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7th International Symposium on Clinical Applications of Free Light Chain and Heavy/Light Chain Analysis

Edinburgh, UK, 16-17 April 2015

Guest Editor: Stephen Harding

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Posters

POSTERS

MONITORING MONOCLONAL GAMMOPATHIES

A1 - HEAVY/LIGHT CHAIN IMMUNOASSAYS MEASURING IgMκ AND IgMλ FOR QUANTIFYING AND MONITORING MONOCLONAL IgM IMMUNOGLOBULINS

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Background. Quantification of monoclonal IgM (M-IgM) using electrophoretic techniques can be challenging. Novel nephelometric/turbidimetric immunoassays measuring IgMκ and IgMλ (heavy/light chain, HLC) may provide a convenient alternative. Calculation of HLC IgMκ/IgMλ ratios can be used as an indicator of clonality and may obviate the need for immunofixation assays. *Objectives.* To compare HLC assays with traditional tests for M-IgM protein quantification in patients with IgM paraproteinemias. *Methods.* We analysed 166 serum samples from 113 patients with IgM paraproteinemias. Median age was 67(35-91) years and M/F ratio 68/45. HLC IgMκ and IgMλ concentrations were measured with Hevylite on the SPAPLUS turbidimetric analyser. Results were compared to CZE and total IgM measurements by turbidimetry. *Results.* 22 patients with diagnostic sera and M-IgM identifiable by CZE had an abnormal IgMκ/IgMλ ratio (19 IgMκ: 81.33 (12.79-1608); 3 IgMλ: 0.04 (0.03-0.14) and elevated levels of involved HLC (iHLC) and total IgM. Median M-IgM concentrations as measured by the three tests were not significantly different (p=0.816, Kruskal-Wallis test): CZE: 11.50 (3.0-31.0) g/L; total IgM: 14.75 (3.35-58.8) g/L; iHLC:15.73 (3.58-73.08) g/L. In all samples, there was good correlation between summated IgMκ+IgMλ HLC and total IgM concentrations (y=1.13x-0.70,R2=0.88). In agreement with Murray *et al.* (Clin Chem 2009) both iHLC (y=1.91x-0.09,R2=0.80) and total IgM measurements (y=1.66x+0.69, R2=0.92) showed a linear, systematically high correlation to CZE for IgM concentrations >8.5g/L. There was no correlation for IgM concentrations <8.5g/L. In 10 Waldenstrom's macroglobulinemia patients with longitudinal samples (median number of samples: 3(2-9), follow-up: 373 (18-713) days), there was good correlation for M-Ig% change relative to baseline between CZE and difference HLC (dHLC: involved-uninvolved HLC; y=0.92x-0.12,R2=0.85) and total IgM (y=0.93x-0.06,R2=0.91). Weighted Kappa (WK) analysis showed substantial agreement between the responses assigned by CZE and dHLC (WK=0.91) and total IgM (WK=0.94). *Conclusions*. HLC immunoassays provide an alternative method of quantifying M-Ig in patients with IgM paraproteinemias. Larger clinical studies are required to confirm the clinical utility of these assays.

A2 - HEAVY/LIGHT CHAIN IMMUNOASSAYS AS AN USEFUL BIOMARKER TO FOLLOW-UP ACCURATE RESPONSE AFTER BORTEZOMIB UPFRONT THERAPY IN MULTIPLE MYELOMA

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Background. The objective of multiple myeloma (MM) therapy is to achieve a deeper and durable response. The routinely follow-up is based in the M protein quantification by serum and urine electrophoresis (SPE, UPE) with immunofixation (IFX). The incorporation of Serum Free Light Chain Assay (FLC), and the quantification of paired clonal and non-clonal immunoglobulins (HLC) in serum, offers the possibility to assess the response to therapy more accurately detecting early biological relapses (EBR). *Aims.* To analyze the usefulness of HLC and FLC during MM follow-up to detect EBR after Bortezomib first line therapy in MM patients in our centre. *Patients and Methods.* Since January 2008 we have incorporate in the protocol for M-components assessment the quantification of FLC, HLC at baseline and in follow-up of MM. We have analyzed these parameters in all consecutive patients diagnosed as secretor MM who underwent Bortezomib upfront therapy between Jan 2008-Jun 2014 in our center. *Results.* 135 patients were registered. Median follow-up 25 months. Females: 40.7%, mean age 69.6 y (32-91). Subtype: IgG-κ: 38.2%, IgG-λ: 9,8%, IgA-κ: 17.9%, IgA-λ: 15,4%, IgDL: 1.6%, Bence-Jones-κ: 6,5%, Bence-Jones-λ: 8,9%, oligosecretor: 1.6%. Stage: IA: 9.8%, IB: 1.6%, II-A: 27.6%, IIB: 8.9%, III-A: 21.1%, III-B: 21.1%. ISS(I: 25.2%, II: 32.5%, III: 23.6%). 21.1% were not included in the analysis of response by uncompleted treatment. Response at end of therapy: minimal response: 5.1%, PR: 44.3%, VGPR: 16.5%, CR: 14.4% SR: 7.2%, Failure: 12.5%. During follow-up 65.7% patients, who achieved at least PR had clinical relapsed/progressed, in 86.9% of them previous EBR were detected (mean 4.4 months before) by FLCr (28.8%), HLCr (13.5%), FLC+SPE (9.6%), FLC+IFX (5.8%), FLC+HLC+SPE (28.8%), FLC+HLC+SPE+UPE (5.7%). Median PFS 20 months (12.8-27.1) Biological PFS: 18 m (12.1-23.8). *Conclusions.* Both FLCr and HLCr are sensitive and precise tools to assess-

ment response in MM detecting 42.3% EBR ahead other techniques.

This work had been partially sponsored by a grant from FEHHA.

A3 - EARLY BIOLOGICAL RELAPSE AFTER ASCT IN MULTIPLE MYELOMA. USEFULNESS OF HEAVY/LIGHT CHAIN IMMUNOASSAYS

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Background. There are new tools for accurate assessment in multiple myeloma (MM). While the Free Light chain immunoassay (FLC) (Bindingsite, Birmingham, UK) is part of the mandatory response assessment according to IMWG-criteria, promissory results have obtained using Heavy/Light Chain (HLC) immunoassay. We hypothesized that the combination of these techniques could permit to detect early biological relapses (EBR). *Aims.* To analyze the usefulness of HLC and FLC to detect EBR in MM after Autologous Stem Cell Transplantation (ASCT) in our hospital. *Patients and Methods.* A retrospective study was performed including all consecutive patients following these criteria: Diagnosed of secretory MM and underwent ASCT between May 2011-Jun 2014, assessed with our protocol including FLC, HLC, serum and urine electrophoresis (SPE, UPE) with immunofixation (IFX), previous to ASCT, after 12 weeks and every 3 months later. *Results.* Fifty-five patients were registered. Median follow-up 21 months. MF ratio: 29/26, mean age 59.5 y (33-71). Immunoglobulin subtype: IgG-κ: 41.8% (23), IgG-λ: 23.6% (13), IgA-κ: 16.4% (9), IgA-λ: 7.3% (4), Bence-Jones-κ: 3.6% (2), Bence-Jones-λ: 7.3% (4). Durie-Salmon Stage: IA: 13.5% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as conditioning regimen, assessment before ASCT: minimal response: 12%, Partial Response (PR): 50.0%, VGPR: 28.0%, CR: 6% SR: 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 13.0% CR, 30.4% VGPR and 39.1% PR. During follow-up 27/50(54.0%) patients, who achieved at least PR after ASCT had clinical relapse/progress, median PFS 24 months (19.8-28.1). EBR were detected in 19/27 patients at median time 7 (2-19) months before symptomatic relapse. The EBR were detected by FLCr (31.6%), HLCr (21.0%), FLC+SPE (10.5%), FLC+IFX (5.2%), FLC+HLC+SPE (15.8%), FLC+HLC+SPE+UPE (15.8%). *Conclusions.* Both FLCr and HLCr are useful tools to detect EBR in more than 50% of patients in our cohort.

This work had been partially sponsored by a grant from FEHHA.

A4 - IMMUNOGLOBULIN HEAVY AND LIGHT CHAIN ISOTYPE PAIRS IN MONITORING MULTIPLE MYELOMA PATIENTS

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Background. Diagnosis and monitoring of disease activity in Multiple Myeloma patients are generally based on quantitative measurements of the monoclonal immunoglobulin (MIg) concentration in plasma and/or urine. Methods for MIg quantitation include densitometric scanning of electrophoresis gels and immunoassay measurements of total polyclonal+monoclonal immunoglobulins (total IgG, IgA or IgM). Hevylite is a more specific method to identify electrophoretically "difficult-to-detect" MIg (*i.e.* IgA) and to more specifically quantify the tumor-derived heavy chain and light chain isotype pair. *Aims.* We compared serial Hevylite measurements to other techniques for MIg quantitation to monitor disease activity in MM patients. *Results.* From December, 2012 to July, 2014, forty-four MM patients were evaluated at various times during therapy. Thirty patients were IgG isotype (20 IgG κ, 10 IgG λ) and 14 IgA isotype (4 IgA κ, 10 IgA λ). Median follow up time was 336 days (range 77-526) for patients with IgG MM and 287 days (range 41-505) for patients with IgA MM. Hevylite IgG λ, IgA λ and IgA κ corresponded well with quantitative immunoglobulin measurements. However, in some patients with high IgG κ monoclonal protein, Hevylite IgG κ (+IgG λ) measured lower than total quantitative IgG. This lower reading appeared to be patient specific, in that the ratio of Hevylite:Total IgG was consistent for a given patient. In monitoring patients, normalization to their own baseline value showed comparable results between Hevylite IgG κ and total IgG. *Conclusions.* In the MM patients studied here, Hevylite corresponded well with total immunoglobulins for IgA λ, IgA κ and IgG λ. Variances in high IgG κ MIg can be normalized to baseline to obtain better agreement with total IgG immunoglobulins. In general, once adopted and a baseline set, results should always be compared from the same method to assess disease progression indicating need for method standardization.

A5 - ROLE OF THE HEAVY/LIGHT CHAIN IMMUNOASSAY IN THE ASSESSMENT OF COMPLETE RESPONSE IN MULTIPLE MYELOMA PATIENTS

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Background. Achieving complete response (CR) and stringent complete response (sCR), defined as negative serum immunofixation analysis and normalization of serum free light chain ratio analysis, has become one of the main objectives in treatment strategies for multiple myeloma patients, since it is associated with longer progression free survival. The recent introduction of a novel heavy/light chain (HLC) immunoassay provides an opportunity to define further the depth of response to myeloma treatment. *Objectives.* To evalute the utility of the novel heavy/light chain immunoassay in the assessment of complete response in multiple myeloma patients. *Methods.* Serum samples of 23 multiple myeloma patients (15 IgG and 8 IgA) in CR or sCR were analysed: standard laboratory procedures (serum protein electrophoresis, serum immunofixation, serum free light chain assay, quantitative immunoglobulins, serum β-2-microglobulin) were performed in addition to heavy/light chain assay (HLC). *Results.* 18 patients were in sCR and 5 were in CR. HLC analysis revealed 3/23 patients had immunoglobulin monoclonal isotypes values above normal (2 IgG and 1 IgA), 1 of whom (IgA) also had abnormal heavy/light chain ratio value. An additional 4/23 patients (3 IgG and 1 IgA) with monoclonal isotype and polyclonal isotype pair values within normal ranges were found to have abnormal HLC ratio values In the follow up period, 2/5 patients with abnormal HLC ratio values experienced relapse. *Conclusions.* The novel heavy/light chain immunoassay, especially the heavy/light chain ratio value, allows accurate detection and measurement of monoclonal immunoglobulins, even in multiple myeloma patients who achieved a complete response. It seems that the new assay is a sensitive tool for detecting minimal residual disease and can detect relapse earlier, compared with traditional methods. As far as its definite role in assessing the depth of response is concerned, further studies on larger groups of patients are needed.

A6 - HEAVY/LIGHT CHAIN IMMUNOASSAYS FOR MONITORING PATIENTS WITH OLIGOSECRETORY MULTIPLE MYELOMA

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Background. Monitoring oligosecretory multiple myeloma (MM) patients whose serum monoclonal free light chains (FLC) levels are low can be challenging. Newly available heavy/light chain (HLC) immunoassays allow quantifying Ig'κ and Ig'λ levels, from which HLC Ig'κ/Ig'λ ratios are calculated to identify clonality. *Objectives.* To evaluate the role of HLC immunoassays for monitoring oligosecretory MM patients. *Methods.* HLC Ig'κ and Ig'λ were measured in sequential sera from 2-IgG and 6-IgA oligosecretory patients with non-measurable disease by FLC. Serum M-protein changes during monitoring as determined by HLC, total immunoglobulin (tIg) and SPEP, were compared. *Results.* At presentation 2/8 patients (both IgA) had non-quantifiable M-Ig levels by SPEP. By contrast HLC ratio was abnormal in all patients. 2/2 IgG patients had tIgG levels within the normal range. In one of these patients HLC ratio normalised after 237 days, whereas SPEP became negative 175 days later. 6/6 IgA patients had tIgA levels above normal. In 4/6 patients HLC ratio remained abnormal throughout monitoring; by contrast in 2/4 patients tIgA normalised, including an IgAκ patient achieving a first response after 284 days when SPEP became (and stayed) negative and tIgA normalised, whilst HLC ratio remained abnormal. Subsequently tIgA and dHLC (involved-uninvolved HLC) levels increased, indicating progression after 452 days. Following a second response, dHLC levels steadily increased signifying another relapse, which was not identified by tIgA until 147 days later (Figure 1a). In another patient (IgAλ) with non-quantifiable M-protein by SPEP, tIgA levels normalised with therapy before increasing to 8 g/L after 426 days; seemingly indicating progression. At this time HLC ratio had normalised and both IgAκ and IgAλ levels were elevated; remaining so throughout most of follow-up and suggesting tIgA elevation was due to polyclonal IgA expansion (Figure 1b). *Conclusions.* HLC immunoassays may aid monitoring oligosecretory MM patients where traditional techniques have limited utility.

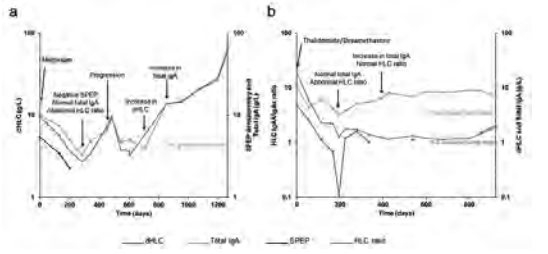

Figure 1.

A7 - FOLLOW UP OF PATIENTS AFTER ASCT BY HEVYLITE AND FREELITE ASSAYS IMPROVE SENSITIVITY IN THE IDENTIFICATION OF SEROLOGICAL RELAPSE

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Introduction. Serological follow up of patients undergoing autologous stem-cell transplantation (ASCT) is currently based on the measurement of the monoclonal com-

ponent using serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE). Hevylite (HLC) is a new assay allowing the quantification of intact heavy/light-bounded chains of a given immunoglobulin. Our study aimed to compare the sensitivity of conventional techniques (SPE, IFE) to nephelometric/turbidimetric assays (HLC and free light chain assay, FLC) in detecting serological relapse in patient after ASCT. *Methods*. Consecutive routine sera submitted to our laboratory during follow up of 21 myeloma patients (16 IgG, 1 IgM, 4 IgA) after ASCT were examined with SPE, IFE, HLC, FLC. 15 patients (4 IgA and 11 IgG) relapsed. Time to serological relapse was determined using conventional methods (SPE, IFE) and compared to relapse indicated by HLC and FLC. *Results.* In 7 patients (3 IgA and 4 IgG) HLC measurements indicated serological relapse earlier than conventional techniques (mean: 4 months earlier). In two of these cases FLC indicated a serological relapse earlier than HLC. In two cases (1 IgA and 1 IgG), which reached serological complete remission, IFE detected the reappearance of the original monoclonal component 1, respectively 2 months earlier than HLC. In all remaining cases serological relapse was detected simultaneously by conventional and nephelometric/turbidimetric techniques. *Conclusions.* HLC and FLC are valuable complementary methods to conventional techniques (IFE, SPE) and improve sensitivity in the identification of serological relapse during follow up of patients after ASCT.

A8 - FREE LIGHT CHAINS ARE NEEDED FOR THE FOLLOW UP OF MULTIPLE MYELOMA

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Abstract. Nonsecretory multiple myeloma (NSMM) is considered a rare variant of the classic form of MM that has a similar clinical and radiologic presentation and lack of M-protein in serum and/or urine on electrophoresis and immunofixation. We describe here, a case of a 29-year-old woman who came in our Hospital in 2010 with back pain and multiple osteolytic bone lesions. This woman had no other symptoms. A CT guide biopsy from the L1 vertebra showed massive infiltration of plasmocytes confirming the diagnosis of suspected NSMM, because she had normal serum and urine protein electrophoresis and immunofixation, without apparent bone marrow involvement. After a chemotherapy induction with VTD and autologous peripheral bone marrow transplant, she obtained an apparent complete remission for two years, when in 2012, routinary blood tests showed normal serum basic analyses but an excess of κ monoclonal free light chains (this parameter was not available at time of diagnosis) One month later, the patient developed an aggressive and diffuse clinical bone relapse and, due to the young age, she was treated with second line chemotherapy and allogeneic bone marrow transplant. There are studies showing that a cytoplasmic M-protein can be identified in nearly 85% of patients initially classified as NSMM. A lot of these patients, even in absence of measurable disease as presently defined, may be monitored by free light chain assay, able to reveal small amounts of monoclonal proteins. The availability of a more sensitive technique for the detection of monoclonal components in biological fluids is quite important, because it may help to make an earlier diagnosis and may represent another parameter of measurable disease, useful to monitor the patients for the optimal drug treatment.

A9 - COMPARISON OF POLYCLONAL AND MONOCLONAL ANTIBODY BASED FREE LIGHT CHAIN ASSAYS FOR MONITORING MULTIPLE MYELOMA PATIENTS IN DIFFERENT CLINICAL SETTINGS

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Background. International guidelines for monitoring serum free light chains (FLC) in patients with monoclonal gammopathies enrolled in clinical trials rely on the polyclonal antibody-based assay Freelite. A novel monoclonal antibody-based assay (N-Latex-FLC) recognising single epitopes has become available for measuring serum FLC. *Objectives.* To compare the suitability of Freelite and N-Latex-FLC for monitoring MM patients according to international guidelines and clinical practice. *Methods.* FLC were measured by Freelite and N-Latex-FLC in sera samples from 59 bortezomib-treated MM patients (21 newly diagnosed, 38 relapsed; median number of samples/patient: 6(3-17); median follow-up: 206(7-819) days). Results were compared to serum protein electrophoresis (SPEP) and total serum immunoglobulin (tIg). IMWG defines measurable disease by serum FLC as monoclonal FLC (iFLC) levels >100mg/L provided FLC ratio is abnormal. iFLC >50mg/L was considered to reflect monoclonal FLC production with clinical utility for monitoring outside trials. *Results.* Following IMWG guidelines, 18(31%) patients had measurable disease by Freelite only, 2(3%) by N-Latex-FLC only, 20(34%) by both assays and 19(32%) by neither. 5/18 patients with measurable disease by Freelite had non-quantifiable paraprotein by SPEP and normal tIg levels, whereas in 13/18 N-Latex-FLC failed to detect monoclonal FLC production as defined. By contrast Freelite reported iFLC>50mg/L in 2 patients with measurable disease by N-Latex-FLC, demonstrating clinical utility. In 19 patients with non-measurable disease by either assay, Freelite reported iFLC>50mg/L in 3 and N-Latex-FLC in none; importantly, 1/3 had neither quantifiable paraprotein nor elevated tIg, making Freelite the only available tool for monitoring. Finally, in 20 patients with measurable disease by both assays, agreement between assays for response assignment was only moderate (Weighted κ=0.54(0.30-0.79)). *Conclusions.* Freelite allowed monitoring of a substantial higher number of MM patients than N-Latex-FLC, both when following guidelines for clinical trials and in clinical practice; suggesting a limitation in recognising epitope variety and/or FLC levels by N-Latex-FLC.

A10 - HEVYLITE MEASUREMENT IS POTENTIALLY HELPFUL FOR DIAGNOSIS AND FOLLOW UP OF PATIENTS WITH POEMS SYNDROME

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Background. POEMS syndrome is an acronym which stands for polyneuropathy (P), organomegaly (O), endocrinopathy (E), monoclonal gammopathy (M) and skin changes (S). The quantification of serum monoclonal proteins (mainly of IgA or IgG λ isotype) is difficult as these components are present at low level, and sometimes could be missed on serum electrophoresis. *Objectives of the study.* Hevylite® was developed by The Binding Site (Birmingham, UK) to quantify the κ- and λ- bounded amounts of serum circulating IgG and IgA. With this method, the involved and uninvolved Ig in the disease could be measured and a ratio named HLCR (heavy light chain ratios) is calculated. The purpose of this work was to investigate the contribution of this measurement and the deriving ratios (HLCR) in a series of patients with POEMS syndrome at diagnosis and/or during the evolution of the disease. *Methods.* We have analyzed retrospectively 136 sera from 18 patients with POEMS syndrome recruited in the Pitié-Salpétrière hospital between 2001 and 2014 and performed Hevylite® testing at different times during the follow up of the disease (before and after relapse, stem cells autologous transplantation or chemotherapy). *Results.* On the 18 patients, 11 have a monoclonal IgA, 7 an IgG. During the curse of their disease, 10/18 (55%) patients have an elevation of the involved Ig level and 13/18 (72%) have an abnormal HLCR. During clinical follow up of the patients, analysis of involved and uninvolved Ig levels and HLCR show kinetic changes of their immunological status for 12/18 (66%) patients and a potential interest of the Hevylite® test. *Conclusions.* Even if Hevylite®applications need more studies, our results were correlated with the curse of the disease in two third of patients. This test could be a biologic weapon to follow patients with POEMS syndrome.

A11 - THE FIRST REPORTED CASE OF HEAVY CHAIN ESCAPE IN A PATIENT WITH FIRST RELAPSE OF MULTIPLE MYELOMA: THE ROLE OF THE HEVYLITE• AND FREELITE• ASSAYS

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We report the first case of heavy chain escape occurring simultaneously with light chain escape in an unusual plasma cell neoplasm. We highlight the role that the Freelite® assay (directly) and the Hevylite® assay (indirectly) had in identifying monoclonal free κ light chains and monoclonal free gamma heavy chains at first relapse. The female patient had been diagnosed two years earlier with Multiple Myeloma and Multiple Plasmacytomas in association with Gamma Heavy Chain Disease. Electrophoretically the patient had a triple gammopathy (IgGκ, 26g/L; IgGκ+Free κ, 4g/L; Free Gamma, 5g/L). The κ serum free light chain was grossly elevated at 24677mg/L. The earliest indicator of relapse was a progressively rising involved free light chain which was identified four months prior to its detection by immunofixation electrophoresis (Figure 1). A skeletal survey showed new lytic lesions in the skull, pelvis and left humerus and the bone marrow aspirate revealed a marked infiltration (50%) of abnormal plasma cells. The emerging role of the Hevylite assay includes its ability to detect the presence of minimal residual disease and early relapse and the measurement of some paraproteins difficult to quantify by electrophoresis. A further role of the assay utilizes its inability to react with free truncated gamma heavy chains (Hevylite antibodies are directed against junctional epitopes on the intact immunoglobulin molecule) and thus the sum of the IgGκ/IgGλ matched pair reflects the concentration of the intact IgG present in the patient's serum. An increasing gamma heavy chain excess was evidenced by the difference between the total IgG and the sum of the IgGκ/IgGλ matched pair. At the peak of biochemical relapse the free κ light chains rose to 6602mg/L and the free gamma heavy chains to 4.2g/L. This strategy could be incorporated into the diagnostic workup of any patient who has suspected Heavy Chain Disease.

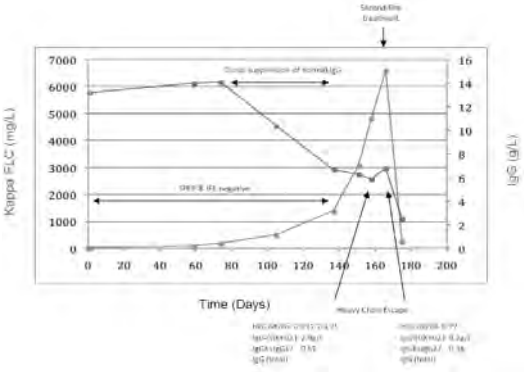

Figure 1.

A12 - BIOLOGICAL ROLE OF IMMUNOGLOBULIN FREE LIGHT CHAINS INTERACTOMES

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Background. Monoclonal Gammopathy of Undetermined Significance (MGUS) is a plasma cell disorder that could be diagnosed incidentally and behave like a benign, asymptomatic entity or progress (1% per year) to different haematologic malignancies such as multiple myeloma (MM). Monoclonal plasma cells often secrete high

amounts of immunoglobulin free light chains (FLCs) that could induce tissue damage. Recently we showed (Di Noto *et al.*, 2013) that FLCs are internalized in endothelial and myocardial cell lines and secreted in extracellular vesicles (EVs). Our data show that MM EVs internalization is FLCs and GAGs mediated and we could demonstrate that MM EVs induce NfkB nuclear translocation. Blocking FLCs with anti FLCs antibodies or masking the GAGs recognition with heparin altered the EVs intracellular uptake and NfkB nuclear translocation (Di Noto *et al.*, 2014). EVs carry determinant information about the onset of MGUS-MM switching which can be exploited for early assessment of MM onset and progression. *Objectives.* We aim to identify binding partners that associate with the FLCs in the blood stream (FLCs interactome) and are involved in the EVs generation and cellular uptake. *Methods.* EVs, purified from serum of control, MGUS and MM patients, were analyzed by a blend of conventional analytical techniques, MALDI TOF analysis and surface plasmon resonance (SPR). *Results and Conclusions.* In a previous proteomic study immuno-purified FLCs isolated from patients with monoclonal gammopathies were characterised and some proteins such as clusterin and albumin associated with FLCs were identified. We then tracked the binding isotherms of EVs to a heparin-coated sensorchips. The results show a marked difference between the binding isotherms of EVs from healthy individuals and from MM and MGUS patients.

A13 - LIGHT CHAIN-BASED MODEL PREDICTING COMPLETE RESPONSE TO INITIAL TREATMENT WITH CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background. KRd is active in NDMM, producing overall response rates of 98%, CR/nCR up to 72%, and stringent CR (sCR) rates up to 55% (Jakubowiak *et al.*, Blood 2012, Jasielec *et al.*, Blood 2013). Achieving CR during initial treatment with KRd is associated with prolonged overall survival (OS) and/or progression-free survival (PFS). Therefore, strategies, such as early modification of therapy in patients unlikely to achieve CR, can be explored as ways of improving treatment outcome in NDMM. *Objectives.* Establish predictive model of CR achievement to KRd treatment based on early changes in involved serum free light chains (iFLC) and serum M-protein levels. *Methods.* We analyzed iFLC and M-protein levels during the first 2 cycles of KRd treatment in 53 NDMM patients from a phase I/II trial using a modified model that predicts achievement of at least very good partial response at the end of induction treatment if at least one of the following has occurred: ≥90% reduction of iFLC, normalization of free light κ/λ ratio (FLCr), or disappearance of M-protein (Dytfeld *et al.*, Leuk Lymphoma, 2012). *Results.* Changes in iFLC and M-protein after 1 cycle of KRd treatment had a positive predictive rate for CR/sCR achievement in 23/32 pts, with false positive rate in 1/19 pts, *i.e.* 72% sensitivity (95% CI 0.53-0.82) and 94% specificity (95% CI 0.72-1.00). After 2 cycles, sensitivity increased to 94% (32/34 CR/sCR pts, 95% CI 0.79-0.99) and specificity remained at 94% (95% CI 0.72-1.00). Updated PFS and OS based on level of response after 4 years of median follow-up further support the role of achievement of CR to KRd and will be presented at the meeting. *Conclusions.* Considering the significance of achieving CR/sCR to initial treatment, this model may be used as proof of principle for future studies to individualize therapy and optimize disease control.

A14 - INVOLVED/UNINVOLVED RATIO CAN BE AN EARLY PREDICTOR OF PROGRESSION IN TRANSPLANTED MULTIPLE MYELOMA PATIENTS

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Methods. Twenty patients with MM of IgG isotype undergoing SCT (8 with IgG-λ and 12 IgG-κ) were included in the study. Patients were followed prospectively for 19±2.1 months. We studied in serum samples collected at variable times during follow-up: protein electrophoresis, immunofixation, total immunoglobulin levels, serum heavy/light chains (HLC) and free light chains (FLC). FLC, HLC and Involved/Uninvolved ratios were calculated. *Results.* Five transplanted patients relapsed, other four showed a stable monoclonal immunoglobulin peak with no clinical progression and 11 achieved complete remission. We studied the best factors to anticipate relapse by measuring all the afore mentioned variables in samples obtained every 3 months. We identified the samples obtained at disease relapse and studied the variable that gave clearer differences in previous samples. The higher differences were found in the I/U ratio. Values of basal and pre-relapse samples were 1.36 (0.98-5.51) *vs* 10.36 (5.8-84.13), median (25-75 percentile), p=0.03. By contrast, this index remained stable in patients with MM in complete remission [1.10 (0.86-1.26) *vs* 1.27 (0.77-1.43), first and last sample obtained during follow-up, p=0.84] or in those with stable monoclonal immunoglobulin peak without clinical progression [4.1 (2.7-4.78) *vs* 4.3 (3.7-5.0)]. *Conclusions.* Our data strongly suggest that quantification of involved and uninvolved chains of the monoclonal isotype can early predictors of progression in MM transplanted patients.

A15 - FREE LIGHT CHAIN ESCAPE IN PATIENTS SUFFERING FROM MULTIPLE MYELOMA: THE EXPERIENCE OF THE TOULOUSE TEACHING HOSPITAL

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Background. Serum free light-chain (FLC) assay is a diagnostic and prognostic factor in the management of patients suffering from multiple myeloma, and is useful for the monitoring of oligosecretory myeloma and for the assessment of a stringent complete response. Some patients, who are stable or in remission from a myeloma secreting intact monoclonal immunoglobulin, relapse with production of FLC only. This "FLC escape" illustrates the fact that multiple myeloma is a heterogeneous disease with intraclonal heterogeneity. FLC escape is observed in 2.5 to 8% of myeloma. *Objectives.* We report the experience of the Toulouse Teaching Hospital for FLC escape in myeloma disease. *Methods.* We retrospectively reviewed our patient' database between 2007 and 2014. *Results.* We identified seven cases of FLC escape: one case (IgA myeloma) occurred after a stringent complete response, four cases (IgA, IgD myeloma) after a partial response, and two cases (IgA, IgG myeloma) during a stable disease. The median age at diagnosis was 58 years. All patients were treated with bortezomib and dexamethasone. Four patients received autologous peripheral blood stem cell transplantation. The median time from initial diagnosis to the occurrence of FLC escape was 35 months. During relapse, the renal impairment increased in parallel with the rise of the involved free light chain. Four patients died of myeloma progression after a median of 2 months from FLC escape and of 79 months from diagnosis. We report the first description of FLC escape in a patient with IgD myeloma. This patient was a 50-year old man, who died one month after FLC escape and 30 months after autograft. *Conclusions.* The experience of the Toulouse Hospital confirms the importance of monitoring FLC in patients with myeloma. FLC monitoring may allow more rapid diagnosis of FLC escape which is of poor prognostic, in order to adjust the different treatment options.

A16 - HEVYLITE® TO MONITOR HYPOGAMMAGLOBULINEMIA, A PREDICTOR OF RESPONSE TO THERAPY IN MULTIPLE MYELOMA

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M. Macro,[4] C. Hulin,[5] B. Onraed,[2] L. Garderet,[6]
M. Roussel,[7] B. Arnulf,[8] B. Pegourie,[9] B. Kolb,[10]
A.-M. Stoppa,[11] S. Brechignac,[12] M. Michallet,[13]
G. Marit,[14] C. Mathiot,[15] A. Banos,[16] M. Tiab,[17] M. Dib,[18]
J.-G. Fuzibet,[19] M.-O. Petillon,[1] P. Rodon,[20]
M. Wetterwald,[21] B. Royer,[22] L. Legros,[19]
L. Benboubker,[23] O. Decaux,[24] D. Caillot,[25]
M. Escoffre-Barbe,[24] J.-P. Fermand,[26] P. Moreau,[27]
M. Attal,[7] H. Avet-Loiseau,[28] T. Facon,[29] X. Leleu[1]

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Background. The depth of hypogammaglobulinemia has been related to adverse prognosis in multiple myeloma (MM), and it has been suggested that its recovery following treatment was associated with good outcome and prolonged survival. However, traditional techniques do not allow precise measurement of isotype-matched hypogammaglobulinemia (*i.e.* concentrations of IgGκ in an IgGλ MM). Recently, a new test quantifying paired clonal and non-clonal immunoglobulins (heavy/light chains HLC *i.e.* IgGκ/IgGλ) in serum was developed. Here we aim to assess the new HLC assay as a tool to assess hypogammaglobulinemia. *Materials and Methods.* 107 MM patients treated with pomalidomide and dexamethasone in two IFM studies were included (IFM2009-02 in end-stage RRMM, and IFM2010-02 in del17p and t(4;14) RRMM). Patients solely measurable on UPEP and sFLC were excluded. All sera were collected centrally before treatment and sequentially every cycle until progression. *Results.* 98 (92%) patients had an abnormal suppressed uninvolved HLC level at baseline and as much as 94 (87%) at the time of best response. The median uninvolved IgG and IgA HLC concentrations at baseline were 0.62 and 0.2 g/L respectively (range: 05-6.9;0.01-5.6). 55% of responders had improved levels (by at least 20%) of the uninvolved paired isotype HLC compared to 18.5% of the non-responders (p=0.001). This data strongly correlated to the depth of response, since 75% of patients in VGPR or better had improved levels of uninvolved

paired isotype HLC by 50% at the time of greatest response, compared to 31% for PR and 13% for SD (p=0.005). *Conclusions.* The mechanism of immunosuppression in MM patients is poorly understood. Here we have shown for the first time that isotype-matched hypogammaglobulinemia correlates to depth of response. Hypogammaglobulinemia is important to assess not only because of its greater risk of infectious complications, often severe in MM, but also as a prognostic factor for response.

