly with increasing CKD stage (both FLCs; $p<0.0001$). Urinary FLC concentrations were positively correlated with their corresponding serum concentration (κ, R=0.63; λ, R=0.65; both $p<0.001$) and urinary albumin creatinine ratio (κ, R=0.58; λ, R=0.65, both $p<0.001$). The proportion of patients with abnormally high urinary FLC concentrations rose with both the CKD stage and the severity of albuminuria. In conclusion, this study demonstrates significant abnormalities of serum and urinary polyclonal FLCs in patients with CKD. This data provides the basis for studies assessing the contribution of polyclonal FLCs to progressive renal injury and systemic inflammation in patients with kidney disease.

E38. CHRONIC KIDNEY DISEASE PATIENTS AND RENAL TRANSPLANT RECIPIENTS HAVE A HIGH PREVALENT RATE OF MGUS

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Purpose. This study assessed the prevalence of monoclonal proteins in patients with chronic kidney disease and renal transplant recipients. *Methods.* Serum protein electrophoresis and immunofixation were used to identifying intact monoclonal gammopathy (M protein) and a highly sensitive immunoassay (Freelite) to quantitatively assess serum free light chains (FLC). We analysed (i) a population of patients with CKD (Group 1; n=289) (ii) a second, comparative CKD cohort (Group 2; n=306) and (iii) a renal transplant follow-up cohort (n=461). *Results.* There was no statistical difference between group 1 and 2 for any demographic or incident data. The M protein prevalence in these groups was 10.6%, indicating an MGUS prevalence over 3x that of an age matched population. 4.9% had an isolated monoclonal FLC. There was a non-significant increase in prevalence with age ($p<0.18$), but not with stage of CKD. In the renal transplant recipient cohort the MGUS prevalence was 8.2%. This was nearly 10 fold that seen in an age matched normal cohort. There was no significant relationship between the age of the renal transplant and the prevalence of MGUS. *Conclusions.* Patients with CKD have a high age-related prevalent rate of MGUS. The MGUS prevalence in renal transplant recipients is very high, over three fold that seen in native CKD. These findings may have important implications for the pathogenesis and natural history of patients with CKD and chronic allograft nephropathy.

E39. SERUM FREE LIGHT CHAIN MEASUREMENT AIDS THE DIAGNOSIS OF MYELOMA IN PATIENTS WITH ACUTE RENAL FAILURE

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Monoclonal free light chains (FLCs) frequently cause rapidly progressive renal failure in patients with multiple myeloma. Immunoassays which provide quantitative measurement of FLCs in serum, have now been adopted into screening algorithms for multiple myeloma and other lymphoproliferative disorders. The assays indicate monoclonal FLC production by the presence of an abnormal κ to λ FLC ratio. Previous work, however, has demonstrated that the FLC ratio can be increased above normal in patients with renal failure, but with no other evidence of monoclonal proteins. This study evaluated the diagnostic sensitivity and specificity of the immunoassays in patients with acute renal failure. Sera from 142 patients with dialysis-dependent acute renal failure were assessed by serum protein electrophoresis, FLC immunoassays and immunofixation electrophoresis. The sensitivity and specificity of utilizing the normal range for the FLC ratio, 0.26-1.65, was compared with a modified range to account for renal failure (0.3-3.0). Forty one patients had a clinical diagnosis of multiple myeloma; all of these patients had abnormal serum FLC ratios. Receiver operating characteristic (ROC) curve analysis showed the modified FLC ratio range increased the specificity (from 93% to 98%) with no loss of sensitivity. Monoclonal FLCs were identified in the urine from 23 of 24 patients assessed; the patient who had no Bence-Jones protein identified had a renal biopsy result of cast nephropathy and 1800 mg/L of FLCs identified in the serum. In conclusion, measurement of serum FLC concentrations and calculation of the serum k/l ratio is a convenient, sensitive and specific method for identifying monoclonal FLC production in patients with multiple myeloma and acute renal failure. Rapid diagnosis in these patients will allow early initiation of disease specific treatment, such as dexamethasone.