A17 - HEVYLITE® TO MONITOR RESPONSE TO THERAPY IN MULTIPLE MYELOMA

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Background. Protein electrophoresis and immunofixation in the serum and urine (SPEP, SIF, UPEP and UIF), and more recently serum free light chain assay (SFLC), are commonly used for monitoring the M protein in Multiple Myeloma (MM). However, these techniques remain disappointing. Recently, a new test quantifying paired clonal and non-clonal immunoglobulins (heavy/light chains HLC *i.e.* IgGκ/IgGλ) in serum was developed. We aimed to assess this new HLC assay (Hevylite®) compared to standard tests during MM monitoring. *Materials and Methods.* 110 MM patients treated with pomalidomide and dexamethasone in two IFM studies (IFM2009-02 in end stage RRMM and IFM2010-02 in del17p and t(4;14) RRMM) were included. All sera were collected centrally before treatment and sequentially every cycle until progression. *Results.* All patients had a measurable disease by standard tests (SPEP and/or UPEP). All patients but one also had a measurable disease with HCL. Approximately 32% of patients had an M-spike below 20g/L and/or an electrophoretic migration in β region meaning in the range of lack of sensitivity of the techniques used, all of whom had a measurable disease using HLC. The ORR using traditional tests was 32%, including 29% PR, no CR, and 44% SD (SD and MR). Using HLC, with the exact same criteria as to the sFLC-based response criteria recommended by IMWG, the ORR was 36%, including 26% PR, 4% CR, and 33% SD (r^2 0.823, p<.0001). Interestingly, 7 patients classified as SD with regular techniques were classified as progressive disease using HLC, anticipating a progression of MM. Similarly, 5 patients classified as SD with regular techniques were classified ≥PR using HLC. *Conclusions.* Our study indicates that HLC may be used as a replacement for traditional tests for diagnosis, prognosis, response to treatment and detection of disease progression, and may offer greater sensitivity in some instances.

A18 - BENEFIT OF USING THE HEVYLITE IMMUNOASSAY IN THE FOLLOW UP OF AN IgA MULTIPLE MYELOMA

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Background. Hevylite (HLC) is an immunoassay that identifies and quantifies both isotypes of the tumor related immunoglobulin. *Objectives.* To present the utility of HLC in the monitoring of a patient with IgA Multiple Myeloma (MM). *Case report.* At diagnosis (Day 0) the proteinogram (SPE) showed a well-defined monoclonal peak (4.34 g/dl) identified by immunofixation (IFE) as IgA-κ. HLC (IgAK=66.604 g/l, IgAL=6.302 g/L, ratio=10.57) identified clonal disease IgA-κ too. The patient began treatment with Bortezomib-Cyclophosphamide-Dexamethasone and the monoclonal protein (MP) was monitored by SPE, IFE and HLC. During the treatment, the MP was decreasing showing a good response with reduction of the peak in SPE; however the HLC ratio remained altered confirming the persist-

ence of the low amounts of MP. At day +58 (after 4th cycle) there was a small peak in SPE (0.18 g/dl), with positive IFE and altered HLC ratio (IgAK=3.566 g/l, IgAL=0.664 g/l, ratio=5.37). At day +68, SPE was negative but the HLC ratio remained altered (IgAK=3.566 g/l, IgAL=0.664 g/l, ratio=5.37) confirming the existence of MP that was verified by IFE. At days +131 (after 5th cycle) and +184 (end of treatment after six cycles) the SPE and IFE were negative but HLC was altered confirming the existence of residual disease. The patient achieved a status of complete remission and he underwent autologous stem cell transplantation (Figure 1). *Conclusions.* Monitoring of IgA MM requires the measures of SPE, IFE and total IgA. The use of HLC presents itself as an alternative with high sensitivity for monitoring these patients, particularly in situations where traditional techniques show limitations (*e.g.* low concentrations, interference of other serum proteins, strong polyclonal background, among others). HLC showed a higher sensitivity than IFE in identifying residual disease. HLC allows typing MP providing equivalent information to IFE with the added value of reporting a quantitative value as SPE.

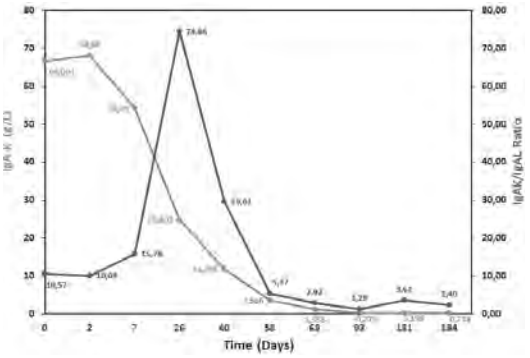

Figure 1.

A19 - IMPACT OF STRINGENT COMPLETE RESPONSE IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH NOVEL AGENTS

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Background. Normalization of serum free light chains (sFLC) ratio in patients with Multiple Myeloma (MM) achieving complete response (CR) may define a deeper response after therapy than obtained by the CR criteria. The stringent CR (sCR) requires normalization of sFLC ratio and absence of clonal plasma cells in bone marrow in addition to the criteria for CR. *Objectives.* To evaluate the impact of sCR in patients with newly diagnosed MM. *Methods.* Thirty patients with MM (14 IgG, 7 IgA, 2 IgD and 7 Bence Jones) achieving CR after therapy with Bortezomib/Dexametasone were included in this study. We studied Disease Free Survival (DFS or time after treatment where disease remains stable) as prognostic factor. DFS was estimated by Kaplan-Meier method and compared by log-rank tests. Serum free light chains were measured by turbidimetry (Freelite) in a SPA PLUS analyzer (The Binding Site Group Ltd, Birmingham, UK). *Results.* The median follow-up of the patients was 19 months (12-32 months). Fifteen patients achieved CR and 15 patients achieved sCR. During the period of study there were 10 relapses, seven in patients achieving CR and three in patients achieving sCR. The median DFS for patients achieving CR was 22 months and not reached for those achieving sCR. Patients achieving CR had a DFS rate of 21% compared with 73% for sCR (HR=4.83; 95% IC 1.16-20.04); p=0.019) (Figure 1). *Conclusions.* The presence of an altered sFLC ratio represents the existence of a persistent clonal population that is secreting very small amounts of monoclonal protein. Our results indicate that sCR represents a deeper response state compared with conventional CR. Analysis of sFLC ratio was able to identify the favorable group of patients and support the inclusion of sFLC ratio as part of the response criteria for MM.

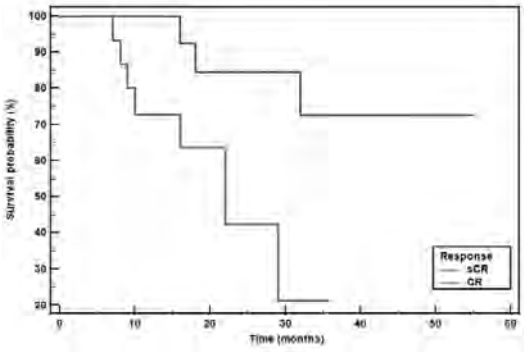

Figure 1.

A20 - CORRELATION OF IgA AND IgG HEAVY/LIGHT CHAIN ANALYSIS WITH M-PROTEIN MEASUREMENT BY CAPILLARY ZONE ELECTROPHORESIS

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Background. Capillary zone electrophoresis (CZE) and total immunoglobulins measurement (TIg) are standard techniques for the quantification of monoclonal protein (M-Ig). Hevylite® allows quantitative discrimination between heavy/light chain (HLC) Igκ and Igλ (involved/uninvolved) immunoglobulin pairs. We compared measurement of M-Ig by CZE and TIg to Hevylite®. *Methods.* 102 serial serum samples from 8 patients with IgA and 180 serial serum samples from 19 IgG intact multiple myeloma patients were prospectively

evaluated. M-Ig was measured using CZE (Sebia Capillarys 2 system) and TIgA, IgAκ/IgAλ and IgGκ/IgGλ HLC concentrations on a SPAPLUS analyser. Passing Bablok fit analysis was used to determine correlation between assays. *Results.* A total of 51/102 samples from IgA MM patients had detectable M-Ig by CZE. HLC analysis was performed on 34/51 samples of which 32/34 (94%) exhibited an abnormal HLC ratio. Measurement of involved HLC (iHLC) (median: 12.45g/L; range: 0.27-44.71g/L) compared well with M-Ig measurement by CZE (median: 11.04g/L; range: 1.2-37.17g/L) y=1.2x +2.40 R^2=0.94. TIgA was measured in 65/102 serial samples. Further, TIgA was above the normal range in 15/65 (23.1%) samples and in all 15 samples the HLC ratio was also abnormal. iHLC (median: 0.88g/L range: 0.05-21.55g/L) correlated well with TIgA (median: 1.44g/L; range: 0.227-21.11g/L l) y=0.85x+-0.85, R^2= 0.98. Changes in iHLC (median: 32.20% range: -96.36-347.8%) correlated with changes in M-Ig (median: 8.13%; range: -96.6-210%) y=1.59x +0.15 R^2= 0.94 and TIgA (iHLC median: -89.56% range: -97.74-80.66%, Median TIgA:-81.98, range: -96.78-57.04% (y=1.06x +0.01 R^2=0.95). For IgG MM patients, 144/180 samples had detectable M-Ig with 132/144 samples analysed for HLC. An abnormal HLC ratio was recorded in 92/132 (70%). Measurement of iHLC (median: 11.32g/L; range: 3.72-60.53g/L) correlated with M-Ig measured by CZE (median: 7.16g/L; range: 0.29-66.64g/L) y=0.83+5.2 R^2=0.81. Changes in iHLC (median: -4.53%; range: -81.62-489.7%) from baseline also correlated with changes to M-protein (median: -43.46%; range: -97.91-748.7%) y=0.90x+0.20 R^2=0.87. *Conclusions.* M-Ig measurement is comparable between Hevylite and CZE for monoclonal protein detection.

A21 - RETROSPECTIVE STUDY COMPARING THE USE OF STANDARD ASSAYS (IMMUNOFIXATION AND PROTEIN ELECTROPHORESIS) TO HEVYLITE ASSAY IN PATIENTS WITH MULTIPLE MYELOMA

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Background and Objectives. The performance of the Hevylite assay (HA) has not been adequately compared to standard assays (SA). The goal of this small retrospective study is to compare results achieved using both assays in the follow up of multiple myeloma (MM) patients. *Methods.* 44 patients who had undergone transplantation at MSKCC for MM had serum samples available, collected at time of relapse/Progression (R/P) and at time points preceding R/P. The samples were analyzed by HA and results compared to those achieved SA. *Results.* Among 44 patients, 25 had achieved CR, 7 VGPR, 4 nCR, and 5 PR by SA. Samples from CR patients collected at Relapse, yielded abnormal results by HA, in 5 out of 5 IgA patients, 5 out of 14 IgG patients, and 2 out of 6 FLC only patients. Samples from CR patients collected prior to relapse yielded abnormal results by HA in 4 out of 5 IgA patients, 1 out of 14 IgG patients, and 1 out of 6 FLC only patients). Among PR patients, 10 out of 16 available samples showed abnormal measurements by HA. The other 6 samples from FLC only patients were normal. Among 7 patients in VGPR, 19 out of 27 available samples showed abnormal measurements by HA; 8 were normal (5 FLC only patients, and 3 IgG patients). *Conclusions.* Although retrospective and limited, this analysis suggests: 1) HA is more sensitive than IF or SPEP in patients with IgA disease, detecting earlier disease relapse; 2) HA is of limited use in patient with FLC disease only, although it may rarely detect intact monoclonal immunoglobulins undetectable by IF or SPEP; 3) HA may be less sensitive than IF to detect relapse in IgG patients; 4) There is a need for further detailed analysis and comparison of HA and SA.

A22 - UTILITY OF HEAVY/LIGHT CHAIN AND FREE LIGHT CHAIN IN THE FOLLOW-UP OF THREE PATIENTS DIAGNOSED WITH WALDENSTRÖM MACROGLOBULINEMIA

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Introduction. There's a growing interest in the use of new serological markers for monitoring and predicting clinical outcome in Waldenström's macroglobulinemia (WM). New laboratory techniques have been developed requiring only a blood test to assess both disease progression and treatment response. The new test Hevylite® is being studied as alternative to the classical quantification of monoclonal peak by protein electrophoresis, a relatively low sensitivity test. Hevylite® is an immunoassay that allows the quantification of specific heavy/light chain immunoglobulin pairs (HLC), *i.e.*, IgMκ and IgMλ. *Objectives.* Evaluate the utility of the HLC IgMκ and IgMλ determinations in the monitoring of 3 WM patients. *Materials and Methods.* 25 samples of 3 MW patients were analyzed (mean follow-up 15 months). Two show the MYD88 L265P mutation. After treatment they reached a partial response. Freelite® (FLC) and Hevylite® were quantified in SPAPLUS turbidimeter (The Binding Site®, UK), monoclonal component (MC) by liquid-phase capillary electrophoresis (Capillarys, Sebia®) and/or agarose gel immunofixation (Hydrasis, Sebia®) and total immunoglobulins by nephelometry (BNII, Siemens®). *Results.* The presence of a MC in the proteinogram and total IgM concentrations above normal (RV: 40-230mg/dL) are observed in all determinations. HLC ratios never normalize and show the following average values (range): Pt1=0.021 (0.010-0.031); Pt2=809 (256-2186); Pt3=26.7 (22.3-34.6). The variations of the involved immunoglobulin levels determined by HLC closely match those determined by total IgM and the proteinogram (Figure 1).
Patient 2 shows a continuous suppression of the uninvolved HLC pair, (*i.e.* the IgM λ pair). *Conclusions.* The good agreement between the results obtained with

Hevylite and the other quantification and typification techniques shows that HLC is an excellent alternative to conventional techniques specially with difficult to quantify monoclonal components (co-migration, deficient migration, low concentrations, etc.) or doubtful results. Further follow-up studies are needed to assess the prognostic value of HLC in these patients.

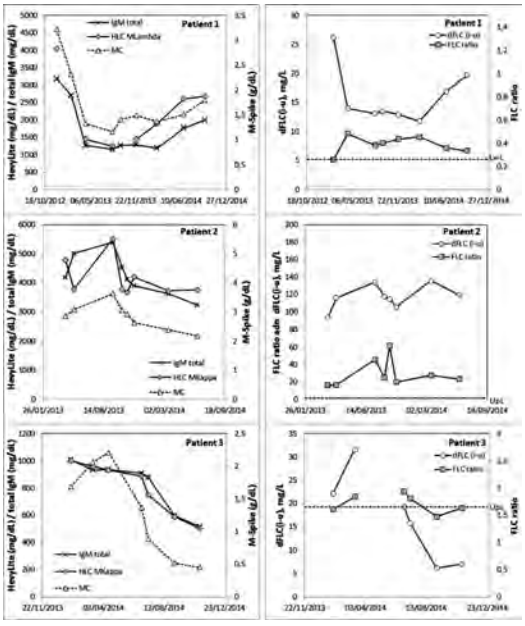

Figure 1. Monitoring of the monoclonal component (MC) of three WM patients using Hevylite (HLC), Freelite (FLC), proteinogram (M-spyke) and total IgM. The horizontal dashed line represents the lower (LwL) and upper (UpL) limits of the FLC ratio normal range (0.26-1.65).

A23 - HEAVY LIGHT CAN PROVIDE ADDITIONAL CLINICAL INSIGHT WHEN USED WITH TRADITIONAL TESTS FOR MONITORING IgA MYELOMA

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Background. HL is a new test to evaluate monoclonal gammopathy patients. *Objectives.* Evaluate 1) HL and FLC measurements in the context of relapse M-spike and/or normal electrophoresis results with abnormal FLC ratio and 2) value added of HL testing for identifying clonality and for monitoring disease progress. *Methods.* Under an approved IRB protocol, samples were collected from 37 patients who were being evaluated for a monoclonal gammopathy. ELP and FLC results were determined at various times during the patients' disease course. The HL values were analyzed in combination with clinical data points, and the ELP and FLC results were evaluated against the information gained from the HL results. *Results.* 24 (71%) of the patients had a MG. When HL was compared to ELP and FLC results 8 (22%) confirmed, defined or further defined the clone(s), 6 (16%) were consistent, 12 (32%) conflicted, and 11 (30%) did not have enough data to compare. Two cases illustrated how the HL assays could provide insight into a better understanding of the clinical presentation and course. In the two cases of Ig A subtype myeloma the HL were elevated despite improvement in or undetectable M-spikes. In one case, the HL increased as the FLC and M-spike decreased with therapy. This patient ultimately relapsed and succumbed to disease within 10 months of diagnosis. In the second case, an undetectable M-spike with normal FLC ratio was observed with an abnormal HL. One month following the abnormal HL levels the FLC ratio and M-spike rose prompting initiation of therapy. *Conclusions.* The HL assays can add value clinically to ELP and FLC testing for patients being evaluated for an MG.

The views expressed in this abstract/manuscript are those of the author(s) and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US Government.

A24 - POLYCLONAL ACTIVATION OF THE IMMUNOGLOBULIN DETERMINED BY FREE-LIGHT CHAIN ASSAY AFTER LENALIDOMIDE-TREATMENT IN PATIENTS WITH MULTIPLE MYELOMA PROLONGS TIME TO TREATMENT FAILURE

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Background. Lenalidomide is an immunomodulatory drug, which induced T-cell and NK-cell activation and is approved for treatment of patients with multiple myeloma. *Objectives of the study.* We investigated the effect of lenalidomide on polyclonal activation of immunoglobuline detected by Free-Light Chain Assay and on its impact on outcome in Multiple Myeloma patients. *Methods.* In this study, we included 61 patients with Multiple Myeloma (male=42, female=19) with a median age of 61 years (range 39-77 years), who received lenalidomide at a median dose of 15mg either as maintenance (n=28) or as salvage therapy (n=33) from 2006 To 2013 in our institution. The median duration of lenalidomide therapy was 14 months (range 1,94-69,09 months). 29,51% (n=18) received lenalidomide as single agent and 70,49% (n=43) in combination with Dexamethasone. All patients were inspected for the presence of polyclonal activation on the basis of free-light chain serum levels. "Polyclonal activation" was defined as an increase of both free-light chain serum levels out of normal range (range κ: 3,3 mg/l-19,4 mg/l; λ: 5,7 mg/l-26,3 mg/l). *Results.* 23 patients (37,70%) had shown a polyclonal activation during lenalidomide-treatment as described above (11/23 Partial Remission, 9/23 Complete Remission, 2/23 Stable Disease, 1/23 n/a). Comparing the time to treatment failure of the polyclonal activation population (n=23) and the non-polyclonal acti-

vation population (n=38), the polyclonal activation population had a significant higher median time to treatment failure (41,03 *vs.* 16,9months, p=0,049). The significance was meanly seen in patients with relapsed Myeloma (58,0 *vs.* 12,94 months, p=0,043) while in maintenance the difference (41,03 *vs.* 26,0 months) was not significant (p=0,40). *Conclusions.* Our study suggests that a polyclonal activation measured by free-light chain synthesis after lenalidomide therapy resulted in a prolonged time to treatment failure, especially for the relapse therapy population.

A25 - CASES OF LIGHT CHAIN ESCAPE FROM A SINGLE INSTITUTION IN POLAND

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Background. Intra-clonal heterogeneity in MM has become a well-accepted phenomenon. One such example is light chain escape (LCE) which is defined as an increase in monoclonal free light chains (FLC) without a corresponding increase in monoclonal intact immunoglobulins. Here we report cases of LCE from a single institution in Poland including progression of a biclonal MGUS. *Aims.* To evaluate routine sFLC measurement to identify intra-clonal heterogeneity in MGUS and MM patients. *Materials and Methods.* One MGUS and six MM patients (4 IgGκ, 2 IgAκ, 1 IgAλ) were serially monitored with SPE, IFE and sFLC (Freelite). *Results.* A biclonal MGUS patient (IgGκ: 3.9g/L, λ FLC: 316mg/L) was treated with methylprednisolone for polymyalgia rheumatica (PMR) that coincidently reduced the IgGκ M-protein (IFE trace) and normalised the κ/λ sFLC ratio. Four months before progression to MM, whilst the IgGκ concentration remained stable, the κ/λ sFLC ratio became abnormal and dFLC level increased to 1052mg/L (κ/λ ratio: 0.008), indicating emergence of a λ FLC clone. The patient progressed with severe renal impairment (creatinine 6.19 mg/dL; eGFR7.03 ml/min/1.73 m2) and 70% clonal bone marrow plasma cells. At this time the dFLC concentration had increased to 9726mg/L and the IgGκ M-protein was not detectable by IFE, indicating progression to λ light chain multiple myeloma. Of six MM patients with LCE, 2 relapsed with renal impairment, 2 with osteolysis, 1 with anaemia and 1 with extramedullary disease. For 3/6 patients, the dFLC concentration increased prior to clinical relapse (median 93.5 days; range 0-378 days) whilst the intact M-protein remained stable or undetectable. *Conclusions.* Intra-clonal heterogeneity may impact on disease progression, including transformation to malignant disease due to the selective outgrowth of a FLC producing clone. Serial monitoring of MGUS and MM patients with sFLC may be beneficial as delayed detection of LCE can result in irreversible organ damage.

A26 - HEAVY/LIGHT CHAIN ANALYSIS FOR RESPONSE MONITORING IN MULTIPLE MYELOMA PATIENTS: COMPARISONS WITH IMMUNOFIXATION, SERUM FREE LIGHT CHAIN, AND MULTICOLOR FLOW-CYTOMETRY ANALYSIS

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Background. Monitoring of MM patients is required to assess and determine treatment response. There are limited data available on the relationship between heavy/light chain (HLC) analysis, immunofixation electrophoresis (IFE), serum free light chain (sFLC) analysis, and multicolor flowcytometry (MFC) for response evaluation in patients with MM. *Aims.* To compare HLC immunoassays with conventional methods of disease evaluation. *Patients and Methods.* Three hundred and three samples from 101 patients with IgG and IgA MM after treatment were retrospectively analyzed with HLC immunoassays. Results were compared to SPE, IFE, sFLC assay, and MFC analysis. MFC negativity was defined as $<10^{-4}$ MM-PCs. HLC-pair suppression was defined as the uninvolved immunoglobulin of the same isotype <50% of the normal range. Overall survival and progression-free survival were calculated by the Kaplan–Meier method. *Results.* The number of patient samples at various responses were: sCR, *n*=86 (28%); CR, *n*=31 (10%); VGPR, *n*=116 (38%); and PR, *n*=70 (23%). A normal HLC ratio (HLCR) was obtained at sCR, CR, and VGPR in 73 (85%), 28 (90%), and 69 (59%) cases, respectively. Discordance between normalization of HLCR and FLC ratio was seen in 42% of patients with ≥CR. Abnormal HLCR was seen more frequently in IgA (19%) than IgG (7.7%) patients that achieved ≥CR. Simultaneous MFC data were available for 70 serum samples from patients with ≥CR. Of the 16 patients that were MFC negative, 12 (75%) had normal HLCR, while 4 were abnormal. Presence of an abnormal HLCR at best response and HLC pair suppression were also associated with poorer survival (Figure 1). *Conclusions.* The findings suggest the potential usefulness of HLC immunoassays for monitoring response in patients with IgA myeloma. Presence of an abnormal HLCR at best response and HLC pair suppression were also associated with poorer survival.

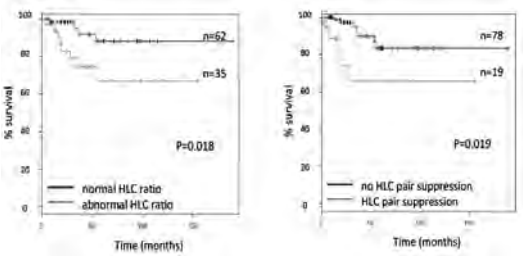

Figure 1. Overall survivals in patients with or without normal HLC ratio (A) and HLC pair suppression (B) at their best responses.

A27 - SERUM HEAVY LIGHT CHAIN RATIOS AMONG MULTIPLE MYELOMA PATIENTS ACHIEVING sCR/CR

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Background. The Heavy/light chain (HLC) assay (Binding Site, Birmingham, UK) is used to measure the ratio of monoclonal to polyclonal immunoglobulins, and may have a role in assessing depth of response and possibly may predict prolonged progression free survival (PFS). *Objectives.* We evaluated the association of normal/abnormal HLC ratios in IMWG defined sCR/CR patients using the standard serological testing. We have also evaluated if HLC ratio normalization may predict for longer PFS. *Methods.* 4179 sequential records of serum HLC, serum FLC , SPEP, SIFX, UPEP, and UIF for 964 multiple myeloma patients from Nov 2011 to May 2014 were analyzed. The normal FLC ratio (FLC ratio; normal range 0.26 to 1.65), and HLC ratios (IgGκ/IgGλ range 1.3 to 3.7 and IgAκ/IgAλ; range 0.7 to 2.2) were used for the analysis. *Results.* 732 patients were included for analysis -IgG κ: 339 pts (46.3%) IgG λ 183 pts (25%); IgA κ (18.2%) and IgA λ (10.5%). Males (58%) and Whites (52.5%) form the majority of the patients. sCR/CR per IMWG criteria were observed in 19.1% patients. A normalization of HLC ratio was observed among 38.1% of the patients. On univariate association with response, HLC ratio was normal in 66.43% of patients who were in serological sCR/CR (140 pts). Abnormal HLC ratio is seen in 33.57% (47 pts) in sCR/CR patients. *Conclusions.* These results suggest that in the era of novel agents, two-thirds of the patients achieving serological sCR/CR have normalization of HLC ratios. Results whether this normalization will result in longer PFS will be presented at the symposium.

A28 - SUCCESSFUL TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED/UNTREATED LIGHT CHAIN MULTIPLE MYELOMA WITH A COMBINATION OF BENDAMUSTINE, PREDNISONE AND BORTEZOMIB

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Background. Patients with light chain myeloma have frequently a light chain tubular cast nephropathy, which can lead to severe renal impairment. *Objectives.* Both bortezomib and bendamustine have been identified as quickly acting, effective and well tolerated drugs and might therefore constitute an adequate combination regimen for patients with newly diagnosed/untreated light chain multiple myeloma. *Methods.* Between September 2009 and March 2014, 20 patients with newly diagnosed/untreated light chain multiple myeloma were treated with bendamustine 60mg/qm on days 1 and 2, bortezomib 1.3 mg/qm on days 1,4,8 and 11, and prednisone 100mg on days 1,2,4,8 and 11 once every 21 days. 5 patients (25%) had a moderate or severe renal dysfunction (eGFR 15–59ml/min) and 9 patients (45%) a renal failure/dialysis (eGFR <15ml/min). *Results.* The median number of the BPV-treatment was 2 (1-5) cycles. 19 patients (95%) responded after at least one cycle of chemotherapy with 3sCR, 4nCR, 5VGPR, and 7PR. The myeloma light chains decreased rapidly, reaching the best response after the first cycle in 8 and after the second cycle in additional 9 patients. 16 patients discontinued therapy after median 2 cycles of BPV treatment to receive autologous (n=13) or autologous/allogeneic SCT (n=3). All together 10/14 patients with at least moderate renal failure improved their renal function (4CRrenal, 2PRrenal, 4MRrenal). 3 of the 6 dialysis-dependent patients became dialysis-independent. With a median follow up of 23 months of the surviving patients, median PFS and OS for patients at 24 months were 90% and 95%, respectively. The most common severe side effect was grade 3-4 leukocytopenia in 25% of the patients. Grade 3-4 thrombocytopenia was observed in 15% of the patients. Moderate to severe infection were seen in 4 patients. *Conclusions.* We conclude that BPV is effective and well tolerated in patients with newly diagnosed/untreated light chain multiple myeloma.

A29 - POLYCLONAL AND MONOCLONAL ANTIBODY BASED FREE LIGHT CHAIN ASSAYS PROVIDE DISCREPANT INFORMATION IN MULTIPLE MYELOMA PATIENTS

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Background. Guidelines for assessment of serum free light chain (FLC) in multiple myeloma (MM) patients are entirely based upon the polyclonal antibody-based Freelite assays. N-Latex-FLC, a new assay utilising monoclonal antisera, has become available. *Objectives.* To assess the analytical performance and usefulness of the new assay for monitoring MM patients. *Methods.* Serum samples from 83 MM patients (40 newly diagnosed, 43 relapsed) treated with bortezomib-containing regimens were analysed retrospectively with Freelite and N-Latex-FLC assays. Results were compared to historic data. *Results.* At baseline 60/83 (72%) patients had an abnormal ratio by Freelite (42 κ, 18 λ) and 58/83(70%) by N-Latex-FLC (38 κ, 20 λ). In 38 patients with an abnormal κ FLC ratio by both assays, FLC κ concentrations reported by either assay were significantly different (Freelite: 281.20 (7.82-8702.00)mg/L, N-Latex-FLC: 47.41 (3.94-4493.00)mg/L; p=0.005) and correlation poor ($y=0.49x+15.87$; $R^2=0.72$). Likewise, FLC λ concentrations in 18 patients with an abnormal λ FLC ratio by both

assays were significantly different (Freelite: 147.80 (18.37-994.80)mg/L, N-Latex-FLC: 68.79 (18.51-298.80)mg/L; p=0.02) and did not correlate (y=0.09x+68.85; R^2=0.14). In 61 patients with at least 2 follow-up samples, Freelite identified clonal disease in 4/61 patients (confirmed by IFE) when the N-Latex-FLC assay ratio had normalised; including a newly diagnosed oligosecretory IgG λ MM patient (M-Ig=1.15g/L by SPEP densitometry) with one year follow-up in which Freelite ratio remained abnormal throughout the patient's disease whilst N-Latex-FLC ratio normalised between days 55 and 279. IFE identified M-Ig at each time-point analysed, which was quantifiable by SPE densitometry (median: 0.94 (0.52-1.36)g/L. Furthermore, 1 patient with progressive disease was identified by the Freelite assay after 586 days, but only at day 670 by the N-Latex-FLC assay. *Conclusions.* MM patient assessment using the Freelite assay has formed the basis of international guidelines. The N-Latex-FLC assay failed to provide similar analytical and clinical information.

A30 - INTERIM RESULTS OF MCRN-001, A CANADIAN TRIAL BASED ON ACHIEVEMENT OF MINIMAL RESIDUAL DISEASE NEGATIVITY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION AND LENALIDOMIDE MAINTENANCE IN MULTIPLE MYELOMA PATIENTS

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Background. Methods other than conventional serum/urine electrophoresis and immunofixation (EP/IFE) are desirable for MM pts undergoing modern therapy. Measurement of bone marrow MRD by multiparameter flow cytometry (MPF) represents one approach; achievement of MRD-negativity correlates with improved outcomes post-ASCT. Also, the serum Hevylite™ chain (HLC) assay offers a more sensitive method to assess the intact monoclonal immunoglobulin (Ig) present in most pts. We initiated a Canadian study in 10 centres in which newly diagnosed MM pts with successful baseline MPF received bortezomib-based induction followed by high-dose melphalan+busulfex and ASCT; len maintenance was commenced on day 100 post-ASCT and continued until progression. *Objectives.* The primary goal was assessment of MRD negativity on day 100 post-ASCT. Secondary goals included determination of the: 1) utility of the HLC assay at day 100 and during maintenance; 2) pattern of marrow MRD during maintenance; 3) progression-free and overall survival rates; 4) toxicity. *Methods.* Marrow was examined using 15-color MPF at diagnosis, before ASCT, day 100 post-ASCT then every 3 months for the first year and every 6 months thereafter; serum HLC assay was performed concomitantly. Conventional serum/urine EP/IFE and free light chain assays were performed monthly after ASCT. *Results.* 58 of a planned target of 78 pts have undergone ASCT. At day 100, 11/33 (30.3%) evaluable pts are MRD-negative (5 CR, 5 VGPR, 1 pending); all 6 with HLC results have normal ratios of the involved/uninvolved protein. Of the 20 MRD-positive pts, HLC ratios were abnormal (6), normal (6) and pending (8). After a median follow-up of 11 months, only 1 pt has relapsed. *Conclusions.* Preliminarily, day 100 MRD-negativity was associated with a normal serum HLC ratio for the involved Ig. Further follow-up is required to determine the relationship between these assays and the clinical course of study pts.