E40. ABOUT A CASE OF LAMBDA FREE LIGHT CHAIN MULTIPLE MYELOMA TREATED BY BORTEZOMIB CHEMOTHERAPY AND LONG HEMODIALYSIS ON POLYMETHYLMETHACRYLATE MEMBRANE

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Background. A 40-year old man is admitted to the Nephrology unit for the treatment of a free light chains (FLC) lambda (λ) multiple myeloma (MM) revealed by bone pain and acute renal failure. Bone marrow showed a 55% infiltration with dysmorphic plasma cells, and beta 2 microglobulin was at 23.3 mg/L. FLC measurement by nephelometry was at 8650 mg/L for λ and 33 mg/L for κ (ratio=0.004). Major proteinuria (7 g/24h essentially composed of λ FLC) confirmed the diagnosis of nephropathy with tubular failure. *Aims*. The aim of the study was to estimate if FLC removal was more efficient by combination of hemodialysis with chemotherapy, and if it is helpful for a better renal function recovery. *Methods.* Combined chemotherapy (CT) comprised 2 courses by the time of our study: bortezomib (1.3 mg/m^2) was administrated by days 1, 4, 8, 11, and repeated 3 weeks later. In addition the patient received 40 mg dexamethasone the first 4 days. A 4 hour hemodialysis (HD) was performed daily, using polymethylmethacrylate (PMMA) membrane (BKF 1-6), which is known to lead to a significant reduction of FLC concentration, not only by permeation, but also absorbtion, while avoiding albumin loss. FLC determinations were performed at the beginning, the end and 1 hour after HD. The second course was similar to the first one, except for HD which was performed only before bortezomib administration. *Results.* The decrease of FLC L observed in this patient is significantly due to bortezomib treatment, especially on D4. During the second course, before D4 bortezomib administration, HD permitted a removal of 23% of FLC L only, and the initial rate at D1 is found again. We can even see an increase of FLC concentration during HD. On the other hand, FLC decreased regularly and significantly during the following days HD. During the 2 courses we observe an increase before D11 and at this time HD seems to be of interest to reduce FLC concentrations. After D11, when FLC is lower, the removal by PMMA membrane seems to be efficient. During the first course, when associated chemotherapy and daily HD were performed, we observe a removal of 91% of FLC L from the beginning to the end of HD. During the second course, from the beginning of the first HD to the end of the last HD (4 days after D11), FLC L removal was 94%. The patient does not recover from renal dysfunction, which necessitates 2 HD per week, but his proteinuria has dropped to less than 1 g/24h, with L FLC and tubular proteins. *Conclusions*. This study shows that the association CT + HD is probably more efficient for FLC removal than CT alone. A randomized clinical trial is necessary for evaluating the usefulness of HD for the recovery of renal function.

E41. EXTENSIVE USE OF SERUM FREE LIGHT CHAIN TEST IN PATIENTS WITH NEPHROPATHY INCREASES THE DIAGNOSIS NUMBER OF AL AMYLOIDOSIS AND LIGHT CHAIN DEPOSITION DISEASE

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Background. A diagnostic process toward AL amyloidosis and light chain deposition disease (AAL/LCDD) is activated every time in patients with nephropathy a monoclonal protein is documented. Because of its higher sensitivity, the addition of sFLC test to routine SPE should result in an increase of the diagnosis number of AAL and LCDD in patients with nephropathy. *Aims*. We compared the frequency of AAL/LCDD diagnosis before and after the addition of sFLC test (January 2006). *Methods.* All the AAL/LCDD diagnosis made between 1992-2005 (analyzed in two years periods) and between 2006-2008/5 were retrieved from the electronic archive of our Department. Starting on January 2006, sFLC search was usually made in patients with: proteinuria w/wo nephrotic syndrome and renal failure or in presence of AAL suggestive symptoms. *Results.* In the period 1992-2005 AAL diagnosis was made in 25 patients (1 every 6.7 months; 5/1000 admissions) and LCDD diagnosis was made in 4 patients (1 every 42 months; 0.8/1000 admissions). In the period 2006-2008/5 AAL diagnosis was made in 10 patients (1 every 2.9 months; 13/1000 admissions) and LCDD diagnosis was made in 6 patients (1 every 4.8 months; 8/1000 admissions). *Summary/Conclusions*. In patients with nephropathy, the addition of sFLC test to routine SPE results in an increase of the diagnosis number of AAL and LCDD. The increase is greater in the diagnosis number of LCDD, where lack of systemic and other organ involvement symptoms, frequently results in a clinically misdiagnosis of a primary glomerulonephritis.

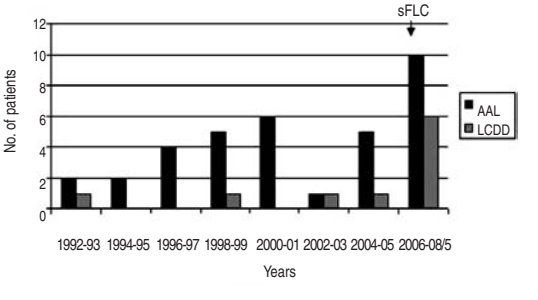

Figure 1.