A31 - SERUM FREE LIGHT CHAINS IN MONOCLONAL GAMMOPATHIES. LOCAL EXPERIENCE

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Introduction. Serum κ and λ light chains quantitative immunoassay (sFLC) is recommended for screening, prognosis and response assesment of monoclonal gammopathies (MG). In our country its determination was not widely available until the development of this project. *Objectives.* To describe the use of sFLC international guidelines for screening and monitoring of GM. To compare sFLC *versus* total serum light chains. *Methods.* sFLC were evaluated in MG patients according to IMWG guidelines. FLCs were performed per manufacturer's instructions. Total light chains were determined by nephelometry. *Results.* 67 sFLC samples were analyzed (39 women/28 men): 15 MGUS, 7 AL, 37 MM, 2MM/AL and 6 SMM. Median age was 61 years (range 43-84). Eight patients (20%) had negative electrophoresis and immunofixation; sFLC detected monoclonality in 6 (3 κ, 3 λ), corresponding to 2 AL and 4 MM. sFLC identified and/or classify 10% of patients. sFLC allowed adequate prognostic stratification in MGUS in low-risk (12) and intermediate-low (3). SMM (6) were stratified as low (2), intermediate (3) and high-risk (1); 4 had abnormal ratio. At 16 months of follow-up none has progressed to MM. 3 patients with AL amyloidosis were analyzed. In 2, sFLC allowed their classification (λ) and in one, sustained increase of FLC ratio determined early re-treatment and transplantation. sFLC was performed

in 39 MM; 47% had renal failure. 4 LCMM (3κ and 1λ) were detected; in 1, abnormal FLC ratio detected early relapse. sFLC confirmed stringent complete response in 5 patients. In 27 cases sFLC was compared to total free light chains; results were discordant in 9/27 (Kendall´s tau-b=0,67). *Comments.* sFLC allowed identification and adequate follow-up of MM and AL according to international guidelines. Total serum light chains should not be used as sFLC surrogate. (MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering MM; AL, systemic AL amyloidosis; LCMM, light chain MM).

A32 - COMPARATIVE ASSESSMENT OF MYELOMA RESPONSE TO INDUCTION TREATMENT IN THE GMMG MM5 STUDY USING IMWG CRITERIA AND HEVYLITE ASSAY

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Background. Treatment of multiple myeloma has substantially improved over the last decade. However response assessment still relies on parameters such as serum protein electropheresis or immunofixation. A new response category of stringent CR was introduced using information on serum free light chains and bone marrow clonality. Recently the hevylite assay measuring κ and λ-restricted IgG and IgA was introduced. *Objectives.* To analyse the value of using the hevylite assay for MM response. *Methods.* The MM5 study of the German GMMG randomised 604 newly diagnosed myeloma-patients to receive bortezomib, dexamethasone and either adriamycin or cyclophosphamide as induction before high-dose chemotherapy. Response after 3 cycles of induction was assessed using IMWG criteria. Clonal IgG and IgA was measured by hevylite in frozen serum samples from study baseline and after induction in 154 patients. 109 patients had IgG-paraprotein with 84 (77%) κ and 25 (23%) λ while 45 patients had IgA with 34 (76%) κ and 11 (24%) λ. Response to induction according to IMWG criteria was CR in 8 (5.2%), nCR in 19 (12.3%), VGPR in 27 (17.5%), PR in 71 (46.1%), MR in 19 (12.3%), SD in 7 (4.5%) and missing in 3 patients (1.9%). No patient had sCR based on free-light chain ratios. The frequencies of normal hevylite κ/λ ratios are shown in Table 1. Persistent pair suppression of non-clonal Ig was found in 85/109 (78%) for IgG and 30/46 (65%) for IgA. *Conclusions.* Triplet induction achieved a CR/nCR rate of nearly 20%, while hevylite κ/λ normalisation occurred in 7.5% (IgG) and 24% (IgA). There was little overlap to IMWG response, thus hevylite response not merely mirrors paraprotein response, but also captures suppression of the non-involved pair. It seems warranted to validate hevylite response after induction treatment with progression-free and overall survival once further follow-up data becomes available.

Table 1.

	CR	*nCR*	*VGPR*	*PR*	*MR*	*SD*	*Missing*
IgG	1/2 (50%)	2/4 (33.3%)	1/20 (4.8%)	3/52 (5.5%)	0/16	1/4 (20%)	0/2
IgA	4/6 (66.7%)	2/13 (15.4%)	2/6 (33.3%)	3/15 (20%)	0/3	0/2	0/0

A33 - IMMUNOGLOBULIN SUPPRESSION IN IFE AND SPE POSITIVE MULTIPLE MYELOMA MONITORING SAMPLES BY HEVYLITE™ AND TOTAL IMMUNOGLOBULIN ASSAYS

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Background. Measuring monoclonal protein (M-protein) concentrations in myeloma patients as monitoring tool can be challenging, especially at low concentrations, due to imprecision inherent in electrophoresis, and the presence of interfering proteins. Immunoparesis, as measured by suppression of the non-tumor immunoglobulin proteins (Ig), has recently been reported as a clinically useful and prognostic tool for evaluation of myeloma patient outlook and treatment. We compared non-tumor Ig by Hevylite™ (HLC) and Total Ig assays in IgG and IgA myeloma monitoring patient samples. *Methods.* The HLC and Total Ig assays were run on a BNII™ nephelometer and published reference ranges were used. Only IFE positive samples (Sebia Hydrasys) with measured monoclonal protein (Sebia Capillarys) of less than 20 g/l (30 IgGκ, 9 IgGλ, 20 IgAκ, 15 IgAλ) were evaluated in this study. *Results.* The mean and range concentrations by HLC (g/L) and Total

(g/L), respectively, for each sample set were; IgGκ 9.04 (3.81-24.3) and 12.4 (4.82-39.3), IgGλ 6.17(3.03-18.9) and 9.85 (4.66-24.5), IgAκ 10.44 (2.78-22.7) and 8.26 (2.32-22.0), IgAλ 5.63 (1.34-18.30) and 5.88 (1.41-18.30). By HLC, 31/39 (80%) of the IgG MM samples and 24/35 (69%) IgA MM samples were below normal concentrations of the uninvolved Ig (*i.e.* Ig'λ in Ig'κ MM samples and Ig'κ in Igλ MM samples). When measuring Ig suppression by the Total Ig assay, 25/39 (64%) of IgG MM samples were below normal for IgA concentrations and 20/39 (52%) were low for IgM. In the 35 IgA MM samples, 29 (83%) were below normal for IgG concentrations and 28 (80%) were low for IgM. *Conclusions.* HLC appears to be a relatively sensitive assay for measuring suppression of the non-tumor Ig, especially in IgG MM patient samples.

A34 - POTENTIAL PITFALLS OF SERUM LIGHT CHAIN ANALYSIS TO ASSESS TREATMENT RESPONSE

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Background. The introduction of serum free light chains has been a tremendous help in assessing response to treatment of myeloma patients. The International Myeloma Working Group proposed uniform response criteria including a new definition of stringent complete remission (sCR). The definition of sCR requires absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence and normalization of free light chain ratio in serum. *Objectives of the study.* To report potential pitfalls with the serum free light chain analysis. *Methods.* Measurement of κ and λ FLC (Freelite, Binding Site, UK), and the calculation of a κ/λ sFLC ratio to provide a sensitive indicator of clonality. *Results.* Serum free light chains may increase on treatment, especially with intensive treatment while patients subsequently show an excellent response to therapy. Post-transplantation, there may be a very pronounced immune deficiency, especially after tandem autologous transplants, where the κ/λ ratio cannot be calculated because one or both serum light chains are below the lower limit of detection. Also, there may be abnormal immune recovery resulting in abnormal κ/λ ratios, while patients are in otherwise a stringent complete remission. This is easy to ignore if the light chain increasing above the normal range is the opposite of the involved light chain. However, if it can happen with the non-involved light chains, it must also happen with the involved light chains increased with multiple sequential abnormal κ/λ ratios over time, while there is no other evidence of disease for periods of more than 12 months. Illustrative examples of such cases will be presented. *Conclusions.* Increase in involved light chains on therapy does not necessarily mean lack of response and need for alternative therapy. An abnormal κ/λ ratio does not necessarily exclude a stringent complete remission.

A35 - SERIAL CHANGES IN SERUM FREE LIGHT CHAINS PRIOR TO THE DIAGNOSIS OF AL AMYLOIDOSIS AND MULTIPLE MYELOMA

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Background. We have previously demonstrated that a monoclonal gammopathy precedes the presentation of all patients with immunoglobulin light chain amyloidosis (AL) and multiple myeloma (MM). Serum free light chains are commonly assayed during the monitoring of monoclonal gammopathy of undetermined significance (MGUS). The serial changes in serum free light chains prior to the development of AL and MM have not been described in detail and may helpful in the monitoring of MGUS. *Methods.* We will analyze the serial changes in serum free light chains obtained from the pre-diagnostic sera of patients with AL and MM. We will present data on serial changes on involved light chains, free light chain differential and serum free light chain ratios. *Results and Conclusions.* To be presented at the meeting.

DIAGNOSTIC ALGORITHMS FOR MULTIPLE MYELOMA

B36 - SLOW ADOPTION OF THE RECOMMENDED TEST COMBINATION FOR MULTIPLE MYELOMA SCREENING

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Background. The International Myeloma Working Group issued consensus guidelines in 2009 recommending the combination of serum protein electrophoresis, serum immunofixation, and serum free light chains for screening for multiple myeloma ("recommended test combination"). The clinical laboratories in Intermountain Healthcare have not made any concerted effort to promote the use of the recommended test combination. *Objectives.* To assess the frequency of the recommended test combination in initial orders for protein electrophoresis and related testing. *Methods.* In this retrospective, observational study, orders for serum and urine protein electrophoresis, immunofixation electrophoresis, and free light chains in the years 2004-2014 were extracted from an enterprise data warehouse. Orders from oncologists and hematologists were excluded. The initial testing episode was identified for each patient. The study set was comprised of orders from initial testing episodes in the years 2009-2014. Orders in the study set were classified as "Recommended test combination" or "Other." The association between the frequency of the recommended test combination and the year was tested using the chi-squared goodness-of-fit test. *Results.* As shown in the Table 1, the number of initial testing episodes was relatively constant, ranging from 4081 in 2013 to 4360 in 2010. The frequency of orders for the recommended test combination increased from 6 (0.1%) in 2009 to 92 (2.2%) in 2014, p <0.0001. *Conclusions.* Between 2009 and 2014, there was a statistically significant increase in the frequency of orders for the recommended test combination; however, the recommended test combination constituted only a small proportion of orders. Interventions need to be identified to promote the use of the recommended test combination.

Table 1. Frequency of recommended test combination in initial orders by year.

Orders in initial testing episodes	*2009*	*2010*	*2011*	*2012*	*2013*	*2014*	*Total*
Recommended test combination	6 (0.1%)	9 (0.2%)	13 (0.3%)	12 (0.3%)	40 (1.0%)	92 (2.2%)	172 (0.7%)
Other	4251	4360	4172	4260	4041	4056	25,140
Total	4257	4369	4185	4272	4081	4148	25,312

p <0.0001

B37 - DIAGNOSIS AND MONITORING OF MYELOMA AND RELATED DISEASES – TWO DISTINCT ANALYTICAL PROBLEMS

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Background. To evaluate the current use of tests available for the diagnosis and monitoring of myeloma and to assess the efficiency and value of individual assays. *Aims.* To establish requesting protocols which ensure efficient diagnosis of disease and safe monitoring of previously identified patients. *Methods.* Audit of the effectiveness of addition of free light chains tests, post electrophoresis reporting following the identification of a paraprotein or hypogammaglobulinaemia. Free light chains were added to all samples with a newly identified paraprotein or hypogammaglobulinaemia. Comparison of the results obtained on patients with known paraproteins, under treatment and surveillance. *Results.* In 45% of the cases with a newly identified paraprotein, elevated free light chains or an abnormal ratio were identified. 35% of new patients with hypogammaglobulinaemia as identified by either electrophoresis or total immunoglobulin assay, (less than 5g/L total Ig) had either elevated free light chains or an abnormal free light chain ratio. Serial results on individual patients showed changes in monoclonal protein concentration could be monitored either by paraprotein concentration, electrophoresis total fraction or total immunoglobulin level although there was poor correlation between paraprotein concentrations and immunoglobulin levels when the IgA or IgM were greater than 35. *Summary/Conclusions.* All patients with suspected myeloma should be screened using electrophoresis, immunoglobulin levels, total protein levels and serum free light chains. Patients with known disease can be monitored using total immunoglobulin and elevated free light chain levels only, until paraprotein production is suppressed, when capillary zone electrophoresis should be reinstated to check for relapse.

B38 - USE OF FREELITE IN RESPONSE TO SPECIFIC SELECTION CRITERIA FOR NEW PATIENT INVESTIGATION

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Background. Most laboratories use serum electrophoresis (SPE) and urine electrophoresis (UPE) for detecting M-proteins. SPE is sensitive for monoclonal intact immunoglobulins (mIg) and UPE is sensitive for monoclonal free light chains (mFLC). Most plasma cell dyscrasias, eg multiple myeloma (MM), will secrete mIg (~80%), ~20% will secrete only mFLC and <1% are non-secretors. Therefore, MM cannot be excluded unless both SPE and UPE are performed. However, mFLC may not always be deposited in the urine and only ~5-52% of routine MM investigations are accompanied by a urine sample. As a result, there is growing evidence to support the use of serum Freelite (sFLC) as an adjunct to SPE and potential replacement of UPE. Some laboratories have

taken a pragmatic approach and will only use sFLC on patients who meet certain criteria that increase the probability of MM. *Methods.* The aim of this study was to evaluate whether sFLC analysis in specifically selected patients would lead to increased identification of MM. Consecutive samples were collected over a 3 month period. Any sample that was SPE negative but had either anaemia, renal insufficiency, hypercalcaemia, bone lesions/fracture or had a specific request for a MM screen, was analysed with Freelite. *Results.* Samples from 136 patients were collected. Four of the 136 patients (3%) had an abnormal sFLC ratio. One was confirmed in a follow-up sample and on repeat testing the urine sample also tested positive for FLC. A second patient had normal sFLC and negative urine Immunofixation on follow-up and was thus assumed to be a false positive. Repeat samples could not be obtained for the remaining two patients. *Conclusions.* This study shows that specific selection criteria can be used to identify patients with sFLC disorders, otherwise not detected by SPE and UPE or when urine is not supplied.

B39 - SERUM FREE LIGHT CHAIN ASSAY INCREASES THE OVERALL SENSITIVITY IN SCREENING OF PLASMA CELL DYSCRASIAS: LABORATORY EXPERIENCE

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Background. The International Myeloma Working Group (IMWG), as the best screening strategy to detect monoclonal component (MC), indicates to perform only serum test (electrophoresis (PE), immunofixation (IF) and free light chain (FLC) ratio) excepted for amyloidosis AL, condition that requires also urine examination. *Methods.* We report 3 cases of our routine that highlighted the usefulness of serum FLC assessment and its ratio in revealing the presence of MC. All these cases arrived from the department of Haematology and the following exams had been required: serum PE and IF (Sebia, Firenze), serum FLC (Freelite ™, Binding Site, Birmingham) and urinary IF (Sebia, Firenze). *Results.* In the two cases we observed normal serum PE, negative serum IF, altered FLC values and its ratio (1st: k 198.06 mg/L, λ 14.13 mg/L, k/λ 14.01; 2nd: k 44.33 mg/L, λ 7.10 mg/L, k/λ 6.23), and k Bence Jones proteinuria (BJp) positive. Considering the unexpected result of serum IF, we performed again serum IF using the analytical approach of IF own of urinary samples (more sensitive). Nevertheless, serum IF did not change. This result suggests that FLC test increases the overall analytical sensitivity. The other case was a woman with serum PE negative, very high FLC levels (k 5617.10 mg/L, λ 0.60 mg/L, k/λ 9361.83) and urinary IF positive (k BJp). We decided to run serum IF (not requested in this case) and the result was negative. Once again, serum IF with the approach of urinary samples was conducted and the result became positive (k light chain only, without heavy chain). *Conclusions.* The cases aforementioned, in agreement with IMWG guideline, strengthen the role of FLC in improving the screening of monoclonal gammopathies in particular in case of micromolecular disease.

B40 - THE HUNT FOR "RARE MGUS" DIAGNOSES: A MODEL FOR BETTER AND EARLIER DETECTION

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Background. Rare MGUS pathologies such as POEMS syndrome, Clarksons disease and AL-amyloidosis, are often diagnosed too late. Small clones of plasmacells produce low quantities of paraproteins with abnormal properties inducing damage in different organ systems. These patients often present with diverse symptoms at non-haematologic departments (cardiology,neurology,dermatology and intensive care-unit). *Objectives.* We implemented a MGUS-consulting model in our non–university hospital , linking the clinical laboratory and involved clinical departments. Our inspiration came from the ASH, State-of-the-art Symposium, Chicago, 2011 September. Prof. Angela Dispenzieri: "Weilding one's way between monoclonal gammopathy-associated neuropathy, nephropathy and dermopathy". "If you don't know them, you will miss them". *Methods.* Our weapons: • bidirectional MGUS-consulting between lab and clinician: MGUS-Neuropathy, MGRS (monoclonal gammopathy of renal significance), cardiology and dermatology; • "monoclonal gammopathy" workshops with involved clinical departments; • capillary-zone-electrophoresis, serum- and urine-protein immunofixation; • measurement of serum free light chain(sFLC); • vascular endothelial growth factor(VEGF) in serum/plasma.

Table 1.

AL AMYLOIDOSIS				*POEMS*				
Patient	κ *(mg/L)*	λ *(mg/L)*	*Ratio*	*Patient*	κ *(mg/L)*	λ *(mg/L)*	*Ratio*	*VEGF (pg/mL)*
1	12.6	471.7	0.03	1	25.2	35.2	0.72	2050
2	2261	1.8	1228.8	2	466.2	16.8	27.70	1200
3	53.3	30.9	1.73	3	27.7	21.0	1.32	1500
4	480	8	60.0	4	27.7	34.2	0.81	1300
5	71.1	40.8	1.73	5	4.3	1730.3	0.002	ND
6	20	181	0.11	*CLARKSON*				
7	11	400	0.03		κ *(mg/L)*	λ *(mg/L)*	*Ratio*	*VEGF (pg/mL)*
8	49.8	19.6	2.55	Attack 1	29.2	20.7	1.41	350
				Attack 2	24.9	19.6	1.27	300
				Attack 3	13.2	12.5	1.05	400

Reference value serum VEGF <500 pg/mL; reference value sFLC ratio 0.26-1.65.

Results. In this prospective application of our model for diagnosis of rare MGUS cases, the harvest exceeded clearly the statistically expected numbers (www.orpha.net). Our population covered +/-150.000 inhabitants (region Ghent, Belgium). Patients were diagnosed between October 2011-October 2014 and are listed below: • AL-amyloidosis: N=8 [Echocardiography and sFLC (Table 1)]; • POEMS syndrome: N= 5 [elevated serum VEGF and normal sFLC in 3/5 cases (Table 1)]; • systemic capillary leak syndrome (Clarkson): N=1 (3 attacks) [intensive care consult: first case (*in vivo*) in Belgium; • a general psoriasiform eruption with exacerbations: N=1 [dermatology consult]. *Conclusions.* 1) a multidisciplinary diagnostic approach led to more and earlier diagnoses; 2) earlier diagnoses led to earlier therapy and subsequent better prognosis.

B41 - USE OF AN OPTIMIZED PROTOCOL AT THE EMERGENCY SERVICE TO AVOID DELAY IN THE DIAGNOSIS OF MULTIPLE MYELOMA

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Background. Recent evidence has shown that myeloma patients experience one of the highest rates of delay in diagnosis amongst cancer patients, with 50% requiring three or more consultations before being referred to a haematologist. Thereby, the presence of incidental clinical findings (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia, acute kidney injury) related to Multiple Myeloma (MM) in Emergency Service and Primary Care should be studied for screening the existence of a possible MM. A quick assay panel based on serum protein electrophoresis (SPE) and quantification of serum free light chains (sFLC) enables sensitive probing of monoclonal components in the study of suspected MM. The application of this screening panel in patients with these incidental clinical finding without other diagnosis can help us to efficiently detect a possible MM in much sorter times. *Objectives.* Show the utility of this screening panel (SPE+sFLC) in patients with incidental clinical findings attending the Emergency Service. *Methods.* We studied eight patients where we found incidental clinical finding characteristic of MM. Sera of the patients were sent to the Immunology Lab for the screening of a monoclonal protein. SPE were performed on Capillarys 2 (Sebia) and sFLC were measured with Freelite (The Binding Site) by turbidimetry. Positive results of the screening panel remit the patient to the Hematology Service to complete the study. *Results.* The results are shown in the Table 1. *Conclusions.* In the context of clinical symptoms (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia) that alerts to a possible MM case in patients without obvious clinical diagnosis, we found the application of this protocol (SPE+sFLC) to be efficient and advisable. The combination of SPE and sFLC yields a fast and highly sensitivity approach in the screening of monoclonal gammopathies which in the context of the emergency service is of particular importance.

Table 1.

Case	Sex	Age (years)	Clinical Finding at Emergency Service	SPE	sFLC	Diagnosis
1	F	67	Hyperproteinemia (12 g/dl)	Large peak (4.18 g/dl)	KL= 10.47 mg/l LL=99.59 mg/l Ratio=0.11	MM IgG Lambda Stage 2 ISS
2	F	65	Hyperproteinemia, hyperviscosity and thrombocytopenia	Large peak (3.28 g/dl)	KL=617 mg/l LL=11.1 mg/l Ratio=55.59	MM IgG Kappa Stage 2 ISS
3	F	64	Intense back pain	Large peak (3.22 g/dl)	KL=3.15 mg/l LL=102 mg/l Ratio=0.031	MM IgA Lambda Stage 3 ISS
4	F	55	Pathological fracture at D12	Two weak peaks (0.15 g/dl)	KL=28600 mg/l LL=5.36 mg/l Ratio=5335.82	Light Chain Kappa MM Stage 3 ISS
5	M	12	Hypercalcemia (16.6 mg/dl)	Very large peak (4.34 g/dl)	KL=229 mg/l LL=1.01 mg/l Ratio=216.83	MM IgA Kappa Stage 3 ISS
6	M	68	Pathological fracture at D11 and L1	Very large peak (5.19 g/dl)	KL=835 mg/l LL=6.74 mg/l Ratio=123.89	MM IgG Kappa Stage 3 ISS
7	F	80	Acute renal injury (creatinine 3.4 mg/dl)	Large peak (4.94 g/dl)	KL=5600 mg/l LL=35.8 mg/l Ratio=354.43	MM IgG Kappa Stage 3 ISS
8	M	54	Anemia and thrombocytopenia	Small peak (1.17 g/dl)	KL=3.22 mg/l LL=4025 mg/l Ratio=0.0008	MM IgD Lambda Stage 3 ISS

B42 - HEALTH ECONOMIC MODEL ON SERUM FREE LIGHT CHAIN ASSESSMENT FOR CAST NEPHROPATHY DETECTION IN PATIENTS PRESENTING WITH UNEXPLAINED ACUTE KIDNEY INJURY

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Background. Renal recovery in acute kidney injury (AKI) secondary to multiple myeloma (MM) relies on early myeloma treatment; hence efficient time to diagnosis. The International Kidney and Monoclonal Gammopathy (IKMG) Research Group recommend serum free light chain (sFLC) assays alongside serum protein electrophoresis (SPE) when screening for AKI secondary to MM. *Objectives.* We report on a health economic model that considers implementing the IKMG recommendations *versus* standard serum and urine electrophoretic techniques. *Methods.* A decision tree structure from presentation to time of diagnosis followed by a Markov model was utilised with a 1-year time horizon. SPE+sFLC was compared to SPE plus urine electrophoresis (SPE+UPE), SPE alone (positive results were followed by serum and urine immunoelectrophoresis [sIFE & uIFE]) and SPE+UPE+sIFE+uIFE in parallel. Survival was estimated on AKI stage and UK data stratified for renal recovery in the diagnostic and treatment pathways, respectively. Probability of renal recovery was based on time to diagnosis. Utility values were estimated using the Modification of Diet in Renal Disease equation and published values for MM, applying a decrement for dialysis-dependence, in the diagnostic and treatment pathways,

respectively. Baseline patient distribution: 10% Stage 2 AKI, 90% Stage 3 AKI (50:50 dialysis dependent/independent), 1% cast nephropathy incidence and 5-day time-penalty applied for false-negative results. Clinician consultation confirmed model structure and assumptions. *Results.* The model predicts sFLC+SPE to be dominant against all comparators accruing least costs and most quality adjusted life years (QALYs; Table 1). Time to diagnose all patients was 8, 13, 12 and 10 days for SPE+sFLC, SPE+UPE, SPE and SPE+UPE+sIFE+uIFE pathways, respectively. Savings were driven by reduced in-patient stay and dialysis costs in the diagnostic phase and reduced dialysis costs in the treatment phase. Probabilistic and one-way sensitivity analyses showed the model to be robust. *Conclusions.* The model shows including sFLC alongside SPE provides patient benefits and is cost effective.

Table 1. Differences in cost effectiveness results for the base case.

Comparator	*Incremental Model Results*	*Difference*		
		Diagnostic phase	*Treatment phase*	*Total*
SPE plus sFLC	Cost per patient tested	-£31.42	-£37.06	-£68.48
vs SPE plus UPE	Cost per patient with cast nephropathy	-£3,141.58	-£3,706.58	-£6,848.16
	QALY (Quality adjusted life years) per patient tested	-0.00023	0.00157	0.00133
SPE plus sFLC	Cost per patient tested	-£6.52	-£33.80	-£40.32
vs SPE	Cost per patient with cast nephropathy	-£652.22	-£3,380.02	-£4,032.25
	QALY per patient tested	-0.00014	0.00113	0.0010
SPE plus sFLC	Cost per patient tested	£5.19	-£40.81	-£35.62
vs SPE, UPE, sIFE & uIFE	Cost per patient with cast nephropathy	£519.11	-£4,081.02	-£3,561.91
	QALY per patient tested	£519.11 -0.00003	-£4,081.02 0.00058	-£3,561.91 0.00055

B43 - INCORPORATING FREELITE IN THE MONOCLONAL PROTEIN SCREENING PANEL FOR IMPROVED DETECTION OF MULTIPLE MYELOMA

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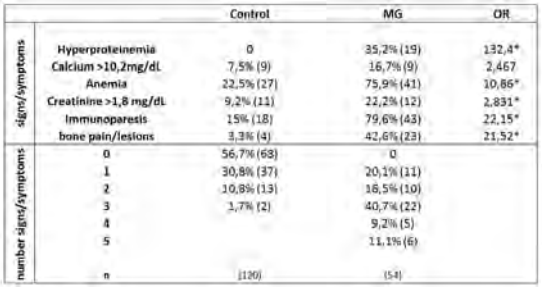
Background. Serum protein electrophoresis (SPE) is the most common technique for query monoclonal proteins (MP); however, isolated, it does not warrant enough sensitivity to identify all monoclonal gammopathies (MG). Adding immunofixation (IFE) and serum Free Light Chains (sFLC) optimizes the diagnostic sensitivity while reducing the need for urinalysis at the screening step. In our laboratory, ≈3000 SPE/month are requested, of which only a fraction corresponds to MG queries. Therefore, there is a need for optimizing the MP detection protocol and the analytical profile that raise the suspicion of MG. *Objectives.* To evaluate the most sensitive techniques for screening MP. *Materials and Methods.* The records of 72 MM/Amiloidosis patients and 120 patients without MG were reviewed. SPE and IFE were performed using Capillarys 2 and Hydrasys (Sebia), and sFLC determined by Freelite (Binding Site). *Results.* SPE, serum and urine IF, sFLC were available for 50 MM, 1 non-secretory MM, 1 amyloidosis and 1 myeloproliferative disease at diagnosis: 42 SPE, 48 IFE, 48 sFLC, and 42 urineIF were positive. SPE combined with sFLC identified 52 cases, SPE+IFE 49 and SPE+urineIF 51. The non-secretory MM was urineIF positive with 1,91 κ/λ sFLC and negative for SPE/serumIFE/UPEP. The following parameters were used for profiling signs of suspected MM: calcium >10,2mg/dL, Creatinine >1,8 mg/dL, anemia, immunoparesis (IgG, IgA or IgM below reference values), hyperproteinemia >8,7 g/dL, bone pain/lesions. 54 MM/amiloidosis patients diagnosed between 1/01/2011-2014 were included. Immunoparesis and anemia were the most frequent symptoms: 79,6% and 75,9%, respectively, compared to 15% and 22,5%, in a control population, respectively. (Table 1) Patients presented most frequently with 3 signs/symptoms, but noteworthy 20,1% presented with only 1 symptom. *Conclusions.* SPE+sFLC was the simplest panel with highest sensitivity for screening MP. In this population, all signs/symptoms but hypercalcemia associated significantly with the presence of MG.

Table 1. Frequency of each signs/symptoms and frequency of accumulation at presentation.



		Control	MG	OR
signs/symptoms	Hyperproteinemia	0	35,2% (19)	132,4*
	Calcium >10,2mg/dL	7,5% (9)	16,7% (9)	2,467
	Anemia	22,5% (27)	75,9% (41)	10,86*
	Creatinine >1,8 mg/dL	9,2% (11)	22,2% (12)	2,831*
	Immunoparesis	15% (18)	79,6% (43)	22,15*
	bone pain/lesions	3,3% (4)	42,6% (23)	21,52*
number signs/symptoms	0	56,7% (63)	0	
	1	30,8% (37)	20,1% (11)	
	2	10,8% (13)	18,5% (10)	
	3	1,7% (2)	40,7% (22)	
	4		9,2% (5)	
	5		11,1% (6)	
	n	(120)	(54)	

*, significant (Fisher's exact test <0,05).

B44 - IMPACT OF FREE LIGHT CHAINS IN DIAGNOSIS OF MONOCLONAL GAMMOPATHIES

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Background. Low sensitivity of electrophoresis tests lead to some difficulties in the diagnosis of monoclonal gammopathies, particularly for free light chains and non-secretory /oligo-secretory diseases. Serum free light chains assay is recommended in screening of plasma cell dyscrasias (PCPD). *Objectives.* To determine the sensitivity of the combination of tests to detect monoclonal gammopathies and the contribution of Free Light Chain (sFLC) assays as part of the screening algorithm. *Methods.* sFLC assay was performed in frozen samples

from 113 patients diagnosed at INEN, with a monoclonal gammopathy as initial diagnosis of PCPD, who had serum / urine protein electrophoresis and immunofixation within 30 days of diagnosis. *Results.* The sensitivity of the tests and combination of tests are shown in Table 1. An abnormal sFLC Ratio was detected in 82.3% of PCPD: 86% of MM, 50% of Plasmacytoma, 83.3% of Light chain MM (LCMM) and 57.1% of Non-secretory MM (NSMM). Using a combination of tests that included sFLC allowed us to detect 2 patients more with PCPD in comparison with the tests that did not use them; these patients had NSMM and Plasmacytoma. Only 2/113 patients with PCPD were negative in all assays. All the patients with Intact Inmunoglobulin MM (IIMM) were detected in any combination of tests. In LCMM patients, the sensitivity was the same with any of the combination of tests. *Conclusions.* The addition of sFLC assays allowed identifying 2 patients that were not detected by electrophoresis assays. Determination of FLC was more sensitive for detecting non-secretory PCPD. Since no additional information was obtained by adding urine studies, a review of technical issues must be done.