E42. MONOCLONAL FREE LIGHT CHAINS AND RENAL DYSFUNCTION IN MULTIPLE MYELOMA

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Introduction. Renal dysfunction is a frequent complication of multiple myeloma, mainly related to renal damage by monoclonal free light chains (mFLCs). The present study evaluated the relationship between concentrations of mFLCs in serum and the presence of Bence Jones protein (BJP) in urine and renal dysfunction. *Materials and Methods.* Atotal of 369 pairs of serum and urine samples of 98 myeloma patients were examined. FLCs were quantified nephelometrically using an automated immunoassay. The qualitative detection of Bence Jones protein (BJP) in the urine was made by immunofixation electrophoresis (IFE). Parameters for detecting renal dysfunctions were serum creatinine, cystatine and β2 microglobulin levels as well as serum creatinine clearance and urinary excretions of albumin and α1 microglobuline for glomerular and tubular impairments, respectively. *Results.* Samples with mFLCs in serum and BJP in urine (Ser^+BJP^+) were found to be associated with renal dysfunctions in 90% (122/135) of cases compared with 40% (51/126) in samples with mFLCs in serum but not detectable BJP in urine using IFE (Ser^+BJP^-) (p<0,01). κ and λ samples with Ser^+BJP^+ did not significantly differ regarding the frequency of renal dysfunctions, but there were considerable differences regarding the type of these impairments. The proportions of pure glomerular (glom), combined glomerular and tubular (glom+tub), pure tubular (tub) or no dysfunctions in κ samples were 1%, 54%, 33% and 12%, respectively, compared with 6%, 91%, 0% and 3% in λ samples (p<0.01). Pure tubular dysfunctions (tub), identified by increased urinary excretion of α1 microglobuline and exclusively found in κ samples, were not usually detected when only serum creatinine levels were used. In κ as well as λ myeloma, renal impairments were significantly more frequent in samples with Ser^+BJP^+ than in those with Ser^+BJP^- (66/75 *vs*. 23/39, p<0.01 and 28/31 *vs*. 5/28, p<0.01, p<0.05, respectively). This was mainly due to significant differences in the subgroups with tub+glom (25/75 *vs*. 5//39, p<0.05 and 28/31 vs 1/28, p<0.01, respectively). Merely tubular dysfunctions (tub) were almost exclusively seen in association with κ mFLC with and without BJP in urine (40/75 vs 18/39, p=0.47), but rarely observed in association with λ mFLC, irrespective of the presence or absence of BJP in urine (0/31 vs 2/28, p=0.13). In κ samples of tub+glom type, the median value of serum mFLC was significantly lower than in samples of merely tub type (29 mg/L *vs*. 293 mg/L, p<0.05), possibly indicating an increased mFLCκ clearance due to the additional glomerular damage. *Conclusions.* Based on these results, excretion of BJP in the urine is associated with increased frequency of renal dysfunctions both for κ as well as for λ mFLCs. The impairments associated with mFLCκ, however, are both of tub and tub+glom types, while those associated with mFLCλ are exclusively of tub+glom type. In case of mFLCκ, the additional glomerular impairment increases their renal clearance.

E43. SERUM FREE LIGHT CHAINS IN PATIENTS WITH RENAL FAILURE STAGE II-V OF NATIVE KIDNEYS OR RENAL TRANSPLANTS

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Background. Free light chains cumulate in serum in renal failure. It is not clear, whether the κ/λ ratio is influenced by this accumulation. There is no data available on the use of free light chain measurement in patients with renal failure after renal transplantation. *Aims*. We evaluated serum free light chain concentrations in renal failure and compared the results between patients with failing native kidney and transplanted kidneys. *Methods.* We measured free κ and λ light chains in serum by Freelite assay (The Binding Site) in 360 patients with renal failure stage II to V (GFR <90 mL/min). 140 patients had a renal transplant with different degrees of reduction of renal function, 220 patients had renal failure of native kidneys. Blood specimens were collected in 360 randomly chosen in and outpatients in our hospital specialised in renal care. Patients with known gammopathy were excluded. Renal function was assessed by serum creatinin, cystatin c and estimated GFR calculated by abbreviated MDRD formula. *Results.* There was a highly significant correlation between serum concentration of free κ and λ chains and parameters of renal function. Correlation between the κ chain concentration and creatinin, cystatin C and calculated GFR was 0.57, 0.657 and -0.618. Correlation of free λ chains with the same parameters was 0.541, 0.641 and -0.573 (p<0.01 for all results). Results were not significantly different in patients with failing native kidneys or transplanted kidneys. There was no significant change of κ/λ ratio with reduced renal function. *Conclusions.* κ and λ free light chains accumulate equally in renal failure. The κ/λ ratio can be used to detect gammopathy in renal failure as in patients with normal renal function. Follow-up examinations of patients with gammopathy should take declining renal function into account. Free light chain measurement can be used in patients with transplanted kidneys as in non transplant patients.