Table 1. Sensitivity of monoclonal gammopathy screening panels.

	n	All 5 tests	SPE and sIFE	SPE; sIFE and uIFE	SPE; sIFE and sFLC	SPE and sFLC	SPE	sIFE	UPE	uIFE	sFLC
Diagnosis, n (%)											
Monoclonal Gammopathy	113	111 (98.2%)	109 (96.5%)	109 (96.5%)	111 (98.2%)	111 (98.2%)	109 (96.5%)	100 (88.5%)	41 (36.3%)	41 (36.3%)	93 (82.3%)
Multiple Myeloma	100	98 (98%)	97 (97%)	97 (97%)	98 (98%)	98 (98%)	97 (97%)	91 (91%)	38 (38%)	38 (38%)	86 (86%)
IIMM	81	81 (100%)	81 (100%)	81 (100%)	81 (100%)	81 (100%)	81 (100%)	81 (100%)	30 (37%)	30 (37%)	72 (88.8%)
LCMM	12	11 (91.7%)	11 (91.7%)	11 (91.7%)	11 (91.7%)	11 (91.7%)	11 (91.7%)	10 (83.3%)	8 (66.6%)	8 (66.6%)	10 (83.3%)
NSMM	7	6 (85.7%)	5 (71.4%)	5 (71.4%)	6 (85.7%)	6 (85.7%)	5 (71.4%)	0 (0%)	0 (0%)	0 (0%)	4 (57.1%)
Plasmacytoma	12	12 (100%)	11 (91.7%)	11 (91.7%)	12 (100%)	12 (100%)	11 (91.7%)	8 (66.6%)	3 (25%)	3 (25%)	6 (50%)
LCDD	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)

IIMM: Intact Immunoglobulin Multiple Myeloma
LCMM: Light Chain Multiple Myeloma
NSMM: Non Secretory Multiple Myeloma
LCDD: Light Chain Deposition Disease
SPE: Serum Protein Electrophoresis
sIFE: Serum Immunofixation
UPE: Urine Protein Electrophoresis
uIFE: Urine Immunofixation
sFLC: Serum Free Light Chain

B45 - AN ALGORITHM BASED ON SERUM FREE LIGHT CHAIN AND HEAVY/LIGHT CHAIN IMMUNOASSAYS FOR THE IDENTIFICATION OF HAEMATOLOGICAL MALIGNANCIES

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Background. Serum free light chain (FLC) and heavy/light chain (HLC) immunoassays provide information on monoclonal immunoglobulin (M-Ig) production and polyclonal immunoglobulin suppression. In addition, summated FLC κ+λ concentrations (cFLC) have recently been shown to have prognostic value for survival; presumably reflecting immune stimulation. *Objectives.* To investigate the utility of the combined use of FLC and HLC immunoassays for identification of clonal disease and as markers of immune dysfunction. *Methods.* FLC and HLC levels were measured in serum samples from 1468 patients referred to hospital for haematological investigation. A FLC/HLC algorithm was derived indicative of the degree of immunological abnormality individually and results compared to those obtained by routine electrophoretic tests (SPEP and IFE). Diagnoses were recorded ~3 months after the analysis and patients followed for up to 3 years. *Results.* 293/1468 (20%) patients had an abnormal SPEP of which 95/293 were confirmed by IFE, including: 10 intact immunoglobulin multiple myeloma (MM), 6 light chain MM (LCMM), 2 Waldenstrom's macroglobulinemia, 1 cryoglobulinemia, 3 CLL, 1 mantle cell lymphoma, 3 other lymphoma, 1 plasmacytoma and 68 MGUS. The FLC/HLC algorithm identified 85/95 IFE-positive patients; of the 10 patients not identified by the algorithm, 3 had oligoclonal banding indicative of infection and 7 had M-Ig secondary to other diagnoses. The algorithm also identified M-Ig production in 15 IFE-negative patients, including: 2 AL amyloid, 1 asymptomatic LCMM, 1 patient with ~1g/L FLCκ (lost to follow-up), 1 follicular lymphoma and 1 CLL; and 9 patients without diagnosis. Furthermore, the FLC/HLC algorithm identified 205/1468 (14%) patients with no clonal production and cFLC levels>50mg/L. In a subset analysis comparing patients with elevated/normal cFLC concentrations, cFLC>50mg/L predicted all-cause mortality and was associated with poorer survival ($p<0.001$). *Conclusions.* FLC/HLC algorithm identified additional haematological malignancies missed by standard methods (SPEP/IFE) and established the association of elevated cFLC with increased risk of mortality.

RISK STRATIFICATION OF MONOCLONAL GAMMOPATHIES

C46 - EARLY RESPONSE TO TREATMENT BY SERUM FREE LIGHT CHAIN ASSAY IS PREDICTIVE OF INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA OUTCOME

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Background and Objectives. Most Multiple Myeloma (MM) cells secrete an intact immunoglobulin (iIg), but also the corresponding serum free light chain (sFLC) in excess having a much shorter half-life. Our study aim to determine if early detection of response by sFLC measurement is predictive of outcome in patients with iIg MM. *Methods.* We prospective studied 30 episodes of treatment among 24 patients in newly-diagnosed or relapsed MM patients with measurable disease. The paraprotein (SPEP) and sFLC (Freelite® assay) were measured before and weekly after initiation of a treatment. Patients were followed until next treatment or death. Response categories were defined as per IMWG criteria and response was defined as at least partial response (PR). *Results.* Response by sFLC preceded response by SPEP by a median of 3 weeks. By the end of cycle 1, ≥PR was achieved in 23% cases by SPEP, *vs* 63% by sFLC. An early response by sFLC was associated with a greater depth of response on treatment by SPEP (p=0.0006). Event-free survival (EFS) was significantly better in patients with an early detection of response by sFLC. In univariate analysis, median EFS for responding patients compared with stable disease (SD) patients on cycle 2 day 1 was 15 *vs* 5 months (p=0.0004), with similar results earlier during the first cycle. Among responding patients by sFLC, patients presenting with an unstable response had a significantly decreased EFS compared with patients with a stable response by sFLC (median EFS 22 *vs* 5 months, p=0.0011), even in multivariate analysis (HR=25, p=0.006). These paraprotein fluctuations within a cycle were not apparent with SPEP, reflecting the longer half-life of iIg. *Conclusions.* Our results suggest that frequent sFLC measurements at initiation of treatment in MM patients with iIg paraprotein are worthwhile for their predictive value.

C47 - HEVYLITE•, FREELITE• AND IMMUNOPHENOTYPIC EVALUATION OF MULTIPLE MYELOMA PATIENTS IN COMPLETE RESPONSE: THREE TOOLS, ONE PURPOSE

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Background. Accurate quantification of M-component is important for diagnosis and response evaluation in multiple myeloma (MM). To this purpose, the International Myeloma Working Group (IMWG) guidelines recommend the use of protein electrophoresis with serum and urine immunofixation, adding free light chain (FLC) assay for response assessment. Flow cytometry immunophenotyping (FCI) is also considered a useful additional tool to detect minimal residual disease. The novel heavy/light chain (HLC) assay has recently become available to measure IgG and IgA MM subsets. *Objectives.* To assess the effect of HLC ratio, FLC ratio and immunophenotypic response (IR) on clinical outcome of MM patients achieving therapeutic complete response (CR) according to IMWG criteria. *Methods.* We studied 25 patients with MM (14 IgG and 11 IgA) in CR after first line treatments including novel agents +/- autologous stem cell transplantation. At CR time, sera samples were tested for FLC and HLC ratio by immunonephelometry (Freelite and Hevylite Assay, respectively, Binding Site, UK), while bone marrow samples were analyzed by FCI for assessing IR, defined as <10 MM plasma cells in the 100000-event file, using a home-made method. Data obtained were correlated to progression free survival *Results.* Overall, neither FLC or HLC assays, nor IR achievement, alone or in combination, significantly influenced PFS, though a trend for a worse outcome was observed in patients with abnormal FCL or HCL ratio, particularly in IgA subtype. Interestingly, PFS at 18 months was 15% lower in patients with both abnormal HLC and FLC than in those with normal HLC and abnormal FLC (40 *vs* 55%, respectively). *Conclusions.* In our numerically still limited experience the recognition of abnormal HLC enhanced the negative effect of abnormal FLC results on PFS estimate in patients with IgG or IgA MM in CR after first line treatments.

C48 - RISK STRATIFICATION IN MGUS: SERUM FREE LIGHT CHAINS AND BONE MARROW PLASMOCYTOSIS

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Background. In Belgium , the serum FLC test (sFLC) is not reimbursed as a tool for risk stratification in MGUS patients and there are no local guidelines regarding the optimal timing of bone marrow analysis in these patients. *Objectives.* To investigate the use of sFLC ratio as an independent predictor of MGUS progression and as a tool for avoiding unnecessary bone marrow examinations. *Methods.* Twenty-three consecutive patients with a monoclonal gammopathy (MGUS, smouldering myeloma (SMM) , multiple myeloma (MM)) , elected by the clinicians to undergo a bone marrow examination were included. ROC curve analysis was performed to determine a cut-off for the detection of plasmacell abnormalities, which were defined as a plasmocytosis (>4% plasmacells) and/of obvious morphologic abnormalities of the plasmacells. *Results.* See Figure 1. *Conclusions.* In MGUS patients with a sFLC ratio <8 it is unlikely to find

a diagnosis of SMM or MM. In our patient population , we found that this ratio cut-off , can be used as a predictive value in the approach of MGUS patients.

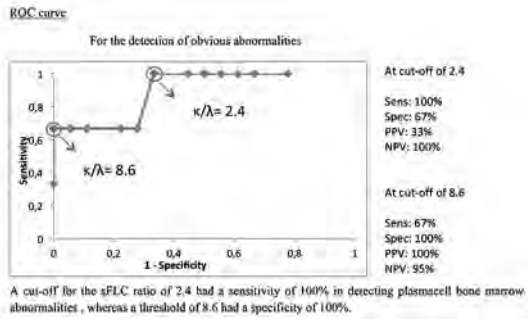

Figure 1.

C49 - HEAVY/LIGHT CHAINS PAIRS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MYELOMA

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Abstract. The risk of progression of monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM) to symptomatic multiple myeloma (MM) is very heterogeneous and, unfortunately, distinguishing the patients at high risk of progression at diagnosis remains difficult. In the last years, suppression of the uninvolved chain of the immunoglobulin by specific heavy/light chain (HLC) pairs in serum has been identified as a new factor that could be incorporated into the risk stratification. We retrospectively evaluated 114 patients diagnosed with SMM (27) and MGUS (87) between 1983 and 2003, with a median follow up for alive patients of 13 years. All specific serum HLC pairs were measured (IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ); 69 patients had one or more sequential samples. Progression to malignant symptomatic gammopathies, mainly to MM, was observed in 13 patients (11%; 8 SMM and 5 MGUS). Risk of progression was 6 times higher in patients with SMM than in those with MGUS (p=0.001, respectively) and 4 times for IgA isotype (p=0.013). IgAκ, IgMκ and IgMλ suppression were associated with poor prognosis (p=0.001; p=0.041 and p=0.025, respectively). Normal or lower than normal HLC ratios for IgG and IgM were associated with longer time to progression to symptomatic disease than higher values (p=0.004 and p=0.022, respectively) (Figure 1). In a multivariate analysis taking into account diagnosis (SMM *vs.* MGUS), IgA isotype *vs.* others, "evolving" pattern and normal or lower than normal HLC ratios for IgG and IgM, only SMM diagnosis, IgA isotype and higher IgG HLC ratio remained significant (HR: 4.6, 6.3 and 3.8 respectively; all p<0.05). In conclusion, findings presented here indicate that HLC ratios seem to be a valuable tool in the risk stratification of patients with SMM and MGUS. Suppression of the uninvolved isotype (*i.e.* uninvolved IgA or IgM HLC in IgG MGUS) is particularly interesting.

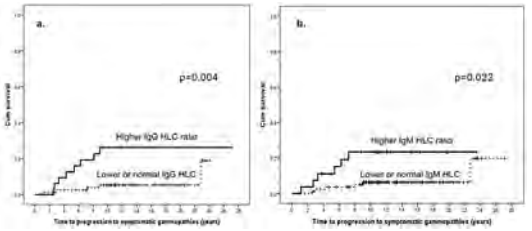

Figure 1. Heavy/light chains (HLC) ratios for IgG (a) and IgM (b) patients, and the risk of progression to symptomatic disease.

C50 - PROGNOSTIC SIGNIFICANCE OF SERUM FREE LIGHT CHAINS RATIO IN A SPANISH POPULATION OF NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background. International Staging System (ISS) is the current standard used for risk stratification of patients with newly diagnosed Multiple Myeloma (MM). Recent studies have evaluated the possibility of using serum free light chains (sFLC) at diagnosis as additional prognostic factor. *Objectives.* To evaluate the prognostic value of serum FLCs ratio (sFLCR) at baseline in newly diagnosed MM in a Spanish population. *Methods.* 170 patients with newly diagnosed MM (135 Intact Immunoglobulin MM and 35 Light Chains MM) were monitored for six years. sFLC were measured by turbidimetry (Freelite™, The Binding Site). sFLC ratio (sFLCR) was calculated with monoclonal light chain as numerator. Median sFLCR was 50 (8.72-360) and patients were divided in group "low" with sFLCR<50 (N=85) and group "high" with sFLCR>50 (N=85). Survival was defined as the time from initial diagnosis to death or the last follow-up and the Overall Survival (OS) was calculated by the method of Kaplan and Meier. *Results.* During the period of study there were sixty disease-related deaths: 18 in "low" group and 42 in "high" group. The 5-years OS was 51% for all patients, 70% for the low group and 23% for the high group (HR=4.64, p<0.0001). Other variables associated with poor survival in univariate analysis were age>65 years (HR=2.10, p=0.015), albumin<3.5 g/dl (HR=2.14, p=0.004), creatinine>2 mg/dl (HR=2.60, p=0.001), haemoglobin<10 g/dl (HR=2.10, p=0.004), β2-Microglobulin>3.5 mg/l

(HR=4.48, p<0.0001) and ISS stages 2 (HR=3.77, p=0.001) and 3 (HR=6.26, p<0.0001). Multivariate Cox analysis identified sFLCR (HR=4.23, p<0.0001), age>65 years (HR=2.14, p=0.015), ISS stages 2 (HR=2.89, p=0.007) and 3 (HR=3.91, p=0.002) as independent risk factors for adverse outcome. *Conclusions.* sFLCR value at diagnosis is an important independent risk factor for poor prognosis in patients with newly diagnosed MM confirming previous observations. In our cohort, a sFLCR>50 predicted very poor outcome with median patient survival of only 37 months.

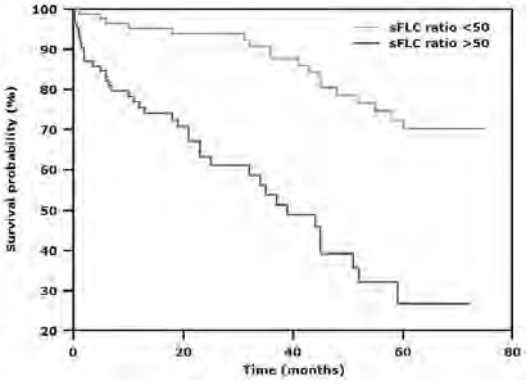

Figure 1.

C51 - HEAVY/LIGHT CHAIN ANALYSIS PROVIDES EARLY PROGNOSTIC INFORMATION PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. Depth of response to induction therapy prior to stem cell transplant is indicative of patient outcome. We sought to determine the prognostic impact of heavy/light chain (HLC) measurements in multiple myeloma (MM) patients following induction therapy. *Methods.* We prospectively evaluated 27 intact immunoglobulin MM patients (12 Female, 15 Male; 19 IgG, and 8 IgA; median age:54 years; range:37-67 years) who received an autologous stem cell transplant (ASCT) subsequent to high dose melphalan. Median follow-up time from ASCT was 448 days (range:168-594 days). Responses were assigned according to international response criteria prior to and 100 days post-ASCT. Serum samples were analysed with Hevylite® prior to ASCT (median:2 days; range:0-106 days). Progression free survival (PFS) was estimated by Kaplan Meier analysis and compared using log rank test. Difference in HLC (dHLC) and involved HLC/uninvolved HLC (iHLC/uHLC) ratio were compared using Mann-Whitney U test. *Results.* Prior to ASCT, 1 patient achieved CR, 13 VGPR, 10 PR, 1 MR and 2 PD. Achievement of ≥VGPR prior to ASCT was not associated with increased PFS (p=0.95) whereas an abnormal HLC ratio (Igκ/Igλ) (17/27 patients) prior to ASCT was associated with significantly poorer PFS (16.7 months *versus* median not reached p=0.015). Addition of a normalised HLC ratio to the assignment of response in patients achieving a ≥VGPR was significantly associated with improved PFS (median not reached *versus* 16.7 months, p=0.013). Achievement of a ≥VGPR 100 days following ASCT was associated with increased PFS (median not reached Vs 12.3 months P=0.004). Patients who achieved ≥VGPR at 100 days had significantly lower dHLC (median:3.36g/L interquartile range (IQR):-0.31-8.05g/L) and iHLC/uHLC ratio (median 1.76 IQR:1.38-6.14) prior to ASCT compared with patients who achieved ≤VGPR (median: 24.26g/L IQR:13.61-44.4g/L p=0.0005 and median:29.68 IQR:7.27-59.30 p=0.002 respectively). *Discussion.* HLC analysis may provide early prognostic markers for PFS in MM patients receiving an ASCT.

C52 - NORMALIZATION OF SERUM FREE LIGHT CHAIN RATIO AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA AS A MAJOR CRITERIA RESPONSE

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Background. Despite serum free light chain (sFLC) ratio has become increasingly adopted into clinical practice, in the contest of response assessment, this ratio is used only in stringent complete remission (sCR) category and in patients with oligosecretory multiple myeloma (MM). No data are available regarding the prognostic significance of normalization of sFLC ratio in patients with a lower grade of response. *Objectives.* To evaluate the impact on outcome of achievement of sFLC ratio normalization post autologous stem cell transplantation (ASCT) in MM patients and to clarify if this normalization may predict for progression free survival (PFS) better than the reduction of M-protein such as defined by International Myeloma Working Group (IMWG) response criteria. *Methods.* We retrospectively reviewed 211 patients with MM (median age 60, range 30-76) who achieved the normalization of sFLC ratio 90 days after ASCT independently by IMWG response. Induction therapy was heterogeneous, but all patients received at least one novel agent. PFS was calculated from ASCT to date of first disease progression. *Results.* We stratified patients into 2 groups: 1) patients who achieved a sCR; 2) patients who obtained a response of lower grade including complete remission (CR), very good partial remission (VGPR) and partial remission (PR). After a median follow-up of 21.4 months, patients in sCR had a median PFS of 34.2 months, while for patients in CR- VGPR-PR, the median PFS was 33 months (p=.813).In Cox regression analysis, no prognostic factor assessed (age, performance status, β-2-microglobulin, ISS staging system, hemoglobin, platelets, creatinine, cytogenetic, presence of

extramedullary disease, lactate dehydrogenase and maintenance therapy) was able to affect the PFS. *Conclusions.* This study demonstrates that normalization of sFLC ratio may predict for PFS more accurately than the reduction of M-protein, contributing to the characterization of sFLC as a response biomarker in the era of new drugs.

C53 - PROGNOSTIC VALUE OF PRE-TRANSPLANT COMPLETE REMISSION BY FREE LIGHT CHAIN AND HEAVY/LIGHT CHAIN RATIOS *VS.* CONVENTIONAL CRITERIA-LONG TERM RESULTS OF THE BMT CTN 0102 STUDY

P. Hari, A. D'Souza, M. Pasquini, B. Logan on behalf of BMT CTN0102 investigators

Medical College of Wisconsin, Milwaukee, WI, USA

Background. We correlated FLC and HLC assays *vs.* conventional response criteria and their prognostic impact over 6 years of follow-up among 497 patients enrolled in the BMT CTN0102 trial of tandem autologous (autoHCT) *vs.* tandem autoHCT-allogeneic (alloHCT) transplantation. *Methods.* Pre-transplant serum samples prior to first autoHCT were analyzed centrally for FLC (Freelite™) and HLC (Hevylite™). Corresponding disease status (IMWG uniform response criteria) was determined by independent data review committee. HLC-CR was defined as normalization of ratios across all 3 measured heavy/light chain pairs or the normalization of clonal isotype with normal ratios of uninvolved pairs. The demographics of the 497 patients with HLC/FLC samples pre-transplant were concordant with the main study (Krishnan *et al.* Lancet Oncol. Dec 2011; 12(13): 1195). *Results.* 56 patients in conventional CR were also in HLC-CR. Comparing HLC-CR *vs* conventional CR, sensitivity was 100%, specificity 39%, PPV and NPV values 17% and 100% respectively. Of 211 pts in a response better than or equal to very good partial response (≥VGPR), 188 were in HLC-CR (Sensitivity=89%). Comparing HLC-CR *vs.* ≥VGPR: specificity was 52%, PPV 58% and NPV 87%. FLC ratio normalization *vs.* ≥VGPR disease state had a sensitivity of 47%, specificity 81% and PPV and NPV of 64% and 67% respectively. Adjusted multivariate models including baseline response, myeloma stage and study arm were used to compare the prognostic utility of HLC-CR and FLC-CR. HLC-CR was an independent predictor of superior PFS (p-0.016); freedom from relapse (p-0.035) and survival (p-0.012) while FLC-CR was not. Since HLC-CR closely correlated with conventional CR, HLC provided additional prognostic information for those not in CR/VGPR. FLC-CR among those in CR/VGPR did not impact outcomes. *Conclusions.* Abnormal HLC pre-transplant has a high negative predictive value (100%) for identifying patients not in CR by uniform response criteria and is associated with inferior long-term outcomes.

C54 - LEVELS OF UNINVOLVED AND INVOLVED IMMUNOGLOBULIN PREDICT CLINICAL STATUS AND PROGRESSION FREE SURVIVAL FOR MULTIPLE MYELOMA PATIENTS

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Introduction. The levels of monoclonal immunoglobulins (M-Igs) are used to monitor multiple myeloma (MM). HevyLite® +(HLC) assays are able to discriminate between uninvolved and M-Ig levels. We evaluated the levels of involved and uninvolved HLC, their ratios and differences and their relationship to outcomes among 189 MM patients. *Materials and Methods.* Serum samples were analyzed using the HLC assays, and results were correlated with clinical status (complete response (CR), ≥partial response (PR), <partial response, and progressive disease (PD)). Comparisons were made using student's t, Mann-Whitney, and Fisher's tests. PFS was calculated using Kaplan-Meier analysis. All tests were double-tailed and P-values determined. *Results.* The patients were 62% IgG and 38% IgA with a median age of 66 years, β2 microglobulin 3.27 mg/L, albumin 3.8 g/dl, and median follow up of 72.5 months. Patients with PD had higher involved HLC levels, lower uninvolved HLC levels, higher ratios of involved/uninvolved HLCs and greater differences between them compared with patients with ≥PR (all with P<0.0001). A higher proportion of patients in ≥PR had normal uninvolved HLC levels than patients with <PR (P<0.0001). Additionally, patients in CR were more likely to have normal uninvolved HLC than those with below normal levels (P <0.0001). Similarly, patients in CR were more likely to have normal HLCs than in PR (P=0.0040). Patients with normal uninvolved HLCs showed a longer PFS (45 months) than those with less than normal levels (11 months; P=0.0019). Patients with normal involved HLC levels had longer PFS (33 months) than patients with involved HLC above the normal range (11 months; P=0.0405). *Conclusios.* We show that involved/uninvolved HLC ratios, differences between them, involved and uninvolved HLC levels correlate with clinical status for MM patients. Patients with normal uninvolved levels or normal involved HLC levels have a longer PFS.

C55 - LIGHT CHAIN MULTIPLE MYELOMA EXHIBITS SIMILAR OUTCOMES COMPARED TO HEAVY CHAIN MULTIPLE MYELOMA IN PATIENTS UNDERGOING SINGLE AUTO-SCT IN THE ERA OF NOVEL AGENTS

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Introduction. About 15-20% of multiple myeloma (MM) patients secrete monoclonal light chains only, without expression of the normal immunoglobulin heavy chain, which constitutes the so-called light-chain multiple myeloma (LCMM). It has been reported that LCMM is a biologically more aggressive disease with worse survival than intact immunoglobulin or heavy chain MM (HCMM)[1-4]. We aimed to investigate whether clinical outcomes are similar between LCMM and HCMM in patients undergoing single auto-SCT in the era of novel agents treated at a single center. *Objectives.* The primary endpoint of the study was to assess the impact of LCMM on response rate as well as overall and progression-free survival (OS and PFS). *Methods.* All consecutive patients who underwent single auto-SCT at Tom Baker Cancer Center (TBCC) from 01/2010 to 10/2014 were evaluated. A p value of <0.05 was considered significant. *Results.* 21 patients with LCMM were evaluated and compared to 111 cases of HCMM. Clinical characteristics are shown in Table 1.

Table 1. Clinical characteristics.

Characteristic	*HCMM, n=111*	*LCMM, N=21*	*p=value*
Age (median)	58	59	0.452
Gender			0.920
Male	70 (63%)	13 (62%)	
Female	41 (37%)	8 (38%)	
Hb (g/L)	112	134	0.160
Calcium (µmol/L)	2.24	2.31	0.916
Creatinine (µmol/L)	78	80	0.645
B2microglobulin (µmol/L)	2.92	2.21	0.352
Albumin (g/L)	31	37	0.183
Stage I	24	10	0.007
Stage II	67	5	
Stage III	20	6	
LDH (IU/L)	177	194	0.306
BMPC (%)	40	31	0.186
Heavy chain:			N/A
IgG	86		
IgA	23		
IgD	1		
Biclonal	1		
Light chain:			0.568
κ	78	12	
λ	32	9	
Biclonal	1		
High risk	32	1	0.020
Standard risk	79	20	
Induction			0.202
CyBORD	38	4	
Bortezomib/Dexamethasone	47	10	
RVD	22	5	
VTD	0	1	
RD	3	1	
CyBORP	1	0	

BMPC, Bone marrow plasma cells; CyBORD, Cyclophosphamide, bortezomib and dexamethasone; RVD, Lenalidomide, bortezomib and dexamethasone; VTD, Bortezomib, thalidomide and dexamethasone; RD, Lenalidomide and dexamethasone; CyBORP, Cyclophosphamide, bortezomib and prednisone.

Patients received a median of 4 cycles of induction before undergoing auto-SCT. At day-100 post auto-SCT, 83% of HCMM and 85% of LCMM patients achieved ≥VGPR (p=0.824). CR was seen in 13% of HCMM and 28% of LCMM patients (p=0.084). At the time of analysis, 13% of cases in the HCMM group and 1 in the LCMM have died (p=0.260). In addition, 31% of HCMM and 33% of LCMM cases have progressed (p=0.871). Median OS has not been reached in both groups and mean survival was similar (43 *vs* 45 months, p=0.254). Also, PFS was similar (mean of 31 months in HCMM compared to 27 months, p=0.706). *Conclusions.* The current study suggests that LCMM exhibits similar clinical outcomes compared to HCMM in the era of novel agents. It also demonstrates that other inherent features associated with disease aggressiveness are more relevant to OS and PFS than expression of light chain paraprotein only.

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C56 - POTENTIAL PROGNOSTIC VALUE OF HEAVY-LIGHT CHAINS RATIO IN SYMPTOMATIC MULTIPLE MYELOMA

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Background. The study of monoclonal protein (MP) in MM is mainly based on a serum electrophoresis (sPE), immmunofixation (sIF) and nephemometry combination. Despite a high sensitivity, limitations such as hard-to-detect hidden MP or small amounts of MP exist. Complementary techniques such as the FLCs assay and the new HLCs technique help to detect lower amounts of disease, improving diagnosis accuracy and response assessment. *Objectives.* To investigate the prognostic impact of HLCr at diagnosis and after treatment in MM patients in the context of two GEM clinical trials. *Patients and Methods.* 87 patients with an IgG/IgA-MM, treated according to the GEM05<65y & GEM05>65y PETHEMA/GEM trial, were included. The HLC assay (HEAVYLITE®, The Binding Site, Birmingham, UK) was performed on an automated nephelometer (BNII, Dade Behring/Siemens, Marburg, Germany). The sPE assay was performed by capillary electrophoresis (V8, Helena Biosciences Europe), and sIFE was performed for γ, α, κ, and λ Ig chains (SAS-3 & SAS-4, Helena Bioscience Europe). *Results.* Establishing several cut-offs, we note that "very pathological" (VP) HLCr values (<0.75 or >60) at diagnosis showed an increased risk of progression (p=0.001) and a lower OS (p=0.008), and the presence of uninvolved pair suppression had no prognostic impact in our series (OS p=0.747; PFS p=0.427). Absolute HLC-involved-Ig values show linear correlation with absolute values of MP by sPE (p= 0,000; Pearson's r=0.708). After treatment, regardless of the achieved response, a normal HLCr appears to associate to a greater PFS (p=0.030). Very few patients in CR had a HLC analysis, therefore we cannot assess whether a normal rHLC implies a better prognosis in this population. *Conclusions.* VP-HLCr values (<0.75 or >60) at diagnosis suggest a greater risk of progression. HLC non-involved pair suppression at diagnosis wasn't related to worse prognosis. Absolute HLC-involved-Ig values show linear correlation with absolute values of MP by sPE.

C57 - SUPPRESSION OF THE UNINVOLVED HEAVY CHAIN/LIGHT CHAIN PAIR CORRELATES WITH SURVIVAL IN NEWLY DIAGNOSED AND RELAPSE/REFRACTORY PATIENTS WITH MYELOMA

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Background. Suppression of polyclonal immunoglobulins has been associated with a poor prognosis in multiple myeloma (MM). New immunoassays measuring both light chain types of the same immunoglobulin class (heavy/light chain, HLC) and from which HLC Ig'κ/Ig'λ ratios can be calculated to determine clonality, allow for the first time quantification of the isotype-matched uninvolved, polyclonal immunoglobulin (HLC-pair suppression; *i.e.* levels of IgGλ in an IgGκ patient). *Objectives.* To compare the prognostic value of HLC-pair suppression with systemic immunoparesis (SI) in relation to outcome in newly diagnosed and relapsed/refractory MM patients. *Methods.* HLC levels were measured in serum samples from 155 newly diagnosed and 47 relapsed/refractory MM patients, and compared to total non-clonal immunoglobulin concentrations. HLC-pair suppression and SI were defined as 50% reduction below the published normal ranges (lower limit normal range: IgGκ=3.84g/L, IgGλ=1.91g/L, IgAκ=0.57g/L, IgAλ=0.44g/L, IgG=6g/L, IgA=0.4g/L, IgM=0.2g/L). β-2-microglobulin (β2M) was measured by standard methods. SPSS_v19 was used to analyse the data. *Results.* All patients had abnormal HLC ratios. HLC-pair suppression was identified in 87/155 (56%) newly diagnosed and 26/46 (57%) relapsed/refractory patients, whereas SI was present in 77/155(50%) and 14/47 (30%) patients, respectively. HLC-pair suppression associated with shorter OS in newly diagnosed IgG (HR(95%CI): 1.55 (0.95-2.55); median OS: 49 months *vs.* NR, p=0.02) and relapsed/refractory IgG and IgA patients (HR(95%CI): 3.67(1.02-13.24); median OS: 23 months *vs.* NR, p=0.03). By contrast SI did not associate with survival in any case. A risk model based on β2M≥5.5mg/L and HLC-pair suppression stratified both newly diagnosed (75% OS: 41, 25 and 16 months for patients with 0, 1 and 2 factors, respectively; p=0.001) and relapsed/refractory patients (75% OS: NR, 21, and 8 months for patients with 0, 1 and 2 risk factors, respectively; p<0.0001). *Conclusions.* HLC-pair suppression associates with poor survival in both newly diagnosed and relapsed/refractory patients. Larger longitudinal studies are required to confirm our findings.

C58 - THE IMPORTANCE OF IMMUNOPARESIS ASSESSMENT IN PATIENTS WITH ASYMPTOMATIC MULTIPLE MYELOMA - FIRST RESULTS OF A NEW CMG PROJECT

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Introduction. Exploring new prognostic factors is important theme in patients with multiple myeloma (MM). The goal is to find simple laboratory examinations that would allow the identification of patients with varying degrees of the risk of transition to symptomatic form. Such tests include immunoparesis determination, which can be assessed through examination concentrations of polyclonal immunoglobulin (Ig) or newly determine the degree of suppression of an alternative pair of monoclonal immunoglobulin (MIG) using the method HevyLite™. *Methods.* Report analyzed in this pilot study included 71 serum samples of patients with asymptomatic MM (34 men and 37 women; 51 patients with IgG and 20 patients with IgA MIG type). For determination of the concentra-

tion of heavy/light chain immunoglobulin pairs and then for assessment of the immunoparesis degree was used HevyLite™ method on turbidimeter SPA Plus platform. The project is supported by The Binding Site research grant. Statistical results and basic parameters analyzed patients from the CMG registry ensure Institute of biostatistics and analysis by Faculty of Medicine MU Brno. *Results.* Of the 71 patients occurred during the processing of the results already in 32 (45,1%) disease progression to symptomatic forms. HevyLite™ method were statistically significantly associated with the risk of progression only in patients with IgG MIG type, in this group of patients was found prognostic value higher (p=0.006) than for the immunoparesis assessment using polyclonal Ig (p=0.017). Conversely, in patients with IgA type MIG when using methods HevyLite™ no statistically significant relationship was proved. *Conclusions.* Assessing the degree of immunoparesis is an important prognostic factor in MM, which is part of the new staging systems of asymptomatic MM. The results of our pilot study confirmed the value of HevyLite™ method as a new tool for immunoparesis assessment till now only in patients with IgG MIG type.

C59 - RETROSPECTIVE ANALYSIS OF CHANGE IN SERUM FREE LIGHT CHAINS AS A PREDICTION OF RESPONSE IN PATIENTS WITH MULTIPLE MYELOMA

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Background. The goal of first line therapy in patients with multiple myeloma is to attain a very good partial response (VGPR) or better. Hansen *et al.*[1] showed that an 80% reduction of the iFLC on day 21 could be an early predictor of VGPR in this patients. The aim of this retrospective study was to recapitulate the findings in our patients. *Patients and Methods.* We report on thirteen patients treated with different chemotherapy regimens including VRD (4), bRAD (5), PAD (2), VCD (1) and VD (1). Serum free light chains analyses were collected on day 1 (+/- 14 days) of the first cycle of chemotherapy and at the beginning of the second cycle of chemotherapy on day 21 (+/- 39 days). Overall response was analyzed after a median of 3 (range 2-4) cycles of chemotherapy. *Results.* After one cycle of chemotherapy, six patients had an initial reduction of >80%, three patients of >50% in iFLC. One patient showed only a reduction of <50%, while three patients had an increase of their iFLC. From patients with an initial reduction of >80%, four achieved a VGPR. From the patients with a reduction <50% or increase in iFLC, one achieved a VGPR, two a PR and one patient had a SD. *Conclusions.* Our findings encourage the results of Hansen *et al.* However, one patient with an early increase in iFLC achieved a VGPR after three cycles of chemotherapy. Further studies are needed to decide if changes in iFLC at day 21 could be an predictor for response in multiple myeloma.

Reference

1) Hansen *et al.*: Evaluation of the serum free light chain (sFLC) analysis in prediction of response in symptomatic multiple myeloma patients: rapid profound reduction in involved FLC predicts achievement of VGPR. European Journal of Haematology 93 (407-413).

C60 - SERUM FREE LIGHT CHAIN AND SOLUBLE SYNDECAN-1 LEVELS ARE MAJOR MARKERS OF DISEASE EVOLUTION OF SMOLDERING MULTIPLE MYELOMA PATIENTS

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Introduction. SMM consists an asymptomatic plasma cell dyscrasia that fulfills the diagnostic criteria of MM, without end organ damage symptoms. Since there is a risk of progression to symptomatic MM in about 10% of the patients, a question arises whether high risk SMM patients would benefit from early initiation of anti-myeloma therapy. Therefore efforts are being made to establish powerful prognostic markers of disease evolution. *Purpose.* To investigate any prognostic markers of disease evolution to symptomatic MM. *Patients and Methods.* We studied 124 SMM patients, diagnosed and followed-upup in our department. Median age was 69 years (31-86) while 60% were males. Seventy percent, 23% and 7% were Durie-Salmon staged 1, 2 and 3 respectively, while 60%, 20% and 20% were ISS staged I, II and III respectively. MM type was IgG in 76%, IgA in 21% and light-chain in 3% of population. Soluble syndecan-1 was measured by ELISA in 77 frozen samples collected at diagnosis. Serum FLCs were estimated by nephelometry (FREELITE, The Binding Site Ltd., Birmingham, UK). Serum FLC ratio (FLCR) κ/λ or λ/κ was calculated with the uninvolved LC as denominator. Statistical analysis was performed with SPSS v21.0. Survival was calculated with Log-rank test and depicted with Kaplan-Meier method. *Results.* Median follow-up was 63 months (1-203). Eighteen% evolved to symptomatic MM within 30 months (10-114) since diagnosis. Adverse prognostic factors for SMM evolution were bone marrow infiltration >60% (p<0.001), FLCR>100 (p<0.001), involved FLC >30 mg/L (p=0,002), non-IgG isotype (p=0,013), pathological FLCR (p=0,035) and s-syndecan-1 levels >120 pg/ml (p=0.001). In multifactor analysis, only FLCR >100, pathological FLCR, non-IgG isotype and s-syndecan-1 levels kept their prognostic value. *Conclusions.* Serum FLC levels and FLCR are important prognostic factors of disease evolution. Syndecan-1 serum levels of SMM patients consist another valuable predictor of disease progression to symptomatic MM.

C61 - RELATIONSHIP BETWEEN IMMUNOPARESIS TYPE AND KAPPA/LAMBDA (κ/λ) RATIO IN PATIENTS WITH IgA OR IgG MONOCLONAL GAMMOPATHY UNDERMINED SIGNIFICANCE

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Backgrounds. In patients (pts) with MGUS, abnormal k/λ ratio and immunoparesis are related and have a poor prognostic significance. The importance of immunoparesis is greater if involves two immunoglobulins (Igs). *Methods.* We measured Free Light Chain (FLC) (Binding Site) and serum Igs in 128 pts with IgG MGUS and in 20 pts with IgA MGUS, evaluating the number and the type of decreased polyclonal immunglobulins and the relationship with k/λ ratio. *Results and Discussion.* In pts with IgA MGUS, 13 showed IgM decreased and 5 IgG decreased. In pts with IgG MGUS, 54 showed IgM decreased and 22 IgA decreased. In 57 pts (39%) one Ig was decreased (25 with normal k/λ ratio and 32 with abnormal one), in 18 pts two Igs were decreased (1 with normal k/λ ratio and 17 with abnormal one) (p=0,002). In pts with IgG MGUS, IgM were decreased in 21 pts with normal k/λ ratio and in 32 pts with abnormal one, IgA were decreased in two pts with normal k/λ ratio and in 19 pts k/λ ratio were altered (p=0,001). In pts with IgA MGUS with normal k/λ ratio IgG were in the normal range, while in 5 pts with k/λ ratio altered they were decreased. IgM were decreased in 4 pts with normal k/λ ratio e in 9 with abnormal k/λ ratio. Investigating all pts, IgM were decreased in 25 with normal k/λ ratio and in 41 with abnormal one; the other Igs were decreased in 2 pts with normal k/λ ratio and in 25 with abnormal one (p=0,003). IgM are the Igs most frequently involved in immunoparesis, but in pts with abnormal k/λ ratio are often involved 2 Igs type. The abnormal k/λ ratio indicates thus a higher immunological impairment in pts with MGUS

C62 - ANALYSIS OF POTENTIAL CONTRIBUTION OF HEVYLITE™ ASSESSMENT IN LIGHT-CHAIN ONLY MULTIPLE MYELOMA

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Background. Determination of heavy/light chain immunoglobulin pairs (HLC) is a promising method in the diagnosis of oligosecretory multiple myeloma (MM) and in the evaluation of the depth of response in MM. *Objectives.* The aim of this study was to evaluate the relationship of PFS and OS to different levels (cut-off=median) of isotypes of HLC in light-chain only (LCO) MM. *Materials and Methods.* Hevylite™ analysis of serum IgG, IgA and IgM pairs was performed in 31 patients with LCO MM. *Results.* In the whole cohort, we found following relationships: for serum IgG-κ (cut-off 2.62 g/l) we found significant relationship to both OS (23.6 *vs* 58.4 months, p=0.046) and PFS (12.7 *vs.* NR; NR=not reached). For serum IgA-λ (0.18 g/l) there were significant differences in survival curves in PFS and OS, and in IgM-κ (0.99 g/l) there were differences in OS.
In the κ group we detected difference of medians and survival curves in the case of IgA-κ (0.22 g/l) for PFS and OS, in IgA-λ (0.16 g/l) for PFS (10.4 *vs.* NR, p=0.04) and OS, and in IgM-κ (cut-off 0.1 g/l) for OS.
In the λ group we identified differences in the case of IgG-λ (1.76 g/l) for OS and in IgG-κ/IgG-λ ratio (1.81) for PFS, in the case of IgA-κ (0.13 g/l) for PFS and OS, in case of IgA-λ (0.20 g/l) in for PFS and OS, and in the case of IgA-κ/IgA-λ ratio (1.1) for PFS and OS, and in IgM-κ/IgM-λ ratio (1.5) for PFS and OS (23.3 *vs.* 58.4; p=0.029). *Conclusions.* Our preliminary analysis shows the potential benefit of serum HLC pairs determination, including their ratio, to estimate PFS and OS even in LCO MM.
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C63 - VERY HIGH SERUM FREE LIGHT CHAIN CONCENTRATIONS (>10,000 MG/L) PREDICT HIGH EARLY MORTALITY FROM MULTIPLE MYELOMA

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Background. The serum free light chain assay is useful test for diagnosis and monitoring of patients with plasma cell dyscrasias. Baseline free light-chain concentration is a major prognostic indicator for plasma cell neoplasms, and the serum free light chain ratio is correlated with elevated serum creatinine, lactate dehydrogenase, extensive marrow infiltration, and light chain type multiple myeloma. Furthermore, a high serum free light chain levels at baseline reflect more aggressive disease, and normalization of free light chain ratio has been reported as a prognostic indicator of favorable outcome. *Objectives.* To determine if >10,000 mg/l light chain levels predicted high early mortality in patients with multiple myeloma. *Methods.* The results of all Free Light Chain Analyses conducted at the Hospital of the University of Pennsylvania from 1/1/12 until 1/8/14 were reviewed and those patients with >10,000 mg/L at anytime in theirs disease course were identified and a retrospective chart review was conducted. *Results.* 60 patients with >10,000 mg/L light chain levels since 1/1/2012 were identified out of >500 individuals tested over this time period. With a median F/U of 1.5 yrs, 33 patients (55%) had died within a median 6 months of the high level measurement, and 27

patients remain alive. *Conclusions.* Very High Serum free light chain concentration is likely correlated with mortality due to light chain deposition and toxicity, as well as a reflection of more aggressive disease. A detailed analysis of this poor prognosis group of patients is underway to help guide early, more effective intervention.

C64 - FEASIBILITY OF HEAVYLITE CHAIN MEASUREMENTS AS A COMPARATIVE PROGNOSTIC TOOL IN A MULTINATIONAL, MULTICENTER SETTING: RESULTS FROM THE EU FP7 PROJECT OPTATIO

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Background. OPTATIO (*OPtimizing TArgets and Therapeutics In high risk and refractOry Multiple Myeloma*) is a project in the framework of the EU FP7 programme (grant agreement 278570; www.optatio.eu). It is the goal of the OPTATIO consortium to seek out novel strategies for the development of novel diagnostic and therapeutic options for multiple myeloma (MM). Part of this project is the comparative appraisal of the Hevylite™ test as a prognostic tool for MGUS progression, MM prognosis and clinical outcome. *Materials and Methods.* Heavylite analysis was performed at 3/6 OPTATIO centers (other partners shipped to nearest cooperating laboratory) on all MGUS,SMM and MM samples from patients for which a complete clinical data set, as well as a bone marrow aspirate for drug testing were available. Furthermore conventional MM risk factors (cytogenetics, ISS, etc.) were collected for a comparative analysis. Test kits were supplied from the same quality controlled batches by partner-6 and test samples circulated to prove test consistency. *Results.* (see Table 1).

Table 1. Real life data from EU FP7 Project OPTATIO.

L Analysis

Center	*Brno/Ostrava*	*Innsbruck*	*Bari*	*Budapest*	*Cumulative*
MGUS	239	23	0	0	262
SMM	58	0	0	0	58
MM	13	152	92	93	350
Pts.	260	175	92	93	620
Samples	310	324	92	93	819

819 samples from 620 patients could be collected between APR 2012 and OCT 2014. Furthermore a web based clinical data bank was instituted. Analytical results were consistent in all participating laboratories and no impact of shipping and storage conditions on analytical stability were noted. The prognostic (and may be) predictive impact of the Hevylite™ test will be analyzed and presented compared to classical myeloma predictive factors. *Conclusions.* The Hevylite™ test is a valuable tool for risk stratification in MGUS, SMM, and MM and easily applicable in a multicenter setting with high analytical reproducibility.

CASE REPORTS

D65 - ACUTE ONSET QUADRIPARESIS IN A CASE OF LIGHT CHAIN MYELOMA AFTER ACHIEVING COMPLETE RESPONSE

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A predominantly-sensory peripheral neuropathy is a recognized complication in patients receiving bortezomib. Here we report a rare case of severe motor neuropathy one month after cessation of treatment. A 76 year-old Asian man was diagnosed with light chain myeloma (ISS stage 2) and treated with bortezomib and dexamethasone. During the second cycle, he was admitted with a fever and postural hypotension. Clinical examination showed a sensory deficit in a glove and stocking distribution and absent joint position sense in the great toes. Motor examination showed distal grade 4 weakness. He was treated with antibiotics, gabapentin for peripheral neuropathy and fludrocortisone for postural hypotension. His neurological symptoms resolved over a period of two weeks; however there was a residual sensory deficit in his feet. His serum free light chain ratio and range normalised. He was re-admitted two weeks later with sudden-onset flaccid weakness in his legs and hands and sensory loss up to the thighs. CT and MRI scans of his entire spine did not show features of cord compression. Nerve conduction studies showed a generalized neuropathy which was predominantly axonal. CSF analysis showed: glucose of 6.8 mmol/L, raised protein of 1.8 g/L, 3 lymphocytes seen in entire cytospin, negative viral screen and negative bacterial cultures. A diagnosis of an inflammatory polyneuropathy was made and confirmed by a neurologist. He was treated with intravenous immunoglobulin 0.4g/kg/day for 5 days and prednisolone 60mg alongside physiotherapy. After two months he was mobilizing independently and had grade 4 power in distal muscles and full-power in all proximal muscles. However, there was a residual sensory deficit in anterior part of his sole. Axonal and demyelinating inflammatory neuropathies have been described in patients treated with bortezomib. Our case adds to this existing literature on this disabling condition.

D66 - MEASUREMENT OF IgAκ/IgAλ HLC RATIO MAY AID ASSESSMENT OF RESPONSE TO TREATMENT

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Background. In the event of IgA paraprotein co-migration, international guidelines recommend the measurement of total IgA (tIgA) to monitor response. Heavy/light chain (HLC) assays have been developed that quantify IgAκ and IgAλ separately. This provides information on both the involved monoclonal immunoglobulin (M-Ig, *e.g.* IgAκ in an IgAκ patient) and the uninvolved polyclonal HLC-pair (uHLC, *e.g.* IgAκ in an IgAλ patient). Here we report a case study illustrating the utility of measuring HLC for assessing response to treatment. *Methods.* Eight sequential serum samples were taken from a male IgAλ multiple myeloma patient 64 years old, on treatment with Cyclosporine/Thalidomide/Dexamethasone (CTD). HLC results were compared retrospectively to tIgA, capillary zone electrophopresis (CZE) and serum free light chains (FLC). Normal ranges: IgA HLC ratio 0.8-2.04, IgAκ 0.48-2.82 g/L, IgAλ 0.36-1.98 g/l, κ/λ FLC ratio 0.26-1.65 and tIgA 0.8-4.0 g/L. *Results.* At presentation the M-Ig was detectable but unquantifiable by CZE due to co-migration with the β region, tIgA=57g/L, κ/λ FLC ratio=0.126, IgA HLC ratio=0.0006, IgAκ=0.029 and IgAλ=50.2g/L. After 4 cycles of CTD, the M-Ig was undetectable by CZE, the tIgA and the κ/λ FLC ratio had both normalised at 4g/L and 0.389 respectively. The concentration of HLC IgAλ decreased to just above normal range from 50.2g/L to 4.07g/L while IgAκ increased slightly from 0.029 to 0.206 but was still below normal range. Together this resulted in an IgA HLC ratio that increased slowly from 0.0006 to 0.05 but remained well below normal range. *Conclusions.* The measurement of IgA HLC in this patient suggested the CTD treatment targeted both the tumour and normal plasma cells and that this effect was not detected by Freelite, CZE or total IgA. Measurement of HLC may therefore aid clinicians to better assess the specific effects of anti-myeloma treatment.

D67 - SERUM FREE LIGHT CHAINS AS AN EARLY BIOMARKER OF RELAPSE IN MULTIPLE MYELOMA PRESENTED AS AN INTRACRANEAL PLASMACYTOMA

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Background. Serum free light chains (sFLC) are used in the diagnosis, prognosis and monitoring therapy of Multiple Myeloma (MM) patients. *Objectives.* To investigate the utility of sFLC to detect early relapse of an IgD-κ MM presented as an intracranial plasmacytoma. *Case report.* A 51 years old man diagnosed of IgD-κ MM achieved a complete remission status in April 2012 after treatment. Three months later, the sFLC ratio becomes progressively more altered, predicting a relapse with values of 2.52 in July, 4.27 in August, 60.23 in October, and a maximum of 135.85 in December. The corresponding monoclonal sFLC κ levels were 67.69, 66.39, 150.2 and 2868 mg/l. However, immunofixation (IFE) was normal from April to September remaining the patient in complete remission. In October 2012, IFE (IgD-κ) and Bence Jones proteinuria (PBJ) become positive for first time in the relapse, MRI scan showed new pathological fractures

at D3 and the bone marrow showed decreased cellularity with 1% of plasma cells. In December 2012, the patient developed diplopia and palpebral ptosis. Computed tomography (CT) scan was performed and an intracranial plasmacytoma was observed in both sides of frontal bone of the cranium that compresses the adjacent brain tissue. The relapse is clinically confirmed and the patient began treatment with VBAD and received one cycle with, nevertheless, increasing plasmacytoma size and sFLC levels (κ 3805.6 mg/l with ratio=460.16 in January '13). *Conclusions.* The quantification of sFLC is a useful key tool in the assessment of response to treatment in MM and it could detect early relapse four months before traditional methods like IFE and PBJ.

D68 - DIAGNOSTIC IMPACT OF HEVYLITE• TESTING IN A CASE OF ACQUIRED VON WILLEBRAND SYNDROME DUE TO MONOCLONAL GAMMOPATHY

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Background. von Willebrand syndrome (AVWS) is a rare and often misdiagnosed disorder of the primary hemostasis. Among other conditions it is found in lymphoproliferative diseases and myelomas. Several pathophysiologic mechanisms in this context can lead to functional and structural disturbances in an otherwise normal von Willebrand factor (VWF), in particular, enhanced adsorption of high molecular weight VWF multimers to myeloma cells. *Objectives.* We report on a previously healthy 66 year old woman with recurrent episodes of severe gastro-intestinal and nasal bleeding requiring transfusion of packed red blood cells. Endoscopic evaluation showed a diffuse bleeding type. There was no familial history of coagulation disorders. *Methods and Results.* Hemostaseologic evaluation demonstrated normal concentrations of factor VIII and VWF with diminished VWF activity and loss of large VWF multimers suggestive of AVWS. The total levels of IgG, A and M as well as both free light chains and the respective ratio (Freelyte®) were in the normal range. Immunofixation, however, revealed a monoclonal IgG κ band. Quantification with Hevilyte® yielded an almost complete repression of the uninvolved chain. Osteolytic lesions and bone marrow infiltration >10% were absent, thus, a diagnosis of monoclonal gammopathy of unknown significance was made. Because of the bleeding complications the patient was in urgent need of therapy although neither the diagnosis of multiple myeloma nor the classical CRAB criteria were fulfilled. Intravenous substitution of IgG reduced the blood loss, paralleled by diminished concentration of Ig G κ and improved coagulation parameters. Since this proved only temporarily successful, the patient is now treated with bortezomib/dexamethasone. *Conclusions.* In atypical cases without elevation of both total immunoglobulins and free light chains quantification of the paraprotein using Hevilyte® is helpful for diagnosis and therapy monitoring.

D69 - FREELITE ASSAY SOLVED A PUZZLING DIAGNOSIS OF NONSECRETORY MULTIPLE MYELOMA: A CASE REPORT

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The serum immunoglobulin free light chain (FLC) Freelite assay measures levels of free κ and λ chains by immunonephelometry and has undoubtedly exhibited clinical utility in the management of plasma cell (PC) disorders. In fact, it has been shown to improve sensitivity at screening, to assess prognosis and treatment response, and to monitor residual disease in oligo-nonsecretory Multiple Myeloma (MM). However, the test has both technical and economic limitations because of potential fluctuations in reproducibility and additional costs, impacting on the actual cost-effectiveness in clinical practice. For these reasons, its use should be carefully weighted and limited to defined clinical situations. We describe a case in which Freelite assay was used to reach a correct diagnosis of nonsecretory MM in a patient who presented with severe renal failure (serum creatinine 2.9 mg/dl, creatinine clearance 22.8 ml/min) and hypogammaglobulinemia (IgA 20, IgG 279 and IgM 7 mg/dl) of uncertain origin. A PC disorder was suspected, but a preliminary screening showed neither measurable monoclonal protein in serum nor Bence Jones proteinuria and the bone marrow biopsy revealed 15% monoclonal κ PC proliferation without amyloid deposition detectable by Congo red staining. On these bases, a diagnosis of MM according to IMWG criteria could not be reached. Meanwhile, a kidney biopsy was performed, revealing a histological picture of focal segmental glomerulosclerosis along with monoclonal κ PC involvement of the renal interstitium. Therefore, Freelite assay was used, which demonstrated an abnormal FLC ratio (7.38) in serum with an involved κ chain of 172 mg/L. This solved the diagnostic uncertainty and allowed to conclude for nonsecretory MM. Moreover, Freelite determination provided a baseline value for subsequent evaluation of IMWG Response Criteria, overall response rate and progression free survival upon initiation of treatment. In conclusion, Freelite assay confirms its powerful role in difficult diagnostic processes of PC disorders.

D70 - ALTERED FREE LIGHT CHAIN LEVEL AND RATIO PREDICTED THE RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: A CASE REPORT

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Background. Serum free light chain (FLC) assay is used for diagnosis and management of plasma cells (PCs) disorder, according to International Myeloma Working

Group guidelines. *Methods.* We present a case of a 53 years-old woman diagnosed for λ light chain myeloma in 2011. She arrived at our department with the following clinical history: diffuse bone pain with lytic lesions (total body X-Ray); hematological evaluation highlighted anemia (Hb 9.4 g/dL) and polyclonal immunoglobulin depression;urinary assay showed λ Bence Jones proteinuria (BJP) along with massive 24h proteinuria (13 g). *Results.* After several investigations (backbone RMN, bone marrow aspirate: 25% PCs) and biochemical assessment (immunofixation (IF): monoclonal free λ chain; FLC k: 20.12 mg/L and FLC λ: 5582 mg/L; turbidimetric method, Freelite™, The Binding Site Ltd, Birmingham, UK) clinicians diagnosed λ light chain myeloma III stage (International Staging System). She was submitted to chemotherapy (Velcade, Thalidomide, Desametasone) and, after CD34+collection, to autologous stem cell transplantation (SCT). After SCT, total body X-Ray and backbone RMN showed no worsening; bone marrow aspirate:<5% plasma cells; serum analysis normalized (IF: negative; FLC k: 10 mg/L, FLC λ: 5.37 mg/L, k/λ ratio: 1.86 [R.I. 0.26-1.65]; BJP: negative; 24h proteinuria: 0.14 g). The treatment lead to a near complete response (nCR). After nine months without bone lesions progression and relevant hematological alterations, FLC λ began to increase (28.94 mg/L) even if FLC ratio (0.44) was still within reference interval; then FLC ratio altered too (FLC k: 8.27 mg/L, FLC λ: 177.22 mg/L, k/λ ratio: 0.04) with BJP still negative and 24h proteinuria of 0.22 g. Three months later, also λ BJP became positive together with glomerular-tubular proteinuria and an increase of 24h proteinuria (1.59 g). *Conclusions.* Based on these results new bone marrow evaluation was conducted (14% PCs), confirmed the relapse and the necessity of a new therapy. In this case report serum FLC level and its ratio were the first altered parameters predicting the relapse and lead clinician to carry out further investigations.

D71 - EARLY DETECTION OF RECURRENCE IN THE MONITORING OF MULTIPLE MYELOMA BY HEVYLITE• CHAIN RATIO

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A significant number of patients with multiple myeloma (MM) have a paraprotein that co-migrates with other serum proteins, such as transferrin, and there is difficulty in quantitation of the M-component by protein electrophoresis. This in turn can affect patient monitoring. Hevylite chain (HLC) testing allows quantitative analysis of the involved monoclonal immunoglobulin and uninvolved polyclonal immunoglobulin of the same immunoglobulin type, expressed as a ratio. This is of particular value when a paraprotein is obscured by co-migration with other serum proteins. HLC antibodies target an epitope that bridges the heavy and light components of the immunoglobulin molecule and so measures the intact immunoglobulin. Here we describe the use of HLC in monitoring a patient with an IgGκ multiple myeloma (MM) where the paraprotein migrated in the β 2 region with other serum proteins. HLC ratio was measured retrospectively over a 16 month period using the Hevylite assay (The Binding Site SPA PLUS) and compared with standard methods for monitoring MM (capillary zone electrophoresis (CZE), total immunoglobulins and serum free light chains [sFLC]). The HLC ratio became abnormal and continued to rise, indicative of a monoclonal progression. At this time CZE showed no evidence that a relapse was occurring. The HLC ratio indicated changes in the M-component three months before detection by CZE was possible. The eventual rise in M-component by CZE was accompanied by an increase in the sFLC ratio and total IgG. In this case study the monitoring of HLC ratio was not considered to have effected the management of the patient. However the HLC ratio could be an effective addition to standard techniques in the monitoring and early detection of relapse in patients with MM, especially where the M-component co-migrates with other serum proteins or is too small to quantify.

D72 - A CASE OF LIGHT CHAIN ESCAPE-MULTIPLE MYELOMA: IMPORTANCE OF SERUM FREE LIGHT CHAIN ASSAY IN RELAPSE DETECTION

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Background. Multiple myeloma cases that at diagnosis produce an intact immunoglobulin chain can relapse, after a period of partial or complete remission, trasforming in light chain producing disease. This phenomenon has been known for a long time and has been defined "light chain escape". Recently, sFLC assay has allowed to measure levels of free k and λ chains and can be used to monitor disease. *Case report.* A 62-year old male was diagnosed with IgGk, stage I (according to ISS) MM (del 13q14) in february 2009. Serum protein electrophoresis (SPEP) revealed a monoclonal protein spike (2,38 g/dl), serum immunofixation (IF) disclosed an IgGk paraprotein, kFLC were 178 mg/l. Bone marrow aspiration showed 35% of plasma cells. Multiple costal fractures were present. Patient received VTD (borteomib, thalidomide, dexametasone) therapy followed by autologous peripheral blood stem cell transplantation (auto PBSCT) and obtained a complete remission with negative SPEP, IF end sFCL. Two years after transplatation patient showed a rapidly progressive sFCL (to a maximum of 13900 mg/l), with negative SPEP. A grade 1 anaemia(10,8 g/dl) was present, neither renal failure nor bone lesions. Plasma cells infiltrating bone marrow were 90%. Patient was treated with VCD (bortezomib, cyclophosphamide, dexametasone), second auto PBSCT, VD consolidation (bortezomib, dexametasone), thereby obtaining a complete remission with negative bone marrox aspiration, IF and sFCL (15,5 mg/l). Seven months later sFCL increased

to 2820 mg/l with a bone marrow infiltration of 40%. Treatment with Rd (lenalidomide, dexametasone) was started and complete remission was induced, which has been lasting for 10 months. *Discussion.* Our patient represents a case of LCE-MM in which serial detection of sFCL permits early recognition of progressive disease due to a subclone expansion not identified by clasiccal diagnostic tools (*i.e.* SPEP); follow-up of sFCL can thus prevent further complications and improve treatment results.

D73 - CLINICAL FEATURES AND PROGNOSIS OF IgD MULTIPLE MYELOMA: A DESCRIPTIVE STUDY OF FIVE CASES IN THE SOUTH AREA OF SPAIN

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Background. IgD MM is a rare variant (2% of all MM) associated with a poor prognosis. *Objectives.* To describe the clinical features and prognosis of IgD MM patients in the South area of Spain. *Methods.* Five patients diagnosed of IgD MM between 2010 and 2014 have been studied. Data were analyzed from the medical record of the patients. *Results.* The main presenting features of the patients were: median age at disease presentation of 63 years, male predominance (80%), bone pain (80%), lytic bone lesions (100%), renal function impairment (100%, median value of creatinine of 3,37 mg/dl), predominance of λ light chain (80%), M-protein undetected by serum protein electrophoresis (40%), serum free light chains ratio abnormal at baseline (100%), hypercalcemia (40%), presence of plasmacytomas (20%), associated amyloidosis (20%) and aggressive clinical course (ISS stage 3, 80%). The median value of plasma cell in bone marrow was 26%. In the patient with IgD κ MM; the value of free κ at diagnosis was 24769 mg/L with free λ of 15.78 mg/L and a ratio of 1570. In IgD λ MM patients; the median value of free λ was 2859 mg/l with median free κ of 7.57 mg/L and a median ratio of 0.002. There were four disease-related deaths with a median survival of 21 months (Table 1). *Conclusions.* IgD MM presents clinical and laboratory findings that defines a distinct entity. In our population, IgD MM is characterized to have poor prognosis with a median survival of 21 months after diagnosis confirming previous studies. The recognition of IgD monoclonal component can be sometimes difficult to detect by SPE and the quantification of serum free light chains are essential for the diagnosis of these patients and improve the management of patients these patients.

Table 1.

Patient	*1*	*2*	*3*	*4*	*5*
Age (years)	83	50	63	54	77
Plasma Cells (%)	28	4	15	60	26
Serum Protein Electroforesis	Positive Large peak	Negative	Negative	Positive Small peak	Positive Small peak
Serum Immunofixation	IgD-L	IgD-K	Negative	IgD-L	IgD-L
Bence Jones Protein	No sample	κ	Negative	λ	λ
Free κ (mg/L)	11.20	24769	1.57	3.15	8.62
Free λ (mg/L)	1410	15.78	3290	4025	2427.5
Ratio κ/λ	0.008	1570	0.0005	0.001	0.0036
β-2-Microglobulin (mg/L)	23.06	21.82	3.0	6.2	18.7
Creatinine (mg/dL)	8.3	14	1.20	4.24	3.37
Diagnosis	IgD-L MM ISS 3	IgD-L MM ISS 3	IgD-L NSMM ISS 1	IgD-L MM ISS 3	IgD-L MM ISS 3
Survival (months)	1½ (Exitus)	21 (Exitus)	48 (Exitus)	10 (Exitus)	5 (Alive)

D74 - A RARE CASE OF PLASMA CELL MYELOMA IN A 12-YEARS OLD PATIENT

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Introduction. The presence of Multiple Myeloma (MM) in young patients aged less than 30 years old is rare. *Objectives.* Report the case of a 12 year old boy diagnosed with a plasma cell myeloma. *Case report.* Twelve years old patient presented asthenia and anorexia of one month duration. He presented hypercalcemia (16.6 mg/dl), increased IgA (4449 mg/dl), hyperproteinenia (12.6 g/dl), anemia (9.5 g/dl hemoglobin) and osteolytic bone lesions. Given the suspicion of plasma cell neoplasia the patient was admitted to our hospital to be studied. New analytic confirmed previous results (IgA=4820 mg/dl, calcium=16.1 mg/dl, hemoglobin=8.0 g/dl) with other significant findings: creatinine 1.61 mg/dl, albumin 3.2 g/dl, β2microglobulin 5.98 mg/l, presence of rouleaux formation of erythrocytes, ESR=134 mm/hour, immunoparesis of the other immunoglobulins (IgG=525 mg/dl and IgM=37 mg/dl) and altered ratios of Freelite (free κ=219 mg/dl, free λ=1.01 mg/dl, ratio=216.83) and Hevylite (IgA-K=6660 mg/dl, IgA-L=630 mg/dl, ratio=10.57). The serum proteinogram showed a well-defined monoclonal peak in gamma region (4.34 g/dl) confirmed by IgA κ immunofixation. Bone marrow biopsy detected neoplastic cell proliferation with a CD138+,

CD56- and CD20- immunophenotype. Although the patient age does not correspond with MM, the symptomatology and laboratory findings confirmed the diagnosis of MM. Patient was treated with six cycles of chemotherapy based on Bortezomib-Cyclophosphamide-Dexamethasone. At the end of the treatment, the patient achieved a status of complete remission with negative immunofixation, <5% of plasma cells in bone marrow and normal ratio of Freelite but altered ratio of Hevylite confirming the existence of residual disease. *Conclusions.* The diagnosis of MM in patients younger than 30 years is rare. However, this case demonstrates that plasma cell neoplasms should be considered in the differential diagnosis of very young patients presenting severe hypercalcemia and destructive bone lesions to rule out the disease despite the very low incidence in these patients.

D75 - USEFULNESS OF SERUM FREE LIGHT CHAINS IN THE EVALUATION OF THE RESPONSE OF A NON SECRETORY MULTIPLE MYELOMA

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Background. Non Secretory Multiple Myeloma (NSMM) is characterized by the absence of detectable monoclonal proteins in serum and urine; therefore, invasive bone marrow examinations are required for monitoring the disease activity. Quantification of serum free light chains (sFLC) allows us a sensitive method to evaluate these patients. *Objectives.* To show the utility of sFLC assay (Freelite, The Binding Site) in the monitoring of NSMM patients. *Case report.* A 63 year old man diagnosed of NSMM was in complete response after treatment and autologous stem cell transplantation (ASCT). During the monitoring of the patient after ASCT (Figure 1), sFLC λ levels began to increase with abnormal sFLC ratio (51.2 mg/L at month+46 with ratio=0.12; 144 mg/L at month+47 with ratio=0.08) suggesting recurrence of NSMM at this moment. In month+50 (λ=572 mg/L with ratio=0.02) ; the bone marrow showed a 4% of plasma cells and the serum protein electrophoresis and Bence Jones proteinuria were negative. The patient began treatment with Lenalidomide/Dexamethasone and received 13 cycles with reduction of sFLC λ to 20.1 mg/L in month+58 with ratio=0.58. Seven months later this treatment, sFLC levels began to increase again with values of 231 mg/L at month+65 (ratio=0.09), 893 mg/L at month +67 (ratio=0.01) predicting a new relapse. At month+69, the patient presented a clinical relapse with presence of new osteolytic lesions. He began a new treatment with Bortezomib/Dexamethasone. *Conclusions.* Freelite is a noninvasive assay that has the potential to be useful for monitoring the disease activity of NSMM. Due to their high sensitivity, this assay can predict a relapse months before evidence of clinical relapse is present improving the precision in monitoring patients with NSMM. Furthermore, Freelite reduces the number of bone marrow biopsies for this group of patients avoiding patient anxiety with fewer bone marrow biopsies.