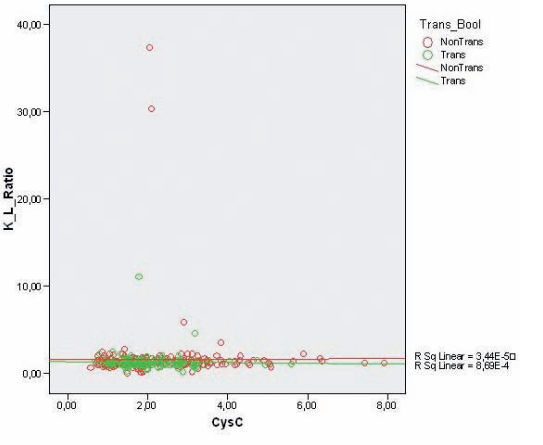

Figure 1.

HEAVY CHAIN/LIGHT CHAIN ASSAYS

F44. NEPHELOMETRIC ASSAYS FOR THE QUANTIFICATION OF IGA KAPPA AND IGA LAMBDA IN HUMAN SERUM

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Specific polyclonal antibodies have been produced which recognise conformational epitopes spanning the junction of the heavy and light chains of the immunoglobulin molecule. Here we describe IgAκ and IgAλ assays for use on the Siemens Healthcare Diagnostics Behring Nephelometer II analyser. The main assay characteristics are summarised in the Table 1.

Table 1.

Assay characteristics	*IgAκ*	*IgAλ*
Calibration curve	6 points	6 points
Sample dilution	1/20	1/20
Measuring range	0.43-13.6 g/L	0.33-10.4 g/L
Sensitivity	0.021 g/L	0.016 g/L
Total assay precision, n=84% CV (mean)		
Low level	8.3% (7.13 g/L)	6.6% (5.84 g/L)
Mid level	6.1% (2.15 g/L)	5.2% (1.94 g/L)
High level	7.2% (0.77 g/L)	7.1% (0.56 g/L)
Linearity	y=0.986x + 0.922, r^2=0.997	y=0.977x + 0.955, r^2=0.996
	Between 1.72-35.7 g/L	Between 1.21-34.5 g/L

IgAκ and IgAλ concentrations were measured in 191 normal (blood donor) sera; median IgAκ 1.27 g/L (SD±0.51, 95 percentile range 0.44-2.36 g/L), median IgAλ 0.87 g/L (SD±0.42, 95 percentile range 0.34-1.85 g/L), median IgAκ/IgAλ ratio of 1.41 (SD±0.50, 95 percentile range 0.58-2.52). Thirty-eight multiple myeloma (MM) patient sera were collected (VIIth UK MRC MM trial) and evaluated for IgAκ and IgAλ, total IgA (using the Behring BNII kit) and serum protein electrophoresis (SPE). Three samples were excluded due to sample degradation, lipaemia or mis-classification (Bence Jones MM). In 3/35 samples there were no monoclonal paraproteins evident, a further 2/35 were non-quantifiable by densitometry and in an additional 13/35 samples the IgA monoclonal protein migrated into the β-region of the SPE. All samples tested gave an abnormal IgAκ/IgAλ ratio. Guidelines[1] for monitoring MM recommend immunofixation and quantification by SPE followed by densitometry. In the case of IgA MM total immunoglobulin measurements can be used for samples that are difficult to interpret by SPE. However this has limitations since it can be difficult to accurately determine disease burden when paraprotein levels drop below 4 g/L (IgA normal range 0.8-4 g/L).[2] In conclusion it is possible to accurately measure IgAκ and IgAλ concentrations in serum. These measurements can be used to calculate IgAκ/IgAλ ratios which have, in this study, been shown to be useful in interpreting IgA MM patients whose paraprotein migrates into the β-region of an SPE.