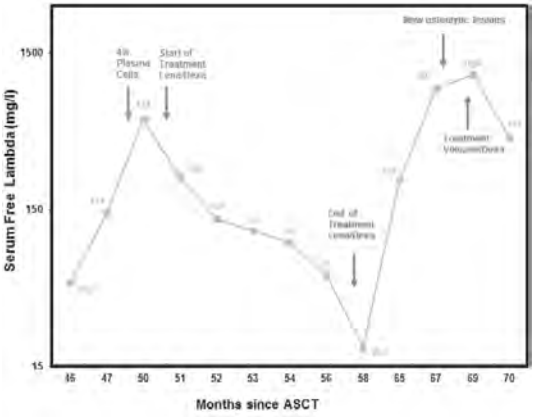

Figure 1.

D76 - CLONAL TIDES OF MULTIPLE MYELOMA: REPORT OF AN INFORMATIVE CASE

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Background/Objective of the study. Clonal heterogeneity is one of the key factors of high risk multiple myeloma (MM). Here we report an informative case of a multiple myeloma patient with a high variability of disease markers indicating several sub-clones. *Methods.* As disease marker serum and urine electrophoresis, total immunoglobulin levels, Freelite™ and Hevylite™ assay were used and correlated to bone marrow cytogenetic analysis including fluorescence *in situ* hybridization (FISH). *Results.* At time of diagnosis in 2007 immunofixation showed three monoclonal proteins but nearly normal free light chains. FISH showed a complex pattern of cytogenetic abnormalities. Four years after first line treatment increasing M protein accompanied by highly elevated free light chains indicated a new sub-clone confirmed by cytogenetic analysis. The light chains decreased to within normal range under the bortezomib based second line regimen. However, monoclonal IgA increased after one year without light chain component suggesting another sub-clone showing clonal evolution and this was confirmed by cytogenetic analysis. After change to lenalidomide all disease parameters declined and this is ongoing. Eventually, treatment had to be stopped for thrombocytopenia and thromboembolic events caused by endocarditis and macrophage activation syndrome. At this time point, bone marrow showed an increase of plasmablastic cells and unusual mastocytosis indicating another non-secretory sub-clone. One year later after relapse treatment with bendamustin, bortezomib and dexamethasone the patient showed plasmablastic MM in addition to secondary myelodysplastic syndrome.

Conclusions. Here, the use of disease markers allowed to detect clonal instability and to identify several sub-clones with different sensitivity to various treatment strategies.

D77 - IMPORTANCE OF FREE LIGHT CHAIN DETERMINATIONS IN MULTIPLE MYELOMA PATIENTS WITH RENAL INVOLVEMENT: CASE REPORT

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One of the main complications of Bence Jones myeloma is renal impairment. The determination of free light chains (FLC) and serum electrophoresis in cases of acute renal failure with no apparent justification allows rapid diagnosis in case of myeloma kidney. Rapid initiation of treatment and reduction of circulating FLC ensures good chances of recovering renal function in most patients. *Case report.* 80 year-old women develops acute renal failure (ARF) after abdominal surgery requiring hemodialysis. The analytical study (May/30/2013) shows hypogammaglobulinemia, and monoclonal λ FLC identified by immunofixation and Freelite® 12000 mg/L, suspecting multiple myeloma with renal involvement due to cast nephropathy. Bone marrow aspiration confirms MM diagnosis (40% plasma cells). Chemotherapy (dexamethasone and bortezomib) is initiated in parallel with high cut-off hemodialysis (Theralite 2.1 m^2) to help reduce FLC levels thus decreasing the nephrotoxic stress. Nine consecutive 6-hour sessions are made, resulting in a decrease of >98% from the first to the last dialysis session: 3200 mg/L *vs* 61.5 mg/L. Creatinine values remain high, typical of Acute Tubular Necrosis (6.5-6.8 mg/dL) until July 11th when a progressive decrease is observed. Presently, the patient is dialysis independent with stable creatinine values of 2.4-2.6 mg/dL, largely compensating the high cost of the filters (€ 700). The rapid decrease of the FLC prevented progression to interstitial fibrosis avoiding irreversible renal damage and life-lasting dialysis dependence (Figure 1).

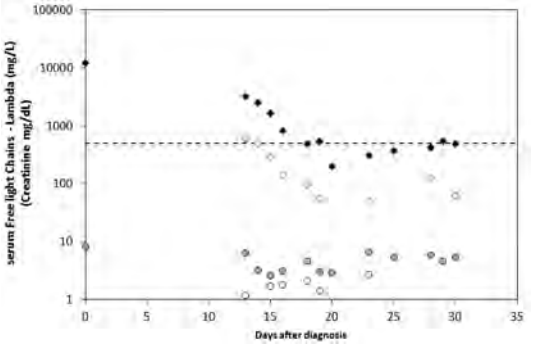

Figure 1. Creatinine (circles) and serum FLC (diamonds) values at diagnosis and during dialysis of high cut-off. Closed and open symbols are pre- and post-dialysis values, respectively. The horizontal dashed line represents the 500mg/L nephrotoxic FLC limit.

Conclusions. Due to ARF, serum FLC determinations were the only way to assess treatment response and efficacy of FLC removal during dialysis. Interestingly, the decrease of FLC levels appears to predict renal recovery earlier than creatinine. Finally, the use of FLC in the study of ARF resulted in the quick diagnosis of MM and myeloma kidney allowing the initiation of specific treatment to avoid permanent renal failure with long-term clinical benefits and a cost-effective use of healthcare resources: ambulatory hemodialysis/month: € 3,610.

D78 - A CASE REPORT OF A HEAVY CHAIN DISEASE RECOGNIZED BY DISCREPANCY BETWEEN RESULTS OF SERUM PROTEIN ELECTROPHORESIS AND HEVYLITE ASSAY

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Background. The heavy chain disease (HCD) is characterized by the presence of an abnormal paraprotein molecule consisting of truncated heavy chains dimmer. The Hevylite assay is used for quantification of the κ/λ-bounded amounts of IgG, IgA, and IgM immunoglobulins. *Aim of the study.* To report a case of HCD associated with multiple myeloma (MM). *Methods.* Serum protein electrophoresis (SPE) was used for densitometric measurement of the paraprotein, the immunofixation was used for identification of the paraprotein. The Hevylite assay was used for the IgGκ and IgGλ quantification. The Freelite assay was used for free light chains (FLC) κ/λ measurement. The sodium dodecyl sulfate electrophoresis (SDSE) was used for separation of proteins according to their molecular mass and the immunostaining was used for the characterization of the abnormal paraprotein. *Results.* SPE showed an M-spike of 44.6 g/L, and M-protein IgGκ was identified using immunofixation. The levels of IgGκ and IgGλ measured using the Hevylite assay were 8.9 g/L and 0.058 g/L, respectively. The levels of FLCκ and FLCλ were 104.3 mg/L and 4.86 mg/L, respectively. The SDSE with immunostaining showed the IgGκ level comparable with NHS serum using the anti-κ antibody; indicating the presence of intact monoclonal IgGκ immunoglobulin. The anti-IgG antibody recognised at least one other protein migrating at around 100kDa, which is consistent with immunoglobulin heavy chain dimmer. *Conclusions.* The monoclonal intact immunoglobulin IgGκ, truncated IgG heavy chain dimmer and FCLκ are concomitantly present in a patient with MM.

D79 - THE HEVYILITE• ASSAY COULD BE USED FOR MONITORING MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA PATIENTS. A CASE REPORT

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Background. The detection of minimal residual disease (MRD) is important for monitoring multiple myeloma patients. Less is known if maintenance therapy could influence the MRD levels and the prognosis. In this case study for MRD detection we quantified the intact immunoglobulins with the Hevylite® assay (HLC) during initial and maintenance therapy to evaluate the impact on outcome. *Objectives/Methods and Patients.* The patient in this case study had an IgG-k myeloma. After first-line induction therapy with Bortezomib the patient received high-dose chemotherapy (HD-PCT Melphalan) and autologous stem cell transplantation (ASCT). Subsequently the patient received maintenance therapy with Bortezomib until progression. HLC ratio (HLCR) and serum free light chains (sFLC) were quantified using polyclonal antisera assays on the BN II nephelometer. *Results.* After initial therapy and ASCT the patients relapsed followed by a second high-dose chemotherapy and ASCT. After this therapy the serum free light chains (sFLC) and β-2-microglobulin levels were normal but based on a pathological HLCR the patient received maintenance therapy with Bortezomib. The HLCR became normal and the patient is in a good condition for more than a year. *Conclusions.* The quantification of the HLCR is helpful and sensitive assay to evaluate the MRD after a therapy and can be used for the decision if a maintenance therapy is sensible. It was more sensitive than the sFLC and the β-2-microglobulin. In our case study the normalization of the HLCR showed a good correlation with the patients outcome.

D80 - THE CERVICAL FRACTURE FIRST SYMPTOM OF MULTIPLE MYELOMA: CASE REPORT

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Background. Multiple myeloma (MM) is a clonal disorder characterized by proliferation and accumulation of malignant plasma cells in the bone marrow. Its annual incidence is 6/100,000 in western countries and in Italy are reported about 2,000 new cases a year. The median age at diagnosis is 65 years[1]. Bone disease occurs in 80% of patients with newly diagnosed MM, and in 70% of the case bone pain is the first symptom to be reported at disease onset. Spine is the bone site that is most frequently affected by MM-related lesions. The cervical spine is the least common site of disease involvement. *Case report.* A 60-year-old female patient was referred to the Department of Neurosurgery of the General Hospital "P. Giaccone" in Palermo, for neck pain. Neurologic examination revealed no abnormalities. A magnetic resonance imaging scan showed a pathological fracture of the fifth cervical vertebra (C5). The results of the laboratory tests showed haemoglobin 11,1 g/l, β_2-microglobulin 3,1 mg/l; serum creatinine and calcaemia were within the normal range. The protein electrophoresis and immunofixation revealed a monoclonal immunoglobulin (Ig) A band with κ chain, total proteins was 9.66 g/dL. The bone marrow examination showed infiltration with 30% of plasma cells, leading to a diagnosis of IgA MM with κ light chains. The patient underwent cervical arthrodesis and started systemic Bortezomib-Thalidomide-Dexamethasone (VTD) combination chemotherapy. During the follow up the general condition of our patient was well with disappearance of pain. *Discussion.* Bone dissolution caused by MM may lead to pathologic fractures, osteopenia, or lytic lesions. The involvement of the cervical spine is not common. We report a case of MM with pathological fracture of C5 discovered accidentally after a traumatic event. This case underlines the importance of suspecting MM in all the cases of compromised bone.

Reference

1) S. Rajkumar. Am.J.Hematol. 89:999-1009, 2014.

D81 - A CASE OF RECURRENT SOLITARY PLASMACYTOMA OF THE BONE PROGRESSING TO MULTIPLE NON-SECRETORY EXTRAMEDULLARY PLAMACYTOMAS

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Here we report a case of a 53-year-old Caucasian male who presented with back pain 3.5 years ago. MRI of the spine confirmed expansile lesion and L3 pathologic fracture but without other abnormalities. Biopsy showed CD138 positive, λ light chain restricted plasma cells. Serum and urine electrophoresis and free light chain studies showed no monoclonal protein. Bone marrow showed trilineage hematopoiesis without plasmacytosis. He was treated with radiation therapy and kyphoplasty. One year and 4 months later he developed chest pain. CT scan showed lytic lesion of 4th right rib. Biopsy showed λ light chain restricted CD138 positive plasma cells. Bone marrow biopsy was normal. Serum and urine studies showed no monoclonal protein. The PET CT was negative. He then received radiation therapy. One year and 6 months later, the patient developed painless neck swelling. PET-CT showed two cervical masses 3.5x1.5 (SUV 3.0) and 1x0.8 (SUV 2.5), a density in the anterior cardiophrenic angle 4x1.8 cm (SUV 2.5) and bilateral pararenal soft tissue densities 2.5x2.4 cm and 2x1cm respectively. Biopsy showed λ light chain restricted CD 138 positive plasma cells. Serum and urine studies showed no monoclonal protein. Bone marrow was without plasmacytosis. After two cycles of bortezomib, cyclophosphamide and dexamethasone, PET-CT showed

complete resolution of the pericardial, pararenal densities, one cervical node and reduction in the size of the second cervical node from 3.5 cm to 1.3 cm (SUV 1.5). Patient received two additional cycles and underwent auto transplant with MEL 200. Non secretory myelomas are rare plasma cell neoplasms. We believe this case is unique because this patient's plasma cells never secreted any monoclonal protein over a period of three years and now proliferate only in extramedullary microenvironment.

D82 - MEASUREMENT OF HEAVY/LIGHT CHAIN PAIRED SUPPRESSION MAY AID ASSESSMENT OF RESPONSE TO TREATMENT

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Background. Quantification of monoclonal immunoglobulins (M-Ig) by serum protein electrophoresis (SPE) and capillary zone electrophoresis (CZE) may be challenging if co-migration occurs with other serum proteins. This is a particular issue with IgA multiple myeloma and when it occurs international guidelines recommend the measurement of total IgA (tIgA). Heavy/light chain (HLC) assays have been developed that quantify IgAκ and IgAλ separately. This provides a more accurate quantification of the involved M-Ig (*e.g.* IgAκ in an IgAκ patient) but also a quantification of the uninvolved polyclonal HLC-pair (uHLC, *e.g.* IgAκ in an IgAλ patient). Here we report a single case study illustrating the utility of uHLC measurement for assessment of response to treatment. *Methods.* From the patient (IgAκ multiple myeloma, male; 83 years old), five sequential serum samples were taken post treatment. HLC levels were measured turbidimetrically at Kings College Hospital, London, UK. Results were compared prospectively to CZE and total IgA. *Results.* Concentration of M-Ig over the five post treatment time points when measured by CZE (median 29.5 g/L; range 29.1-30.5 g/L) and tIgA (median 36.2 g/L; range 32.2-37.7 g/L) remained constant. Measurement of IgAκ/IgAλ HLC ratio however, showed a decrease from 289 to 189 (median 188; range 189-332). The concentration of iHLC (IgAκ) was constant during monitoring (median 34.7 g/L; range 34.7 g/L-39.5 g/L) and correlated well with tIgA (Mann Witney, 34.7 g/L *vs* 36.2 g/L, p=1.0) whereas the concentration of uHLC increased in response to treatment (median 0.12g/L; range 0.1-0.18 g/L). *Conclusions.* The measurement of IgA HLC ratios and uHLC in this patient indicated a response to treatment, which decreased tumour specific HLC pair suppression, but was not detected by CZE or measuring tIgA. Measurement of uHLC tumour specific isotype paired suppression in MM patients may aid the clinician assess patients response to treatment.

D83 - HEVYLITE AND FREELITE ASSAYS PROVIDE ADDITIONAL CLINICALLY-RELEVANT INFORMATION WHEN ASSESSING MULTIPLE MYELOMA AND OTHER PATIENTS WITH PLASMA CELL DISORDERS

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Background. Freelite assays have been used for years in patients with PCD. Hevylite is a newer test that allows measurement of clonality in intact immunoglobulin pairs such as IgA-κ and IgA-λ. Minimal residual disease (MRD) is starting to be measured on bone marrow analysis in MM patients. Some patients may not have normal Hevylite and Freelite values when other traditional tests have normalized so these assays may be able to be used to detect forms of MRD. *Objectives.* Determine if Hevylite and Freelite assays can add value in the assessment of PCD patients when other standard tests are not useful. *Methods.* Perform Freelite and Hevylite assays on patients in whom standard tests are not useful. *Results.* Several cases have been found where Freelite and/or Hevylite added additional value to standard tests. In a 52 year old patient with high risk MM, the κ light chains prior to autologous stem cell transplant (ASCT) remained elevated at 155 mg/dL (normal range 0.33-1.94 mg/dL) while the M protein on conventional testing (SPEP) was too low to be followed clinically (0.09 g/dL). The bone marrow biopsy showed 25% plasma cells. At day 100 post ASCT, the κ free light chains (2.53 mg/dL), and κ/λ ratio (2.09, normal range 0.26-1.65) remained elevated. The bone marrow biopsy revealed 2-3% plasma cells with persistent cytogenetic abnormalities. *Conclusions.* The Freelite assays in the above patient provided additional clinically relevant information. Serological tests like Freelite and Hevylite are less expensive and easier to perform than a bone marrow analysis. As long as the Freelite and/or Hevylite tests are abnormal, it may be a sign that there is still an abnormal clone present, and it may not make sense to perform MRD testing until these tests are normal.

D84 - MONITORING OF MULTIPLE MYELOMA PATIENTS WITH HEVYLITE® MIGHT BE ADVANTAGEOUS TO DETECT RELAPSES EARLIER THAN OTHER CONVENTIONAL ASSAYS

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Background. Common assays for identification and monitoring of monoclonal gammopathy are the combination of serum protein electrophoresis (SPEP), serum and urine immunofixation electrophoresis (IFE) and the determination of the total immunoglobulins and the serum free light chains (FLC). The development of the Hevylite® assay (HLC) allows identifying and quantifying of the different

light chain types of each immunoglobulin (Ig) class (for example IgA-κ and IgA-λ). The calculation of the ratio (HLCR) enables us to define the monoclonal amount of the immunoglobulins. The HLC-assays is used for diagnosis, response assessment, for monitoring and for prognostication of monoclonal gammopathies. *Objectives/Methods and Patients.* In this case study we made serial measurements of HLC and FLC during follow-up of two patients to determine and evaluate the sensitivity of the assays and compared them with the total immunoglobulins. With these assays we want to estimate the response to therapy and to detect relapses. HLC and FLC were quantified using polyclonal antisera assays on the BN II nephelometer. Absolute values of IgG, IgA and IgM were analyzed on the BN II nephelometer. *Results.* After initial therapy the two patients had reached normal total IgA respectively values. At this time point the HLCR was pathological. This could suggest that there was a minimal residue disease. The HLC and the FLC-assays indicated a relapse before the total immunoglobulins and SPEP became abnormal. *Conclusions.* The measurement of FLC and HLC and the determination of the ratio could provide additional information about the clonality. In our case studies we could detect the relapse earlier with HLC than with SPEP. The occurrence of a pathological HLCR earlier than the relapse could be evidence for a residual disease.

D85 - USE OF BIOLOGICAL VARIATION FOR THE INTERPRETATION OF FREE LIGHT CHAIN RESULTS: A CASE REPORT

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Background. Biological variation components for FLC have been determined (Clin Chim Acta 2012;414:10-11). The index of individuality for κ, λ and κ/λ are low indicating that the establishment of an individual's baseline will be more meaningful than comparing results to population based reference ranges. The reference change value (RCV), above which a change is statistically significant is 23, 20, and 13%. An individual's own baseline result during health is rarely known for plasma cell disorders. *Objectives.* Determine if use of FL BV data is useful when monitoring MM patients. *Methods,* Five serial serum samples from an IgAκ multiple myeloma patient who had been treated, but was in remission, were tested using serum protein electrophoresis (SPE), (Sebia, Norcross, GA) and FLC (Binding Site, Birmingham, UK). *Results.* The first 2 samples had a normal SPE (Figure 1A,B), FLC were within the reference range, and the slight difference in serial FLC results did not exceed the RCV. The 3rd sample showed an increase in the free κ (0.78 to 1.7 mg/dL) and λ (0.79 to 1.6), exceeding the RCV. The free κ/λ ratio did not, and the SPE was normal (Figure 1C). The 4th sample showed an increase in κ (2.56 mg/dL), a decrease in λ (1.10 mg/dL), an abnormal κ/λ (2.33), and a small monoclonal SPE band (Figure 1D). The change between samples 3 and 4 exceeded RCV for κ, λ and k/λ.The 5th sample showed more significant changes in FLC and a large monoclonal band (Figure 1E). *Conclusions.* Using RCV this patient showed a significant increase in free κ and λ 3 months before results were abnormal against a population-based reference range, and before a band was evident by SPE. It was another month before the appearance of an overt monoclonal band. Use of RCV for FLC may increase their clinical usefulness.

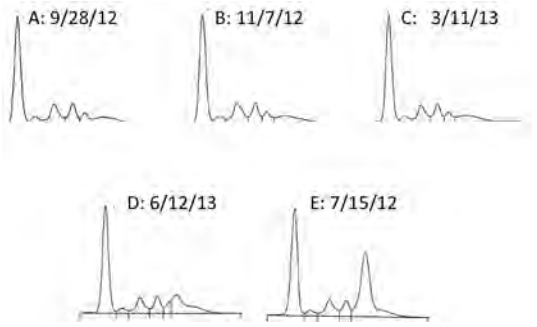

Figure 1.

AMYLOID

E86 - HEAVY/LIGHT CHAIN ANALYSIS IN 200 NEWLY DIAGNOSED PATIENTS WITH AL AMYLOIDOSIS

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Background. Detection of a free light chain λ or κ (the precursor of AL amyloid) or an M-protein is important in the evaluation of AL amyloidosis. The quantitative free light chain (Freelite®) assay has dramatically improved detection and follow-up of patients with AL amyloidosis. *Objectives.* To study additional utility of the quantitative heavy/light chain (Hevylite®) assay to the free light chain assessment for detection of AL amyloidosis. *Methods.* Serum from 200 consecutive patients with AL amyloidosis (104 men and 96 women, median age 63, range 33-88 years) was studied using first the free light chain assay followed by the heavy/light chain assay. Results were compared with serum protein electrophoresis, immunofixation, and urine electrophoresis. *Results.* Hundred and nineteen patients were diagnosed AL-λ (both increased free λ concentration and decreased κ/λ ratio) whereas 53 patients were diagnosed AL-κ (both increased free κ concentration and increased κ/λ ratio). Ten (5%) of the remaining 28 patients (14%) had a quantifiable heavy/light protein (6 IgG-λ, 1 IgG-κ, 1 IgA-λ, 1 IgA-κ and 1 IgM-κ) with both increased concentration and abnormal κ/λ ratio. Identical M-proteins were found in these 10 patients using serum immunofixation. Eighteen patients (9%) were not identified using the quantitative free light chain and heavy/light chain assays. Ten of these 18 patients had an M-protein or light chain using serum immunofixation (5 IgG-λ, 2 IgA-λ, 1 IgM-λ, 1 IgG-κ, and 1 BJ-λ) and 11 had a light chain or M-protein using urine immunofixation (9 BJ-λ, 1 IgA-λ, and 1 IgG-κ). No M-protein or light chain was detected in 5 patients (2.5%) whatever detection method was used. *Conclusions.* Quantification of free light and heavy/light chains is useful to detect 91% of AL patients, though qualitative detection using immunofixation of serum and urine is still necessary to identify another 6.5% of AL patients.

E87 - PHASE 2 STUDY OF BENDAMUSTINE IN COMBINATION WITH DEXAMETHASONE IN PATIENTS WITH PREVIOUSLY-TREATED SYSTEMIC LIGHT-CHAIN AL AMYLOIDOSIS

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Background. Relapse of AL amyloidosis patients after treatment with the alkylating agent melphalan plus dexamethasone is a significant problem. Bendamustine is a bifunctional alkylating agent with efficacy in CLL, NHL, and MM but its safety and efficacy in AL amyloidosis is not known. In an effort to improve the outcome of patients with relapsed AL we conducted a multi-center, Phase 2 study of Ben/Dex in AL (NCT01222260). *Objectives.* To determine the partial hematologic response rate (PR) of patients who received at least 2 cycles of therapy. Secondary objectives included overall hematologic response (OHR) rate (incorporating Freelite® measurements), organ response rate (OrRR), time to failure (TTF), toxicities and overall survival (OS), and the expression of genes associated with ER stress. *Methods.* A two-stage optimal Simon design was used to enroll relapsed patients (median of 2 prior therapies, creatinine clearance [CrCl] ≥15mL/min). Patients with very advanced cardiac involvement (NYHA Class IIIB/IV) or active renal failure were excluded. Dosing is described in Table 1. *Results.* Fifteen patients have received treatment. Median age was 66.5 (range 43-76). Four patients discontinued treatment: 1 due to disease progression, 3 due to AEs. Drug-related AEs included fatigue (67%), nausea (53%), anemia, constipation and dyspnea (47% each). Grade ≥3 AE occurring in >25% of patients were only fatigue and chronic kidney disease (27% each). No deaths and no increase in NT-proBNP were observed. Of 11 patients eligible for response evaluation, 5 (45%) responded hematologically (≥PR 36%, CR 9%). The median PFS is 11.2 months with a range of 0.4-15.7 months. Median OS has not been reached yet. *Conclusions*. Bendamustine in combination with dexamethasone is feasible and effective in heavily pretreated AL amyloidosis with impaired organ function (NYHA IIIB and creatinine clearance of 30-15 mL/min were allowed).

Table 1.

CrCl (mL/min)	*Bendamustine (mg/m²) Days 1, 2 (IV)*
≥60	100
59-30	90
30-15	70

Dexamethasone was started at 20-40 mg weekly based on performance status.

E88 - IMMUNOGLOBULIN FREE LIGHT CHAINS AND NT-PROBNP ALLOW EARLY CARDIAC DAMAGE DETECTION IN PATIENTS WITH PLASMA CELL DISORDERS

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Background and Aims. In immunoglobulin light chain amyloidosis a plasma cell clone synthesizes an unstable

misfolded light chain which is prone to aggregate and forms amyloid fibrils in target organ; clinical manifestations reflect advanced organ damage. In this study we exploited if serum free light chain and NT-proBNP may be sensitive biomarkers of presymptomatic early cardiac involvement. *Methods.* During routine clinical practice we observed 43 patients with a serum monoclonal protein (43- 91 years of age; 27 men and 16 women; 20 patients had IgG κ, 13 IgG λ, 4 IgA κ, 4 IgA λ, 2 IgM κ) who were evaluated with the current well standardized biochemical and imaging tests. Serum free κ and λ isotype levels (FLC) were measured using an immunoturbidimetric assay on the Roche Modular P analyzer (Freelite kit, The Binding Site Group Ltd, Birmingham, UK); NT-proBNP was measured with an automated electrochemiluminescence assay on Roche Elecsys analyzer. *Results.* All patients except one had high κ or lamba FLC with abnormal κ/λ ratio; at first visit, 18 patients (41,8%) had unexplained NT-proBNP level higher than the age and sex-specific reference range. AL amyloidosis was successively diagnosed in 8 of these patients (19,1%) with standard procedure; all these patients had elevated difference between involved and uninvolved light chain (FLC-diff >18 mg/dl). *Discussion and Conclusions.* Elevated FLC level with abnormal κ/lamba ratios and FLC-diff in combination with high level of NT-proBNP may allow early detection of cardiac disfunction caused by amyloidogenetic light chain and, therefore, should promptly trigger procedures to diagnose these insidious diseases.

E89 - FREE LIGHT CHAIN BURDEN AND ELEVATED ALKALINE PHOSPHATASE IDENTIFY PATIENTS WITH NON-CARDIAC AL AMYLOIDOSIS AND POOR OUTCOME

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Background. Heart involvement is the main prognostic determinant in AL amyloidosis. The outcome of patients with non-cardiac disease, who are generally considered low-risk, has not been systematically studied. *Objectives.* To identify factors affecting survival in non-cardiac AL amyloidosis. *Methods.* We selected 135 newly-diagnosed stage I patients without cardiac involvement from the Pavia database. *Results.* Involved organs were kidney (82%), soft tissues (16%), liver (13%), peripheral nervous system (10%), gastrointestinal (GI) tract (10, 7%), and 79% had >1 organ involved. Fifty-two patients (38%) received melphalan/dexamethasone (MDex), 42 (31%) bortezomib-based regimens, 14 (10%) stem cell transplant (ASCT), and 13 (10%) thalidomide-based regimens. After a median follow-up of 51 months, 23 patients (17%) died (5-year survival: 82%). Death was cardiac in 12 patients (52%) who had developed cardiac involvement. All had baseline dFLC >100 mg/L, 7 had failed to respond to therapy, and the remaining had relapsed. Eight patients (35%) died of liver failure. Six had liver involvement at baseline, and 2 developed hepatic amyloidosis during the follow-up, but had elevated alkaline phosphatase (ALP) at baseline (1.3 and 1.4 times the u.r.l., respectively). Five were non-responders and 3 had relapsed. One patient died due to GI bleeding during ASCT, 1 due to myelodysplasia (5.9 years after 6 cycles of MDex), and 1 due to colorectal cancer (6.5 years after diagnosis). Only the two latter not disease-related deaths occurred in patients who presented with normal ALP and dFLC <100 mg/L. Elevated ALP (HR 5.11, P<0.001) and dFLC (HR 4.79, P<0.001) were independent prognostic factors. *Conclusions.* In Stage I AL amyloidosis, fatal cardiac involvement develops only in patients with baseline dFLC >100 mg/L, and liver involvement emerges as a significant cause of death predicted by elevated baseline ALP. These patients should be treated aggressively to prevent terminal organ damage.

E90 - PROTEIN STUDIES IN LIGHT-CHAIN AMYLOIDOSIS

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Backround. Light-chain amyloidosis (AL) is a plasmocellular dyscrasia with deposition of fibrils consisting of monoclonal immunoglobulin light chains, leading to malfunction of the affected organs. Analysis of monoclonal immunoglobulin (MIg) is among the most important aspects in the diagnosis and monitoring of the disease. *Objectives.* The aim of the study was the detailed analysis of MIg in patients with AL. *Methods.* The analyzed group comprised serum and urine samples from 27 patients with AL, examined at the time of diagnosis. In all samples we performed SPE, sIFE, free light chain (FLC), UPEP and uIFE analysis. In 13 patients we provided determination of heavy/light chain (HLC) pairs and also quantification of IgG and IgA subclasses of immunoglobulins. For the analysis, SPAplus turbidimeter was used. *Results.* SPE and IFE identified MIg in 17/27 patients (4xIgGλ, 4xIgAλ, 1xIgGκ, 1xIgDλ, 10xλonly). UPEP and uIFE detected urine MIg in 15/27 patients (11xλonly, 1xκonly, 2xIgGλ+λ, 1xIgAλ+λ). In the case of FLC we detected abnormality in 25/27 patients with pathology of κ/λ ratio. HLC and subclasses analysis was performed in 13 patients (4xIgAλ, 2xIgGλ, 3xλonly, 4xIFEnegative). In case of IgA isotype the pathology of HLC and HLC ratio was detected in 3/4 patients, in 3/4 patients the elevation of IgA1 was found. In case of the IgG, pathology of HLC was observed in 2/2 patients, in one case IgG1, in the second IgG4. In λonly and IFEnegative samples we found pathology of HLC levels and HLC ratio in 6/7 patients, but only in IgG class. *Conclusions.* Our results support the importance of FLC examination as a fundamental parameter for the diagnosis and monitoring of patients with AL. To determine the significance of HLC analysis we need an extension of the existing group of patients.

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E91 - HEAVY/LIGHT CHAIN QUANTIFICATION IDENTIFIES CLONAL PLASMA CELL DISEASE IN PATIENTS WITH AL AMYLOIDOSIS AND NORMAL SERUM FREE LIGHT CHAIN RATIO

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Background. Serum and urine immunofixation electrophoreses (SIFE/UIFE) are used for detection of clonal immunoglobulins in AL amyloidosis. Serum free light chain (sFLC) testing provides a quantitative measure of clonal disease; however, up to 20% of patients may have uninformative sFLC values. *Objectives.* The Hevylite® assay was used to assess the potential role of serum heavy/light chain (sHLC) pairs in quantification of clonal disease in AL amyloidosis. *Methods.* The study included 199 AL patients. Serum samples were obtained at initial evaluation and stored at -20°C. SIFE/UIFE were performed on site. sHLC pairs were assessed by Hevylite® assay at the Binding Site; normal ratios were:1.12-3.21 for IgGκ/IgGλ; 0.78-1.94 for IgAκ/IgAλ; and 1.18-2.74 for IgMκ/IgMλ. sFLCs were assessed by Freelite® assay. In 103 cases, sFLC testing was performed on site; 96 cases were tested at The Binding Site. *Results.* Of 199 AL sera, the sFLC and sHLC ratios were abnormal in 163 (81.9%) and 105 (52.8%) of cases, respectively. Clonal SIFE/UIFE bands were detected in 187 (94%) cases. An abnormal sHLC ratio was found in 25 of 36 (69.4%) samples with a normal sFLC ratio and in 2 of 12 (16.7%) samples with no SIFE/UIFE band. Of 105 patients with an abnormal sHLC ratio, 78 (74.3%) had a single abnormal HLC pair, while 27 (25.7%) had multiple abnormal HLC pairs. A discrepancy between sHLC and SIFE/UIFE was found in four cases. *Conclusions.* All patients in this series had clonal disease by sFLC, sHLC, and/or IFE. The combined use of the sFLC and sHLC ratio yielded quantifiable information in 94.5% of cases. The significance of multiple abnormal sHLC pairs requires further investigation: it may reflect reduced clearance due to renal insufficiency; incomplete immunoglobulin class switching; or oligoclonal disease. The Hevylite® assay has potential for quantification of disease in AL patients with a normal sFLC ratio.

E92 - HOW DO HEMATOLOGIST EVALUATE AND TREAT SYSTEMIC AL AMYLOIDOSIS? CURRENT DATA FROM URUGUAY AND ARGENTINA

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Background. AL amyloidosis is a rare disease caused by deposition of abnormal proteins causing damage and disfunction of different organs or tissues. It is a plasma cell dyscrasia in which a monoclonal protein is detectable in urine and/or serum in >95 percent of affected patients by immunofixation and measurement of serum free light chains (sFLC). sFLC have improved diagnosis and follow up of this disease. *Objectives.* To describe current practice in Argentina and Uruguay concerning diagnosis, evaluation and treatment of systemic AL amyloidosis. To evaluate access to sFLC measurement in clinical practice. *Methods.* A predefined written survey was delivered via email to certified hematologists, through the Argentine Society of Hematology and Uruguayan Society of Hematology. Completion of the Survey was anonymous and voluntary. *Results.* Bone marrow biopsy and Congo Red staining are performed in over 90% of patients at diagnosis. sFLC assays are evaluated in around 50% of the patients in each country. Between 50 and 60% of hematologists include BNP or ProBNP and cardiac MRI in the usual workup. 25% of physicians in Uruguay have never diagnosed a patient with amyloidosis and did not fill treatment options (Table 1). Treatments preferred are those based on Bortezomib or Melfalan+corticosteroids, either for candidates and non-candidates to transplant. Few hematologists have indicated bone marrow transplant for amyloidosis patients (35% in Argentina and 34% in Uruguay) (Figure 1). *Conclusions.* Even though most AL amyloidosis patients receive Bortezomib-based treatments, we still need to define the standard of care considering current guidelines and Bortezomib reimbursement policies. The access to sFLC measurements is limited and variable between centers in both countries and also needs to be improved.

Table 1: Descriptive analysis of AL amyloidosis evaluation

	Uruguay	Argentina
Nº surveys answered/total nº hematologist	42 / 100	127 / 796
Work at Public/Private/Both (%)	19 / 45 / 36	51 / 49
BM biopsy (yes/no) (%)	92 / 8	94 / 6
Congo Red (%) (yes/no/unknown)	83 / 2 / 15	94 / 6
sFLC (%) (yes/no/unknown)	50 / 17 / 33	54 / 46
BNP or proBNP (%) (yes/no/unknown)	49 / 17 / 34	57 / 43
Cardiac MRI (%) (yes/no/unknown)	69 / 5 / 26	——

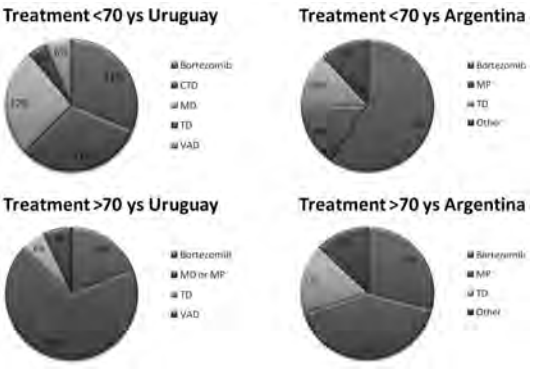

Figure 1.

E93 - CLINICAL AND CYTOGENETIC FEATURES OF LIGHT CHAIN AMYLOIDOSIS PATIENTS WITH A dFLC BELOW 50 MG/L AT FIRST DIAGNOSIS

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Background. Amyloid light chain (AL) amyloidosis is a rare and life-threatening protein-misfolding disorder. A major challenge is the quantification of the underlying clonal B or plasma cell disorder in patients with very low free light chains in serum (difference of κ and λ FLC below 50 mg/l). In most clinical trials dFLC<50 is an exclusion criterion; additionally, these patients are also underrepresented in many retrospective analyses because their serologic/hematologic response cannot be reliably measured. *Objectives.* Characterization of AL amyloidosis patients with dFLC<50. *Methods.* We retrospectively analysed 359 newly diagnosed (between 2003-2014) AL patients with first-line treatment of either melphalan-dexamethasone (Mel-dex) or bortezomib or high-dose melphalan followed by autologous stem-cell transplantation (HDM) who had received cytogenetic evaluation by iFISH. *Results.* Clinical characteristics and results are depicted in Table 1.

Table 1.

Parameter	All patients n=359	dFLC < 50 mg/l n=50	dFLC ≥ 50 mg/l n=309	P values
Age in years, median, range	59 (36-77)	60	59	ns.
Sex, female	146	23	123	ns.
Treatment groups, no. of pts / median follow-up in months				
M-dex	103 / 51	14	89	
Bortezomib	133 / 17	11	122	
HDM	123 / 61	25	98	
Lambda light chain restriction, no. of pts	293	44	249	ns.
BM Plasma cell count in %, median, range	10 (1-62)	7	12	<0.001
iFISH results, no. of pts				
t(11;14)	216	28	188	ns.
del 13q14	114	17	97	ns.
gain 1q21	82	11	71	ns.
Hyperdiploidy (Wuilkcne Score)	55	8	47	ns.
High-risk (del 17p13, t(4;14), t(14;16)	28	4	24	ns.
Organ involvement				
Heart, no. of pts	273	21	25	<0.001
KI, median; range	80 (40-100)	85	80	<0.01
Cardiac Mayo Score 2004 median; range	2 (1-3)	1	2	<0.001
Kidney, no. of pts	221	45	176	<0.001
MDRD, median; range	80 (3-386)	85	80	ns.
Number of organs median; range	2 (1-5)	2	2	ns.

Patients with cardiac involvement (42 *vs.* 82%) and higher Mayo Score (median 1 *vs.* 2) were underrepresented in patient with dFLC<50. Consecutively, KI was higher (median 85 *vs.* 80) and kidney involvement (90% *vs.* 60%) was more common. All analysed chromosomal aberrations were not associated with dFLC<50 (all p values >0.5), although plasma cell count of the bone marrow was lower (7% *vs.* 12%). Overall survival (OS) was better in patients with dFLC<50 regardless of treatment type: Mel-dex (median OS 19 months *vs.* not reached, p=0.02), Bortezomib group (median OS 16 months *vs.* not reached, p= 0.03) and HDM (median OS 97 months *vs.* not reached, p=0.03). *Conclusions.* AL patients with a dFLC <50 mg/l at first diagnosis represent a distinct clinical entity with a small plasma cell clone irrespective of iFISH results. This entity is associated with a low Mayo Score and has a very favourable OS. Results of prospective clinical trials might be adversely influenced by the exclusion of those patients.

E94 - SERIAL MEASUREMENT OF FREE LIGHT CHAIN DETECTS POOR RESPONSE TO THERAPY EARLY IN PATIENTS WITH AL AMYLOIDOSIS

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Background. Serum free light chain (sFLC) assays are important in diagnosis and monitoring of patients with multiple myeloma (MM). The short half-life of κ and λ FLC means that serum FLC assays may provide a more rapid indication of the response to treatment. We have previously demonstrated that close serial monitoring of FLC twice weekly during therapy is useful to evaluate the response to chemotherapy in patients with newly diagnosed MM, and earlier disease assessment by serum FLC assays may be of value in detecting poorly responding patients who need alternative forms of therapy (IJH 96:664, 2012). *Patients and Methods.* sFLC was measured using a FLC assay kit (The Biding Site Ltd, Birmingham, England) once weekly in two patients with AL amyloidosis treated with bortezomib and dexamethasone (BD). *Results.* Case #1: A 67-years-old man with AL amyloidosis (λ) had amyloid deposition in heart, kidney, stomach and liver. He was treated with 2 courses of melphalan and dexamethasone (MD), followed by 2 courses of BD. Involved sFLC decreased but rapidly increased in the latter part of each MD chemotherapy cycle before the next cycle started, suggesting that the response to MD may not be enough for induction therapy in this patient. During BD phase, rebound of sFLC level in the latter part of each chemotherapy cycle was not observed. After 2 courses of BD, he achieved VGPR. Case #2: A 68-years-old man with AL amyloidosis (κ) had amyloid deposition in heart, kidney and stomach. He was treated with high-dose dexamethasone followed by BD. During BD phase, rebound of sFLC level in the latter part of each chemotherapy cycle was minimal, and he achieved CR after 4 courses of BD. *Conclusions.* Serial weekly monitoring of FLC might be useful to evaluate the response to chemotherapy in AL amyloidosis.

METHOD EVALUATION AND COMPARISON

F95 - EFFECTIVE USE OF HEVYLITE®: JUDGEMENT BASED ON OPERATING EXPERIENCE

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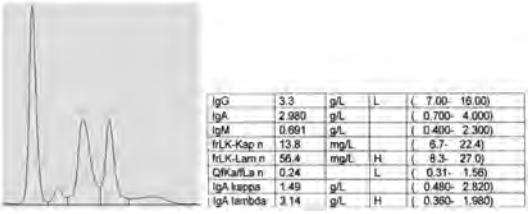
Background. Quantifying of paraproteins in gammopathies has so far usually been done by measuring the M-gradients of the serum-protein-electrophoresis (SPE). Together with the quantitative determination of the immunoglobulins (Ig), in fact the real percentage of the monoclonal (mc) Ig can only be estimated. Additional particular problems are caused by the hidden M-gradients, slight M-gradients and a simultaneous polyclonal state. Moreover, the nephelometric determination of the total–Ig cannot always be regarded as unproblematic (influenced by the plasma-volume, concentration depended IgG half lives). By means of Hevylite® the correspondent κ- and λ-subtypes of the Ig can be identified in quantitative terms and thus the relation of the involved and non-involved Ig-concentration can be calculated. *Objectives.* Objective quantification of problematic M-gradients and their effective use in Patient-Monitoring. *Methods.* Hevylite®, serum-protein-electrophoresis (SPE) and total-immunoglobulins (Ig). *Results.* - Hevylite® provides valuable assistance in quantification of M-Proteins in special cases (Figure 1); - the "Pairsuppression" is a valuable predicting parameter; - individually considered data also within the known reference values may be of importance. *Conclusions.* Hevylite® is a valuable method in quantification of M-Proteins: - it is a specifying tool, especially in problematic cases; - in some setting replacement of SPE and/or Ig is possible or even worthwhile; - and last but not least it may give us additional information (risk assessment).



IgG	3.3	g/L	L	(7.00- 16.00)
IgA	2.980	g/L		(0.700- 4.000)
IgM	0.691	g/L		(0.400- 2.300)
frLK-Kap n	13.8	mg/L		(6.7- 22.4)
frLK-Lam n	56.4	mg/L	H	(8.3- 27.0)
QfKa/fLa n	0.24		L	(0.31- 1.56)
IgA kappa	1.49	g/L		(0.480- 2.820)
IgA lambda	3.14	g/L	H	(0.360- 1.980)

Figure 1. Pat. L.W.: Monoclonal IgA/λ (in β)+Nephrotic Syndrome.

F96 - EVALUATION OF HEVYLITE® ASSAYS FOR THE QUANTIFICATION OF IgA MONOCLONAL COMPONENTS

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Background. In patients with Monoclonal Gammopathies (MG) monoclonal component (MC) quantification is necessary for differential diagnosis, risk stratification and assessment of response to therapy. Serum Protein Electrophoresis (SPE) is the recommended technique to identify and measure MC. When MC is not quantifiable by densitometry, nephelometric assays for intact immunoglobulins may be used. However, such assays can be limited by measurement of background polyclonal immunoglobulins, lack of antigen recognition and lack of parallelism with polyclonal calibrators. Hevylite® immunoassays (Binding Site, UK) allows to quantify immunoglobulin Heavy-Light Chain (HLC) pairs, providing also an index of clonality by means of Ig'k/Ig'λ ratios. *Objectives.* To evaluate HLC assay for the quantification of MC in IgA MG patients. *Methods.* 132 sera from 122 patients, selected on the basis of IgA positive serum immunofixation (sIFE), were analysed. Total IgA values were compared with the sum of HLC IgAk and IgAλ. In 34 (26%) samples the MC was quantifiable by densitometry and results were compared with the involved HLC value. *Results.* A good correlation between total IgA and IgAk+IgAλ values ($y=-0.482+1.070x$; $R2=0.95$; $p<0.0001$) was found and also Bland-Altman analysis did not show significant differences (mean difference: 0.12 g/L, CI 95%: -4.37; +4.60) . The correlation between densitometric MC measurement and involved HLC value was acceptable ($n=34$; $y=-0.519+1.162x$; $R2=0.98$; $p<0.0001$), but Bland-Altman analysis showed a higher mean difference (1.04 g/L, CI 95%: -4.69; +6.78), probably due to the different analytical principles. It is worth noting that in 26 samples (20%) in which a small IgA MC was identified by sIFE, IgA HLC ratios were within the normal range. *Conclusions.* Our results suggests that HLC measurement can be useful to support the quantification of IgA MC not quantifiable by SPE.

F97 - COMPARISON OF WALDENSTRÖM MACROGLOBULINEMIA RESPONSES USING IMMUNOGLOBULIN HEAVY/LIGHT CHAIN ANALYSIS AND CONVENTIONAL ELECTROPHORESIS TECHNIQUES

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Background. Response assessment in Waldenstrom's macroglobulinaemia (WM) is reliant upon the reductions in the monoclonal IgM (m-IgM). This may be challenging to measure with conventional electrophoresis (SPEP) and immunofixation but may be improved by the heavy/light chain assay. *Methods.* We compared the efficacy of 3 techniques in WM disease assessment among 78 patients at diagnosis with an additional assessment during follow-up in 25 patients. Sera were analysed retrospectively using the IgM Hevylite immunoassay (Binding Site, UK) on a SPA_{PLUS} (Binding Site, UK) analyser. Results were compared to SPEP and Immunofixation (Sebia, Hydrasys II), tIgM (Binding Site, UK) and established normal ranges (IgMκ: 0.19-1.63 g/L; IgMλ: 0.12-1.01 g/L; IgMk/IgMl ratio: 1.18-2.74; total IgM: 0.5-2 g/L). dHLC (monoclonal isotype IgM HLC–uninvolved isotype IgM HLC) was used as an assessment of m-IgM. *Results.* At presentation all WM patients had an abnormal IgMκ / IgMλ ratio, 2/48 IgMκ and 9/30 IgMλ patients were not quantifiable by SPEP and 4 patients had m-IgM within normal range on tIgM. There were considerable differences in the m-IgM concentration measured by the three tests. However, responses assigned by the test during follow-up were remarkably consistent (Table 1). Interestingly, IgMκ/IgMλ ratios indicated residual disease in 5/25 patients in whom electrophoresis indicated a CR, in 2/5 of these patients the abnormal ratio was driven by a suppression of the uninvolved HLC IgM. Furthermore, in 7/25 patients tIgM values fell within the normal range during monitoring, and IFE was required to confirm m-IgM. In 7/7 patients IgMκ/IgMλ ratios remained abnormal. *Conclusions.* We demonstrate that Heavylight assay me be used to quantify accurately WM both at diagnosis and during follow-up.

Table 1. Comparison of response assignment using electrophoretic techniques (SPEP/IFE), total IgM/IFE (tIgM/IFE) to IgM HLC.

		SPEP/IFE Assigned Response						Total
		PD	SD	MR	PR	VGPR	CR	
IgM HLC (dHLC & IgMκ/IgMλ ratio) Assigned Response	PD	7	0	0	0	0	0	7
	SD	1	7	0	0	0	0	8
	MR	0	0	3	0	0	0	3
	PR	0	0	1	9	0	1	12
	VGPR	0	0	0	1	1	3	4
	CR	0	0	0	0	0	0	0
Total		8	7	4	10	1	4	34
		tIgM /IFE Assigned Response						Total
		PD	SD	MR	PR	VGPR	CR	
IgM HLC (dHLC & IgMκ/IgMλ ratio) Assigned Response	PD	9	0	0	0	0	0	9
	SD	1	7	1	0	0	0	9
	MR	0	0	5	0	0	0	5
	PR	0	0	1	11	1	1	14
	VGPR	0	0	0	1	2	3	6
	CR	0	0	0	0	0	0	0
Total		10	7	7	12	3	4	43

F98 - QUANTIFICATION OF FREE LIGHT CHAINS: COMPARISON OF TWO NEPHELOMETRIC ASSAYS

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Serum free light chains (FLC) quantification has become an important tool for the diagnosis and monitoring of monoclonal gammopathies. In the present study, we compare the analytical performance of the monoclonal antibody-based assay N-Latex (Siemens) to the established polyclonal antisera-based assay Freelite (The Binding Site). According to the manufacturer, the N Latex assay is able to detect an excess of antigen when combined with the ProSpec(®) nephelometer. To confirm this feature, we analyzed four samples known to induce a prozone effect. At an initial dilution of 1:100, the Freelite assay gave falsely low results while the N Latex was able to detect the excess and automatically re-analyze the sample at higher dilution. Methods comparison was performed on thirty selected samples spanning a wide range of free light chains values. All samples were obtained from patients with monoclonal gammopathy and analyzed on the BN ProSpec(®) nephelometer, according to the manufacturers' instructions. We found good correlation for FLC κ (r2=0.99) but not for FLC λ (r2=0.75). However, this poor correspondence appeared to have no clinical significance, since good agreement in interpretation was observed for κ, λ and the κ/λ ratios. To assess if therapy could be monitored indifferently on both assays, we extended our study to fourty-three serum taken from thirteen oncologic patients over the course of the year. According to our preliminary observations, N-Latex assay gave significantly higher FLC λ values leading to lower κ/λ ratios. Switching from one assay to the other would result in clinical misinterpretation. Moreover, the N-Latex assay gave discordant results for two patients with monoclonal FLC κ. Our results show that the two assays perform differently in clinical practice, and that they are not easily interchangeable. Therefore, treated monoclonal gammopathy should always be monitored by the same assay in a single laboratory.

F99 - EVALUATION OF HEVYLITE™ FOR THE DIAGNOSIS OF MONOCLONAL IMMUNOGLOBULIN HEAVY CHAINS

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Background. Heavy chain diseases are characterized by the production of mononoclonal immunoglobulin heavy chains without corresponding light chains. The laboratory diagnosis of this condition relies on serum protein electrophoresis (SPEP), electrophoresis-immunofixation (IFE) and immunoselection (IS). *Objectives.* In 2009, Bradwell *et al.* developed immunoglobulin heavy/light chain immunoassays, HevyLite™ allowing the specific measurement of serum IgA, G or M-k and IgA, G or M-

λ concentrations. We postulated that the summation of IgGκ and IgGλ or IgAκ and IgAλ compared to the concentration of IgG or IgA might be indicative of monoclonal heavy chain proteins (HC). *Methods.* Capillary zone electrophoresis was used to screen for M-components which were either typed by immunodisplacement on the capillary system or with a semi-automated IFE technique. The presence of monoclonal HC proteins was ascertained using IS according to Radl (1970). Immunoglobulins were quantified by immunonephelometry. Serum IgGκ and IgGλ or IgAκ and IgAλ were quantified by immunoturbidimetry on SPAplus™ using the kit IgG and IgA Hevylite™. The difference D between measured IgG or IgA and the summation of IgGκ and IgGλ or IgAκ and IgAλ was calculated and a ratio established between D and IgG or IgA, R=D/IgG or IgA. *Results.* 22 sera issued from 14 patients were diagnosed with monoclonal g (19/22) or a (3/22) HC using IS. SPEP results were normal in 8 sera. In 13, 7 and 2 sera, IFE results were positive, dubious and negative respectively. Using HevyLite™, 17 sera clearly demonstrated the presence of g or a-HC (78%) with R <85%. In the 5 remaining sera the results were dubious and R ≥85%. *Conclusions.* HevyLite™ provides information similar to the combination of SPEP and IFE or with IS alone with a sensitivity of 100% when R<85% for the diagnosis of HC proteins.

F100 - EVALUATION OF A METHOD TO DETECT PROZONE/HOOK EFFECT/ANTIGEN EXCESS PHENOMENON FOR FREE LIGHT CHAIN QUANTIFICATION USING A SIMPLE POOLING PROTOCOL

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Background and Objectives. Antigen excess is an important issue that can lead to underestimation of patient results and misdiagnosis. With patient samples which can range from less than 1mg/L to over 100 000mg/L, serum free light chain (FLC) measurement is especially prone to this interference. *Methods.* We propose a methodology based on sample pooling for a fast and cost effective method to detect of antigen excess phenomenon for serum free light chain quantification. Our strategy was evaluated on the Immage (Beckman) and on the BNII (Siemens) nephelometers using the Freelite® assay (The Binding Site). *Results.* First, we evaluated the sensitivity of our strategy using a pool of 15 mixed samples spiked with different concentrations of κ free light chain. Secondly, patients with antigen excess ranging from 1099,3 to 89174 mg/L of κ or λ free light chain were efficiently detected using our strategy. Our data also suggest that pools as many as 43 different patients could be used for antigen excess detection. Finally, we report real cases where our pooling protocol identified antigen excess on the Immage and BNII in routine analyses. *Conclusions.* We described and validated a strategy based on sample pooling for detection of antigen excess on free light chain measurement. This approach could be suitable for any laboratory measuring serum free light chain that does not currently have an instrument platform for detection of antigen excess.

F101 - DETECTION OF URINARY FREE IMMUNOGLOBULIN LIGHT CHAINS BY TWO NEPHELOMETRY-BASED METHODS. COMPARISON WITH URINE IMMUNOFIXATION

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Background. Electrophoresis and immunofixation are useful in the diagnosis, prognosis and monitorization of monoclonal gammopathies. Serum free light chains analysis has proved to be valuable for its screening and managing. Bence Jones proteins (BJP) nephelometric measurement is presently not recommended, it is still requested in some laboratories. *Objectives.* To evaluate the usefulness of urinary free light chains (UFLC) measurement by nephelometry in the detection of BJP as an alternative method to urinary immunofixation (UIMF), and to compare two different UFLC quantification kits. *Methods.* Urine samples from 91 patients were collected. UIMF and BJP measurement was realised. UFLC quantification was done in a BN™II (Siemens) analyzer, using two different kits: BNA.FRK.FRL (New Scientific (NS), cut-off: κ=1 mg/dL; λ=0.7 mg/dL) and Freelite™ (Binding Site (BS), cut-off: κ=2.4 mg/dL; λ=0.7 mg/dL). Negative or positive results were reported according to the presence/absence of monoclonal bands (UIMF), and κ or λ UFLC concentrations compared to the cut-off values. *Results.* Cohen's κ coefficient (CKC) between both UFLC quantification methods was 0.69 (*substantial*) for κ BJP and 0.86 (*almost perfect*) for λ BJP. When κ and λ UFLC together were compared to UIMF, CKC was *substantial* for both methods: 0.69 for BS and 0.66 for NS. Sensitivity and specificity using BS were 92.5% and 78.4%, respectively, whereas they were 100% and 68.2%, respectively, for NS (Table 1). *Conclusions.* In the detection of BJP, the high CKC detected between two nephelometry-based methods and UIMF suggests its use as a previous screening. Despite of a higher sensitivity obtained with NS kit, BS kit showed better specificity and slightly higher CKC. In addition, BS reagents can be equally used with serum or urine samples. In conclusion, when UFLC detection is needed, BS reagents shows several advantages for its introduction in the laboratory.

Table 1. Detection of UFLC by immunofixation and two nephelometry-based methods.

Kappa	Negative (NS)	Positive (NS)
Negative (BS)	43	11
Positive (BS)	3	34

Kappa and lambda	Negative (BS)	Positive (BS)
Negative (UIMF)	40	11
Positive (UIMF)	3	37

Lambda	Negative (NS)	Positive (NS)
Negative (BS)	63	5
Positive (BS)	0	23

Kappa and lambda	Negative (NS)	Positive (NS)
Negative (UIMF)	35	16
Positive (UIMF)	0	40

F102 - IgA SUBTYPES: A SUPPLEMENT TO M-PROTEIN QUANTIFICATION BY ELECTROPHORETIC METHODS IN MONITORING PATIENTS WITH MULTIPLE MYELOMA

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Background. For monitoring patients with multiple myeloma (MM), the quantification of the M-protein is recommended by electrophoretic methods. Monoclonal IgA (mIgA) often migrates into the β-fraction, leading to difficulties in determining the M-gradient. Alternatively IgA subtypes can be quantified by Hevylite®, an immunoassay measuring both light-chain restricted immunoglobulins separately (IgAκ and IgAλ). *Objectives.* We investigated whether the quantification of mIgA by standard electrophoretic method can be improved by using Hevylite. Additionally in a case study the determination of the IgA subtypes reflecting the proportion of mIgA with a definable M-gradient by electrophoresis during monitoring was estimated. *Methods.* In sera from 88 patients with IgA monoclonal gammopathy the monoclonal component was determined using electrophoretic tests (SPEP, serum protein electrophoresis; CE, capillary electrophoresis; IFE, immunofixation; Sebia, Lisses, France) Freelite® (FLC, free light chains) and Hevylite® (HLC, IgAκ/IgAλ; The Binding Site, Schwetzingen, Germany). In a MM patient (IgAλ) SPEP, CE, IFE, FLC and HLC were examined during therapy (n=18). The mIgAλ was calculated [mIgAλ=IgAλ-(IgAκ/1.18)] and compared to the quantified M-protein (SPEP). *Results.* In the 88 patients with IgA monoclonal gammopathy the M-protein was detectable in 69.3% and 87.5% using SPEP and CE, respectively. FLC was abnormal in 68.2%. In contrast HLC ratio was abnormal in 93.2%. Combination of the HLC ratio and SPEP or CE increased the sensitivity to 96.5%. The M-protein (SPEP) from the patient with MM ranged from 0.8-37.1g/L during monitoring of the therapy. The HLC ratio and the calculated mIgAλ correlated with the M-Protein during the entire treatment period (r^2=0.997). *Conclusions.* In IgA monoclonal gammopathies the calculated monoclonal IgA using Hevylite® test is a reliable method with a higher sensitivity than SPEP and CE in our cohort, especially for patients with M-proteins migrating into the β-fraction.

F103 - MANAGING CARRYOVER EFFECT IN THE FREELITE ASSAY ON THE SPA PLUS TURBIDIMETER

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Background. Levels of free immunoglobulin κ and λ light chains in serum are an important component in diagnosis, prognosis and monitoring of patients with monoclonal gammopathies. A clinical laboratory providing testing for a Hematology/Oncology service with a myeloma specialty routinely encounters a wide range of results including levels many hundred times the normal physiological range. The SPAplus turbidimeter used to measure free light chains employs a sampling probe and adequate probe washing is essential to avoid carryover of high concentration specimens into neighboring samples causing falsely elevated results. *Objectives.* To determine the significance of carryover using the SPA plus turbidimeter and to attempt to minimize the effect on the accuracy of results. *Results.* Significant levels of carryover have been noted when samples containing very high levels of free light chains are run directly preceding samples of lower concentration *e.g.* a specimen with a free κ value of 11,511mg/L produced an elevation in the adjacent sample from 5.7mg/L to 9mg/L (58% increase) and a free λ value of 107,649mg/L produced an elevation in the following sample from 5.6mg/L to 28.1mg/L (400% increase). These findings have obvious clinical significance for diagnosis and patient monitoring. To attempt to reduce the carryover effect, an additional 2% alkaline wash for the sample probe was programmed between each sample. This step adds only a minimal increase in time for analysis of each specimen and has significantly reduced or, in some cases, eliminated carryover. *Conclusions.* Careful review of data is necessary to ensure the accuracy of results following samples with high levels of free light chains (>10,000 mg/L). Regular checking of the instrument for carryover effects should be performed. Re-analysis of samples following very high results may be indicated. Addition of a 2% alkaline wash step to the routine instrument programming is highly recommended.

F104 - COMPARISON OF HEVYLITE• RESULTS TO IMMUNOFIXATION AND SERUM PROTEIN ELECTROPHORESIS IN CLINICAL SAMPLES

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Background. Multiple myeloma and plasma cell dyscrasias are identified using serum protein electrophoresis (SPE), Freelite® and immunofixation (IFX). IFX is not effective in every instance, as there are cases where a patient can be negative by IFX, but express a low level monoclonal protein (M-protein), a free κ (κ), or a free λ (λ) M-protein. Additionally, IFX is not quantitative. SPE may give variable results at low levels of M-protein (<10g/L). Further, antibody saturation can cause high levels of M-protein (>30g/K) to appear lower than actually present. *Objectives and Methods.* Evaluate the Hevylite® assay to determine if it provides an advantage in the detection of low level M-Protein and identification of patients with plasma cell disorders. In the present study, samples from patients with a history of a M-protein were screened and combined with an IFX

result to determine if Hevylite® could confer an increase in the quantification of high and low M-proteins and identification of residual disease in samples with a negative IFX result. *Results.* The data revealed that the majority of samples screened had a higher M-protein constituent as quantitated using Hevylite® *versus* the SPE/densitometric data (63/77). A subset of the samples had an additional abnormality identified using Hevylite® compared to the IFX result (10/77). Further, when there was no abnormality in the Hevylite® values (*i.e.*, no elevation in IgG κ/λ, IgA κ/λ, or IgM κ/λ), the majority of these samples had an abnormal ratio between κ and λ (18/23 or 78%). *Conclusions.* Further study is necessary to determine whether the abnormalities identified using Hevylite® translate to a change in diagnosis. Additional work should be conducted to determine whether clinical recommendations regarding disease monitoring/treatment would be improved using Hevylite® quantification compared to existing densitometric data.

F105 - POSITIVE URINE FREE LIGHT CHAINS WITH NEGATIVE SERUM FREELITE–RENAL CATABOLISM OR MISSING EPITOPES?

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Background. Occasional patients show urinary free light chains by immunofixation but normal serum free light chain ratio (LCR). These are usually low levels (less than 50mg/L) and match the light chain type of an intact serum monoclonal immunoglobulin. We hypothesise that this results from renal catabolism of intact monoclonal immunoglobulin. *Objectives.* To establish the incidence of these results and if they could result from renal breakdown or poor sensitivity of the assays for some epitopes. *Methods.* (i) Between January 2007 and December 2014 we identified all patients in whom serum free light chain ratio (sLCR) was normal but free light chain was identified in the urine by immunofixation. Free light chain levels in serum and urine were measured by Freelite (SPAplus, The Binding Site). Serum and urine electrophoresis and immunotyping were performed by caplllary zone or gel electrophoresis (SEBIA Capillarys or Hydrasys systems). (ii) We measured urine light chain levels in a selected group of these patients to establish if the Freelite method was able to detect them. *Results.* (i) 305 patients with urinary free light chain were identified in which serum and urine were tested within 90 days of each other. 50 had normal sLCR. Of these, 11 were normal on repeat testing (fresh urine sample). Urine light chain concentration by densitometry was <50 mg/L in all cases. (ii) Urine was available for analysis in two patients. In both patients the measured levels were consistent with those expected from the densitometry results. *Conclusions.* Very low levels of urinary free light chain are common in patients with normal sLCR. In the two patients examined, the ability of the Freelite assay to measure urine levels consistent with densitometry suggests that this is due to renal catabolism and not due to missing epitopes in the assays.

F106 - COMPARISON OF FREELITE AND N LATEX METHODS FOR QUANTIFICATION OF SERUM FREE LIGHT CHAINS

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Background. Serum free light chains (FLC) are recommended as markers of plasma cell dyscrasias. This study analysed FLC concentrations in a group of patients by the polyclonal Freelite (Binding Site) and monoclonal N Latex FLC (Siemens) methods. Due to the large variation in FLC generation, it is hypothesised that N Latex may not be able to recognise all available FLC epitopes. *Methods.* The study consisted of 36 (24 F, 12 M) attended haematology clinic in our local hospital. The average age was 72 (47-95). *Results.* Despite this small number, discrepancies could still be seen which may affect patient monitoring. Linear regression analysis demonstrated R^2 values for κ, λ and the ratio of 0.9961, 0.3262 and 0.9831 respectively. This demonstrates that major discrepancies were seen particularly with the λ quantification. This is not unexpected as λFLC has the largest diversity. There were 7 patients with large differences in the FLC quantification. In one case, a monitored light-chain only patient, the N Latex ratio was normal indicating lack of monoclonal disease yet the Freelite ratio remained abnormal due to raised κFLC levels. This result would affect response assessment. In 2 further patients, Freelite indicated an abnormal ratio with κ monoclonality, which matched with κ light chains in the urine, yet in both cases N Latex indicated a normal ratio. Both of these samples were diagnostic. One patient was asymptomatic and so this difference would have affected the risk stratification when looking at risk of progression to myeloma as an abnormal FLC ratio puts the patient at increased progression risk and may require increased monitoring by the haematologist. *Conclusions.* this small study adds to existing evidence that the 2 FLC tests differ in their ability to quantify FLC levels in the sera of monoclonal gammopathy patients and cannot be used interchangeably.