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F45. RESPONSE TO TREATMENT IN MULTIPLE MYELOMA MAY BE MONITORED MORE SENSITIVELY USING NOVEL IgGκ AND IgGλ NEPHELOMETRIC ASSAYS

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Background. Measurement of monoclonal (M) protein by densitometry has been the preferred method of response assessment in patients with multiple myeloma (MM). The production of specific polyclonal antibodies which recognise epitopes spanning the junction of the heavy and light chains of the immunoglobulin has allowed us to develop sensitive nephelometric immunoassays on the Dade Behring BNII analyser which can determine the serum ratios IgGκ/IgGλ. *Methods.* IgGκ/IgGλ ratios were measured in 109 normal (blood donor) sera to generate a normal range. Total IgG (Dade Behring) was measured on all normal and clinical samples. The MM sera analysed were archived samples collected in the VIIth UK Medical Research Council myeloma trial and sera collected from Heartlands Hospital (Birmingham, UK). Presentation samples were analysed from 19 patients (9 IgGκ /10 IgGλ), with serial sample analysis being completed on 9 (4 IgGκ /5 IgGλ) patients through the course of their disease. *Results.* The sum of the IgGκ+IgGλ measurements correlated well with total IgG in normal (Pearsons Correlation 0.8 $p<0.01$) and monoclonal disease sera (Pearsons Correlation 0.74 $p<0.01$). In the multiple myeloma patients all 19 presentation sera that had a positive immunofixation (IFE) had elevated concentrations of the relevant immunoglobulin and an abnormal IgGκ/IgGλ ratio. For the 9 patients followed through the course of their disease, in all cases the changes in IgGκ / IgGλ ratio reflected the clinical assessments. Four of the 9 did not achieve complete response (CR) and the ratio remained abnormal throughout. In 3 out of the remaining 5 patients relapse from CR was indicated by a change in ratio earlier than by serum protein electrophoresis or IFE. *Conclusions.* This preliminary data indicates it is possible to type monoclonal immunoglobulins using IgGκ /IgGλ ratios. Furthermore, the agreement of the summation indicates it is possible to accurately measure IgGκ

and IgGλ in normal and disease-state sera. Analysing the IgGκ/IgGλ ratio gives a more sensitive indication of relapse in some patients.

F46. SERIAL SAMPLE ANALYSIS OF 3 IGA MULTIPLE MYELOMA PATIENTS USING A NOVEL IMMUNOASSAY MEASURING IGA KAPPA AND IGA LAMBDA

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Background. The standard for measuring paraprotein in patients with multiple myeloma (MM) to assess tumour burden is the quantification protein bands by serum protein electrophoresis (SPE). There are several limitations with this method, such as large proportion of IgA monoclonal proteins migrate into the β region where they can be obscured by other proteins making quantification subjective. A novel nephelometric immunoassay relies on the specific identification of epitopes spanning the junction of the heavy and light chains on the immunoglobulin molecule. Measurement of the involved and uninvolved heavy-light chains in serum may provide an accurate and quantifiable measurement of residual disease. *Aims.* To use a novel nephelometric assay to perform retrospective analysis of tumour burden in patients with IgA multiple myeloma. *Methods.* Samples from MRC MM trial were collected and analysed, results were compared to total IgA and SPE densitometry. Thirty-five presentation sera were analysed and 3 patients were followed throughout the course of their disease. Normal ranges for IgAκ/IgAλ ratios were derived from 191 blood donor sera. *Results.* In 35 IgA MM patient samples analysed at presentation, 14.3% (5/35) did not have a quantifiable SPE band, whereas IgAκ/IgAλ ratios were reported in each case. Patient A was treated with conventional chemotherapy (CVAMP) and received an autologous stem cell transplant resulting in a complete response. Relapse was reported using hevylite IgAκ/IgAλ ratio 596 days prior to conventional SPE and IFE methods. In sera from patient B quantification of IgA monoclonal protein by SPE densitometry was difficult due to other proteins in the β region throughout the course of the disease. IgAκ/IgAλ ratio did not normalise until 518 days after IFE became negative. Patient C received conventional chemotherapy (ABCM) and complete response was reported after 175 days, relapse occurred after 491 days. SPE and IFE became negative with IgA concentration falling into the normal range after treatment. However, the IgAκ/IgAλ ratio remained abnormal throughout. *Summary*. The use of IgAκ/IgAλ ratios correctly identified 35/35 patients tested, including patients where the SPE was ambiguous. Furthermore, analysis of IgAκ/IgAλ ratios in 3 IgA MM patients followed through the course of their disease proved a more sensitive marker of tumour burden resulting in a significantly earlier detection of residual disease when compared to traditional methods.

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