F107 - EVALUATION OF HEVYLITE™ FOR THE DIAGNOSIS OF MONOCLONAL IgAs

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Background. Serum protein electrophoresis (SPEP) and immunofixation (IFE) are routinely used to identify, type and quantify monoclonal immunoglobulins. However in some instances, it is difficult to detect M-components because they may comigrate with transferrin or C3 spikes. Also, M-IgAs appear as a complex figure of several spikes making difficult their quantification. *Objectives.* We therefore undertook the evaluation of the IgA HevyLite™ assay which is based upon the quantification of IgAκ and IgAλ and the calculation of the ratio IgAκ/IgAλ (IgA HLCr). *Methods.* Capillary zone electrophoresis was used to screen for M-components which were either typed by immunodisplacement on the Capillary system or with a semi-automated IFE tech-

nique. Immunoglobulins were quantified by immunonephelometry. Serum IgAκ and IgAλ were quantified by immunoturbidimetry on SPAplus™ using the kit IgA HevyLite™. *Results.* 157 sera from a collection of stored samples issued from 122 patients followed for M-IgA components were studied. They presented with multiple myeloma (55), MGUS (58), AL amyloidosis (5) and miscellaneous (4). IgAκ+IgAλ correlated significantly (R=0.96) with total IgA. Likewise, IgAκ and IgAλ electrophoretic peaks correlated significantly (respectively R=0.97 and R=0.92) with the concentrations of IgAκ and IgAλ respectively. 35 samples with no M-IgA peaks were positive with IFE, of which 8 only had a positive IgA HLCr. Of the 16 samples with a peak <2 g/L, 5 were positive for the IgA HLCr. With a peak ≥2 g/L the sensitivity reached 100%. *Conclusions.* This study demonstrates that IgA HevyLite™ provides a method with 100% sensitivity for identifying M-IgAs when there is an M-spike with a concentration ≥2g/L. While for samples with no M-spike IFE remains a more sensitive technique, the IgA HLCr provides a more precise and accurate estimation of the monoclonal component.

F108 - A POLYCLONAL V MONOCLONAL APPROACH TO FLC MEASUREMENT

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The analysis of serum free light chains (FLC) by immunochemical analysis has been with us for around 15 years. The established assay (Freelite) used polyclonal antisera in nephelometric and turbidimetric assays. There are known difficulties with the assay in terms of potential antigen excess and non-linearity. More recently a second nephelometric assay has come to market using monoclonal antisera to free light chains (N Latex FLC). A third assay, using multiplex technology and monoclonal antisera, has also been described but has not yet come to market. A summary of data from our multicentre study comparing the Freelite and N Latex FLC assays is provided (see reference for full details). These data are compared with other studies in the literature. Our main concern at the time of the evaluation was that we considered it unlikely that all monoclonal proteins would behave identically in the two assays. In general terms the two assays perform similarly. It is noted that in the studies published to date (November 2013, at time of writing) there are cases where the monoclonal assay (N Latex FLC) failed to detect serum FLC in patients with B lymphoproliferative disorders, including light chain myeloma, which were detected by the polyclonal assay (Freelite). There are sufficient data to suggest the assays are not equivalent and cannot be used interchangeably. The data support our contention that some clones might be missed by the more limited epitope specificity expected of monoclonal antisera. It should also be noted that the UK myeloma guides have been written with the experience of Freelite and, in view of the differences in the assays, may not be directly cross applicable to N Latex FLC.

Reference

Lock *et al.*, Ann Clin Biochem 2013;50:255. A multicentre study comparing two methods for serum free light chain analysis.

F109 - EVALUATION OF HEVYLITE™ FOR THE DIAGNOSIS OF MONOCLONAL IgGs

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Background. Serum protein electrophoresis (SPEP) and immunofixation (IFE) are routinely used to identify, type and quantify monoclonal spike of immunoglobulins. In 2009, Bradwell *et al.* developed HevyLite (HLC)™ allowing the specific measurement of serum Ig Gκ and IgGλ concentrations. *Objectives.* We therefore undertook the evaluation of the IgG Hevylite™ assay which is based upon the quantification of IgGκ and IgGλ and the calculation of the ratio IgGκ/IgGλ (IgG HLCr). *Methods.* Capillary zone electrophoresis was used to screen for M-components which were either typed by immunodisplacement on the Capillary system or with a semi-automated IFE technique. Immunoglobulins were quantified by immunonephelometry. Serum IgGκ and IgGλ were quantified by immunoturbidimetry on SPAplus™ using the kit IgG Hevylite™. *Results.* 95 sera from a collection of stored samples issued from 85 patients followed for M-IgG components were studied. They presented with multiple myeloma (60), MGUS (12), and miscellaneous (13). IgGκ+IgGλ correlated significantly with total IgG (r=0.958). Likewise, IgGκ or IgGλ electrophoretic peaks correlated significantly with the respective concentration of IgGκ (r=0.964) and IgGλ (r=0.939). 20 samples had no M-IgG peak: 16 were positive with IFE, of which none had a positive IgG HLCr; Of the 37 samples with a peak <5 g/L, 10 were positive for the IgG HLCr. With a peak ≥5 g/L the sensitivity reached 100%. We also studied the influence of the polyclonal background: when the ratio between the concentration of the peak and the concentration of the gamma fraction is >37%, IgG HLCr reaches a sensitivity of 100%. *Conclusions.* This study demonstrates that IgG HevyLite™ provides a method with 100% sensitivity for identifying M-IgG when there is an M-spike with a concentration ≥5 g/L or when the ratio between the concentration of the peak and the gamma fraction is >37%.

F110 - IS IT POSSIBLE TO MONITOR MONOCLONAL GAMMOPATHY WITH N LATEX FLC AS WELL AS FREELITE™?

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Background. The use of free light chain (FLC) assays in the diagnostic and the monitoring of patients suspected of monoclonal gammopathy is well established and advocated in international guidelines. *Objectives.* To

compare the monitoring of two patients suffering of multiple myeloma with two methods for serum free light chains analysis. *Methods.* Serum samples were analysed retrospectively with Freelite™ on SPA+ (Binding Site) by turbidimetry and with N Latex FLC on BNII (Siemens) by nephelometry. *Results.* The first patient presented a κ free light chains myeloma in remission and a renal impairment. The results obtained with Freelite™ are: κ=26.4 mg/L, λ=11 mg/L, difference FLC=15.4 mg/L and the ratio=2.4; in comparison with N Latex FLC results: κ=25.3 mg/L, λ=38.6 mg/L, difference FLC=-13.3 mg/L and the ratio=0.66! The second patient suffered from λ free light chains myeloma. Higher results of λ free light chains are observed with Freelite™. K showed good concordance. Freelite™ identified progressive disease earlier than N Latex FLC. *Conclusions.* Our first case showed that N latex FLC detected higher concentrations of λ free light chains in renal impairment. The monitoring of such patients could be distorted. We also noticed, in the second case, that N latex FLC assay failed to provide similar clinical information about the progression of the disease. In conclusion, some patients can't be monitored if methods are used indifferently. Many of the recommendations are based on Freelite™ assay and can't be directly transferable to N Latex FLC assay.

F111 - COMPARISON OF HEAVY/LIGHT CHAIN ANALYSIS TO THE MEASUREMENT OF MONOCLONAL PROTEINS USING CAPILLARY ZONE ELECTROPHORESIS AND TOTAL IMMUNOGLOBULINS IN IgA MULTIPLE MYELOMA PATIENTS

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Background. In the event of co-migration of IgA monoclonal immunoglobulins (M-Ig) with other serum proteins, quantification capillary zone electrophoresis (CZE) is challenging. In such instances international guidelines recommend the measurement of total IgA. Heavy/light chain (HLC) assays have been developed that quantify κ and λ immunoglobulin isotypes separately, enabling calculation of κ/λ ratios and typing of M-Ig. Unlike the measurement of total immunoglobulins which summates the concentration of monoclonal and polyclonal isotypic immunoglobulins, HLC provides information on both the involved M-Ig (*e.g.* IgAκ in an IgAκ patient) and the uninvolved polyclonal HLC-pair (uHLC, *e.g.* IgAκ in an IgAλ patient). Here we report the comparison between HLC and the measurement of monoclonal proteins using CZE and total immunoglobulins (tIgA) in IgA Multiple Myeloma (MM) patients. *Methods.* HLC levels were measured turbidimetrically on 90 serum samples (58 IgAκ, 32 IgAλ) from 31 IgA MM patients (17M:14F) at various stages of treatment. Results were compared retrospectively with CZE and total IgA (tIgA). *Results.* Using linear regression analysis, summated IgAk/IgAl or the individual isotypes (IgAk or IgAl) correlated well with CZE (IgAk/IgAl n=88, y=0.9038x+0.6945, R^2=0.93, p<0.0001; IgAk n=32; y=1.071x-0.2653, R^2=0.93, p<0.0001; IgAl n=40; y=0.8615x-0.9164, R^2=0.97, p<0.0001) and tIgA (IgAk/IgAl n=90, y=0.8654x+0.7155, R^2=0.91, p<0.0001; IgAk n=32; y=0.9991x+0.3258, R^2=0.91, p<0.0001; IgAl n=43; y=0.8158x-0.4244, R^2=0.93, p<0.0001). *Conclusions.* There was excellent correlation between the concentrations of M-Ig measured using summated IgAk/IgAl and individual isotypes (IgAk or IgAl) and those measured with CZE and tIgA. Heavy/light chain analysis could replace the measurement of total immunoglobulins by CZE and tIgA when assessing suspected multiple myeloma.

F112 - IMMUNOHISTOCHEMICAL IDENTIFICATION OF PATHOLOGICAL B-CELL POPULATION IN MONOCLONAL GAMMOPATHIES USING HEVYLITE REAGENTS-FIRST EXPERIENCE

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Backround. Several methods for identification of clonal B-cell population in bone marrow samples in monoclonal gammopathies (MG) have been used. The most common are the methods of undirect immunohistochemistry (IHC) with the separate use of anti-heavy and light immunoglobulin chain antibodies. Currently available diagnostic system HevyLite allows simultaneous determination of pairs of heavy/light chain (HLC) of immunoglobulin. *Objectives.* The aim of the study was to perform IHC identification of monoclonal population in bone marrow samples using HLC antibodies. *Methods.* We evaluated 6 decalcified formalin-fixed paraffin-embedded (FFPE) bone marrow samples from patients with multiple myeloma (IgGκ, IgGλ, IgAκ, IgAλ) and Waldenström macroglobulinemia (IgMκ). In all samples, IHC was performed using anti-heavy, anti-light chain and HLC antibodies (IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ), for identification of myeloma and lymphoplasmocytoma cells, CD138 resp. CD20 and CD79a antibodies were used. *Results.* In all samples, IHC using HLC antibodies recognized clonal cells, which were identified separately by staining using heavy and light chain antibodies. The results show that the use of HLC antibodies by IHC allows differentiating pathologic population from non cancer background, which may exhibit a positive result, when using heavy and light chain antibodies separately. Preliminary results also indicate the successful use of HLC antibodies in the case of decalcified FFPE samples. *Conclusions.* Our preliminary results support the potential benefit of using HLC antibodies for IHC detection and typing of pathological B-cell populations in MG patients.

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F113 - USING IgM HEVYLITE TO DISTINGUISH MGUS FROM MALIGNANT DISORDERS WITH CLONAL IgM PARAPROTEIN

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Background. Monoclonal IgM (mIgM) can be found in elderly patients without evidence of any haematological disorder but also in patients with lymphomas (NHL), or myeloma (MM). Both show a positive immunofixation but have a quite different prognosis and management. Therefore it is crucial to have diagnostic tools available that can rapidly distinguish these two scenarios. *Objectives.* To analyse the value of using the IgM Hevylite® κ and λ assays (IgM-HLC, Binding Site) to distinguish MGUS from lymphoma or myeloma. *Methods.* From 04/2011 to 11/2012 aliquots of serum samples taken for routine immunofixation were stored at the Institute for Clinical Chemistry at the University Hospital Cologne. For patients with positive mIgM κ or λ immunofixation the IgM-HLC was performed by Binding Site. The analysis was approved by the ethics committee of the University of Cologne. *Results.* 64 patients were identified with mIgM (39 κ, 25 λ) and assessed for IgM-HLC. 3 patients had missing clinical information, therefore 61 patients (36 MGUS, 22 NHL, 3 MM) could be analysed. An abnormal HLC κ/λ-ratio (rHLC) was found in 33/36 (91.7%) MGUS and 24/25 (96.0%) NHL/MM patients (not significant). The median rHLC for IgM κ was 11.1 in MGUS and 142.7 in NHL/MM patients and for IgM λ 0.54 and 0.03, respectively. Both differences were highly significant (p=0.001, Mann-Whitney-U test). A suppressed non-involved IgM κ or λ was present in 3/36 (8.3%) MGUS and 16/25 (64.0%) NHL/MM patients (p<0.001, Chi-square-test). *Conclusions.* While an abnormal IgM-rHLC is present in most cases with a positive IgM immunofixation, both the numerical value of the rHLC and the suppression of the non-involved IgM seem to distinguish malignant from non-malignant conditions presenting with a mIgM. IgM-HLC therefore deserves further investigation in the diagnostic workup of patients with mIgM.

F114 - APPROACH FOR ACCREDITATION AND METHOD VERIFICATION OF THE κ AND λ FREE LIGHT CHAINS ASSAY

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Background. Our laboratory is engaged in an accreditation process according to the European standard EN ISO 15189. In this context, we performed the method validation of κ and λ Free Light Chain (FLC) quantification (Freelite), according to the reference document COFRAC SH-FORM 43. We present our results here and in the absence of consensual guidelines we propose a target for acceptable reproducibility limits for these tests. *Objectives.* Serum free light chains of immunoglobulins κ and λ - method validation. *Methods.* Quantification of κ and λ FLC (Freelite) on a BN ProSpec analyser. *Results.* The following items were studied: description of the method, critical points to master and mastery modalities, performance evaluation of the method : repeatability (0.7 to 5.6%), reproducibility (5.4 to 6.0%), accuracy. The measurement uncertainty evaluation was performed by the method involving the use of internal quality control results and external quality assessment. Measurement intervals and interferences have been described. *Conclusions.* Our approach allowed us to obtain accreditation of the FLC assay (Freelite) according to the NF EN ISO 15189 norm. In the absence of available data for the κ and λ FLC in the standards/repositories of the *Société Française de Biologie Clinique* or of *Ricos* analytical quality specifications for imprecision database, we propose for these tests a 7% maximum variation coefficient for reproducibility, which can be used for the validation of internal quality controls in clinical biology.

F115 - CORRELATION OF IgA AND IgM HEAVY CHAIN/LIGHT CHAIN IMMUNOASSAYS WITH NEPHELOMETRIC TOTAL IMMUNOGLOBULIN MEASUREMENTS

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Background. The measurement of total immunoglobulins (tIg) is a front line test for investigating suspected immune system abnormalities and is the summation of both monoclonal and polyclonal isotype populations (*e.g.* IgAκ and IgAλ). Heavy/light chain (HLC) immunoassays have been developed to quantify the k and l isotypes separately, enabling calculation of isotypic κ/λ ratios. Imbalanced κ/λ ratios are the signature of plasma cell disorders such as multiple myeloma. The aim of this study was to assess the correlation between the IgA and IgM HLC immunoassays and the measurement of tIgA and tIgM. *Methods.* Serum samples from 58 randomly selected patients (median 65 years; range 10-88 years) were assessed. HLC concentrations were measured by nephelometry on the BNII Dade Behringer analyser and while tIgA and tIgM concentrations were quantitated by immunoturbidimetry on the Roche Modular P analyser. Correlation and agreement between assays was assessed using the coefficient of determination (R^2) and Pearsons Coefficient Correlation. *Results.* IgM samples (n=33), 25 had normal (median 1.55; range 1.05-2.15; median IgMk/IgMl summation 1.1g/L; range 0.2-7.5g/L) and 8 abnormal HLC IgMk/IgMl ratios (median 2.6; range 0.73-11.9; median IgMk/IgMl summation 0.6g/L; range 0.2-1.5g/L). IgA samples (n=25), 17 had normal (median 1.3; range 0.86-1.74; median IgAκ/IgAλ summation 3.42g/L; range 0.9-12.0g/L) and 8 abnormal HLC IgAκ/IgAλ ratios (median 2.2; range 0.6-100; medi-

an IgAκ/IgAλ summation 2.1g/L; range 0.2-5.5g/L). Linear regression analysis showed excellent correlation between HLC IgMκ+IgMλ summation and total IgM levels (y=-0.072+1.28x, R^2=0.99 (95% CI: 0.99-1.00);p<0.0001) and HLC IgAκ+IgAλ summation and total IgA levels (y=0.073+1.07x, R^2=0.98 (95% CI: 0.94-0.99);p<0.0001). *Conclusions.* In this randomly selected cohort, our study showed excellent correlation between the summated HLC isotype pairs, IgAκ+IgAλ and IgMκ+IgMλ, their respective tIg. HLC analysis could therefore replace tIg to evaluate monoclonality and monoclonal Ig levels as cost effective monitoring tool in selected patients.

F116 - FAMILIAL BIOLOGICAL VARIATION OF FREE LIGHT CHAINS AND HEAVY CHAIN-LIGHT CHAIN PAIRS MIGHT ALLOW FAMILY MEMBERS TO ACT AS CONTROLS FOR MYELOMA PATIENTS

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Background. An important attribute for all laboratory tests is an assessment of biological variation. There are several components to BV: analytical imprecision, within-subject variation over time, and between-subject variation for a group of subjects. From these measured variables, the index of individuality, reference change value, and number of samples to establish a homeostatic set point can be calculated. Previous results have shown that the index of individuality for FL and HL assays are very low, indicating that the establishment of an individual's baseline will be more meaningful than comparing results population based reference ranges (Clin Chim Acta 2014;436:68-71). An established baseline will not likely be available for a patient exhibiting a newly diagnosed monoclonal gammopathy. If the biological variation attributes for FL and HL assays are genetically linked, real time testing of healthy family members may be a surrogate for the patient's baseline value. *Objectives.* Determine the biological variation for FL and HL among family members. *Methods.* Blood will be collected from 10 pairs of healthy donors (siblings, parent or child) every other week for 6-8 weeks, and tested with the HL and FL assays. Statistical calculations will be conducted as previously described. *Results.* If the various biological variation attributes are similar from pairs of related subjects but differ from unrelated pairs, genetics may be the determinant factor for establishing the homeostatic setpoint. If results within a family are not different from the overall population, the environment may be the determinant factor. *Conclusions.* If the biological variation attributes for FL and HL assays are found to be genetically linked, testing of healthy family members might be used to estimate the patient's baseline value. However, if the biological variation is not genetically linked, the diagnosis may not be evident until results exceed the population-based reference range.

MONOCLONAL FREE LIGHT CHAINS AND RENAL IMPLICATIONS

G117 - FREE LIGHT CHAIN REMOVAL EFFICACY BY POST DILUTION HEMODIAFILTRATION AND HAEMODIALYSIS IN CHRONIC DIALYSIS PATIENTS

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Background. Up to now, removal of middle weight protein is only evaluated by B2µGlobulin (12kDa). Polyclonal free immunoglobulin κ (25 kDa) and λ (50 kDa) light chains (FLCs) represent relevant candidates to evaluate removal of a panel of middle weight protein. *Objectives.* The purpose of this study was to evaluate the efficacy of hemodiafiltration *versus* haemodialysis with 4 different membranes to remove sFLC κ and λ markers. *Methods.* We analyzed 8 stable patients on chronic dialysis. Patients with monoclonal gammopathy, or chronic inflammatory disease were excluded. Every patient underwent successively the 8 protocols. Serum concentrations of polyclonal FLCs were measured before and after dialysis using the Freelite™ assay (Binding Site) on the BN ProSpec system. Removal percentage of either κ or λ FLC were measured for each membrane (Cordiax1000 *(Fresenius)*, Xenium+H21 *(Baxter)*, APS-21H *(AsahiKasei)*, TS-2.1SL *(Toray)*, in post dilution hemodiafiltration (HDF) and haemodialysis. *Results.* Post dilution HDF allowed a greater reduction fraction of λ FLCs than haemodialysis for 3 membranes (Xenium+H21, TS-2.1SL, APS-21H) and of κ FLCs for 3 membranes (Cordiax1000, Xenium+H21, APS-21H) ($p<0.05$), whereas no significant difference were reported for λ FLC removal with Cordiax1000 and κ FLC removal for TS-2.1SL. The removal of λ sFLC was three times more effective with post dilution HDF than haemodialysis. *Conclusions.* Post dilution HDF allows a more efficient epuration of middle molecular weight protein compared to haemodialysis especially for the λ sFLC. Our study did not report different efficacy between tested membranes. The measurement of sFLC could thus represent a good marker for monitoring middle molecular weight removal in chronic dialysis patients.

G118 - ABNORMAL SERUM LIGHT CHAIN RATIO IN THE DIAGNOSIS AND MANAGEMENT OF PATIENTS WITH RENAL MANIFESTATIONS ASSOCIATED WITH UNDERLYING LYMPHOPLASMACYTIC DISORDERS

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Background. Currently, serum free κ to λ light chain ratio is used by nephrologists as a "screening test" for patients older than 50 years with unexplained acute or slowly progressive renal failure, to evaluate a subset of patients with proteinuria (nephrotic and non-nephrotic range) of unclear etiology, and to assess whether patients with MGUS (monoclonal gammopathy of undetermined significance) may have renal injury resulting from the plasma cell disorder. *Objectives.* Renal biopsy files for the last 2 and a half year were searched to identify cases in which an abnormal serum free light chain ratio triggered a renal biopsy and to find out the pathology encountered. *Results.* We documented 36 cases (3260 renal biopsies performed during that timeframe) representing 80% of all patients with a final diagnosis of light chain-related renal disease and 1.2% of all renal biopsies where an abnormal serum free κ to λ light chain ratio was the trigger to perform a biopsy. All but 6 of these patients demonstrated renal findings related to an underlying lymphoplasmacytic disorder. The renal pathology that was detected included light chain cast nephropathy (n=3), proximal tubulopathies related to monoclonal light chains (n=13), AL-amyloidosis (n=16), proliferative glomerulonephritis with monoclonal IgG deposits (n=2), and light chain deposition disease (n=2). The diagnosis uncovered was generally totally unsuspected clinically. In all the cases included in this series a subsequent bone marrow biopsy confirmed the presence of a neoplastic clone of cells responsible for the renal injury. *Conclusions.* The great majority of patients with an abnormal free serum light chain ratio and renal manifestations are diagnosed with a condition associated with an underlying neoplastic lymphoplasmacytic disorder, if a renal biopsy is performed. Early detection results in intervention before irreversible changes have taken placed preserving renal function.

G119 - EXOGENOUS MESENCHYMAL STEM CELLS: FUTURE THERAPY TO REPAIR GLOMERULAR DAMAGE? INSIGHTS FROM A UNIQUE IMMUNOGLOBULIN LIGHT CHAIN-MEDIATED MESANGIAL INJURY EXPERIMENTAL MODEL

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Background. Bone marrow stem cells and progenitor stem cells found in the renal parenchyma can help in restructuring minor mesangial alterations to maintain mesangial homeostasis, but are unable to effectively and efficiently repair significant mesangial damage. *Objectives.* Using a unique model of mesangial injury by glomerulopathic light chains (LCs) (Light chain deposition (LCDD) and light chain amyloidosis –(AL-Am) LCs, the possibility of repair of the damaged mesangium by mesenchymal stem cells (MSCs) was explored. LCs extracted and purified from the urine of renal biopsy-proven LCDD and AL-amyloidosis were used in the experiments. Tubulopathic LCs were used as controls.
METHODS: The first platform used MCs cultured on a

matrix and incubated with glomerulopathic light chains for 4 days to produce mesangial injury and then MSCs were introduced into the culture system. A 6D live cell imaging system was used to evaluate the sequence of events taking place. Sequential photos were taken at prescribed time frames of 6 designated areas. The second platform used an ex-vivo kidney which was perfused through the renal artery with glomerulopathic LCs and after damage was confirmed, MSCs were perfused. Tissue samples were obtained for light, immunofluorescence, and transmission electron microscopy. *Results.* In both platforms, MSCs migrated to the damaged areas and proceeded to clean the damaged mesangium by phagocytosing amyloid fibrils and other deposited mesangial matrix constituents. These MSCs differentiated into a macrophage phenotype to perform the cleaning function. In the LCDD model, the increased mesangial matrix could not be adequately digested by the mesenchymal cells and had to be "primed" prior to dissolution and removal. Exogenous MSCs transformed into MCs. *Conclusions.* Both platforms provided key information to understand how exogenously administered MSCs repair the damaged mesangium. Experimentally, MSCs are able to repair damaged mesangium in an admirable manner offering a promising novel therapeutic option.

G120 - RENAL FAILURE IN MULTIPLE MYELOMA. EXPERIENCE AT HOSPITAL DE CLINICAS, MONTEVIDEO, URUGUAY

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Introduction. Renal impairment (RI) is frequent in multiple myeloma (MM) and its etiologies, multifactorial. Early treatment improves renal function. *Objectives*. To analyze the features of MM patients with RI at diagnosis, treatment indicated and response. *Materials and Methods*. Descriptive analysis of MM patients diagnosed between 2007-2014. Patients with creatinine ≥2 mg/dl attributable to MM were included. *Results.* 43 MM were diagnosed, 12 (27.9%) had RI. Median age was 69 years, median creatinine was 5.34 mg/dl (2.1-14.8), 75% had GFR <30 ml/min. Ig subtype was IgG in 4, 4 light chains, 3 IgA and 1 IgM; 8/12 (66.7%) were λ light chains. All were in advanced stage (IIIB), ISS score 3. Cytogenetics was normal in 11, FISH was normal in all. Median bone marrow plasma cell was 52%, median monoclonal spike was 1.7 g/dl. Mild-moderate hypercalcemia was present in 25%. All had anemia (median Hb 6.7 g/dl); 8 had proteinuria. In 7 patients MM was suspected >1 week from admission. All patients received corticosteroids at diagnosis and completed the first cycle of chemotherapy within 1 week. Nine received Bortezomib-based plans (6 CyBorD, 1 BD, 1VTD, 1 MPV). Six required urgent hemodialysis (HD), 2 normalized creatinine. Six required chronic HD, all had a delayed diagnosis. Six achieved stable creatinine <2 mg/dl. Overall, RI reversed in 50%. Treatments were well tolerated, 5/12 developed polyneuropathy. Treatment response was: 3 CR, 2 VGPR, 7 PR. Three patients received autologous transplantation, without complications. One patient died within 2 years of diagnosis. *Conclusions.* RI in patients with MM was associated with advanced disease, high tumor burden and λ subtype. Late initiation of treatment was associated with need of chronic HD. Bortezomib and dexamethasone are safe and well tolerated. Although the number of patients is small, observations are consistent with those of international studies.

G121 - HIGH-CUT OFF MEMBRANE IN ACUTE RENAL FAILURE DUE TO MULTIPLE MYELOMA. DATA FROM CZECH REPUBLIC

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Background. Renal failure due to myeloma kidney represents severe comorbidity in patients with multiple myeloma. High-cut off (HCO) dialysis membrane has been implemented into the therapeutic armamentarium as a new possibility which is able to remove high amount of free light chains (FLC) from the sera of affected patients. *Objectives and Methods.* 22 patients with acute renal failure due to cast nephropathy were treated in 5 centres in Czech Republic during the years 2012-2013. There were13 men and 9 women, mean age was 63.9 years (37-78). Renal biopsy was performed in 12/22 patients. FLC κ were detected in 12 patients, λ in 9 and 1 patient produced both types of FLC. Median of HCO dialysis was 8.5 (2-16). *Results.* Mean serum creatinine before and after the treatment with HCO dialysis was: 617.7±165.9 *vs.* 400.5±238.3 μmol/l (P=0,0012). Median of serum FLC concentrations before and after the treatment with HCO membrane was: 6626.5 *vs.* 396.5 mg/l (P=0,027). Except of two patients the others response to the treatment with adequate decrease in FLC concentration (>50-75% reduction). Immediate renal recovery was achieved in 13 from 22 patients, other two response afterthought. The treatment was not effective in 3 patients and three other died due to the progression of multiple myeloma or complications of chemotherapy. *Conclusions.* Dialysis with HCO membrane altogether with adequate chemotherapy significantly increases the chance of patients with cast nephropathy for renal recovery and better survival.

G122 - PATIENTS WITH MULTIPLE MYELOMA WHO RECOVER FROM DIALYSIS-DEPENDENT ACUTE KIDNEY INJURY HAVE GOOD LONG-TERM OUTCOMES

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Background. Over 70% of patients with multiple myeloma (MM) and dialysis-dependent acute kidney injury (AKI) remain on dialysis and have poor long-term survival (<1-year). However the overall survival and determinants of survival of patients with MM and AKI who recover renal function are unknown. *Methods.* This is a single-centre retrospective study of patients with MM and dialysis-dependent AKI who recovered renal function between 2005-2012. We assessed survival and long-term renal outcomes. Cox regression modelling was used to assess determinants of survival. *Results.* 27 patients fulfilled criteria for inclusion. The mean age was 61.4 years; 70.4% were males. The median follow-up was 54.06 months. Light chain (LC) only myeloma was seen in 11 (40.7%); IgG in nine (33.3%), IgA in four (14.8%), IgD in two (7.4%) and IgM in one (3.7%). A κ free LC (FLC) clone was present in 14 (51.9%) and a λ FLC clone in 13 (48.1%) patients. 23 patients had cast nephropathy. All patients received high dose dexamethasone; 25 were treated with regimens including thalidomide/bortezomib. The median time on dialysis was 27 days (IQR 16-53). 51.8% of patients were alive at analysis; the overall median survival was 64.1 months. 25 patients maintained renal recovery; two restarted dialysis after eight and 67 months from initial renal recovery and died within 3-months of restarting dialysis. The independent determinants of worse survival were a known history of CKD (p=0.021) and presence of a λ FLC clone (p=0.022). Shorter length of time on dialysis and higher percentage clonal FLC reduction from baseline at day 21 predicted a better eGFR at 6-months (p 0.029, R^2 0.332). *Conclusions.* In this series 92.5% of patients with MM and dialysis-dependent AKI who recover renal function have no requirement for further dialysis. Survival following recovery of renal function is good. Early variables are independently associated with renal function and survival.

OTHER USES OF FREE LIGHT CHAIN AND HEAVY LIGHT CHAIN

H123 - HEAVY LIGHT CHAINS *VS* VASCULAR ENDOTHELIAL GROWTH FACTOR MEASUREMENTS IN MONITORING OF POEMS SYNDROME

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POEMS syndrome is a rare plasma cell dyscrasia characterized by Polyneuropathy, Organomegaly, Endocrinopathy, Skin changes and Monoclonal Gammopathy (MG). Serum Vascular Endothelial Growth Factor (sVEGF) levels correlate with disease activity; in this study they were compared to the heavy/light chain (HLC) of the immunoglobulin involved in MG. HLC and sVEGF (reference range, RR <707 ng/L) were measured in the same samples from two POEMS patients. Patient 1: A 54 years old man was diagnosed with IgA/λ osteosclerotic myeloma and POEMS syndrome. High-dose dexamethasone leds to a complete haematological remission. During this period sVEGF levels decreased from 1,250 to 698 ng/L and HLC IgA/λ from 2.67 to 0.88 g/L (RR 0.80-2.04 g/L). Two years later the disease relapsed: sVEGF levels progressively increased to 1,820 ng/L and HLC IgA/λ to 6.13 g/L. sVEGF and HLC IgA/λ concentrations in 9 samples collected during monitoring significantly correlated (r=0.85; 95% CI: 0.43-0.97). Patient 2: A 49 years old woman was diagnosed as IgG/λ and λ MG, POEMS syndrome and primary amyloidosis with heart involvement. The chemotherapy with melphalan-dexamethasone for 1 year leds to a complete haematological remission. During this period sVEGF levels decreased from 1,800 to 260 ng/L, λ serum free light chain (sFLC) from 85.10 to 4.89 mg/L and HLC IgG/λ from 13.2 to 2.99 g/L (RR 0.98-2.75 g/L). In the following 23 months, the patient continued to be in complete haematological remission with sVEGF concentrations always <250 ng/L, λ sFLC <6.17 mg/L and the HLC IgG/λ <4 g/L. sVEGF and HLC IgG/λ concentrations in 6 samples collected during monitoring showed a statistically significant positive correlation (r=0.99; 95% CI: 0.93-1.00). *Conclusions.* In these patients, IgA/λ and IgG/λ POEMS, HLC showed the same trend of sVEGF and correlated to the disease activity.

H124 - PREVALENCE OF THE ALTERATION OF THE κ/λ RATIO BY FREELITE™ IN BLOOD DONORS OVER FORTY YEARS AT SANTA CASA DE SÃO PAULO: PRELIMINARY ANALYSIS

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Introduction. Monoclonal gammopathy of undetermined significance (MGUS) is defined by the presence of M protein in the serum <3.0 g/dL and/or in the urine <1g/24h, medullary plasma cell infiltration less than 10% and absence of damage to organs and tissues. The discovery of monoclonal gammopathy detected in the electrophoresis of healthy adults is not a rare event and it increases with age. Historically, serum (SPEP) and urine (UPEP) electrophoresis were considered the gold standard for identifying intact M-Ig and free light chain (FLC) respectively. In 2001 the introduction of the Freelite test changed the diagnostic and the monitoring paradigm. This test has not been used for healthy adults. *Objectives.* To identify the prevalence of the alteration of the κ/λ ratio by Freelite™ in blood donors over 40 years at Santa Casa of São Paulo blood bank. *Materials and Methods.* From October 2011 to September 2014, 1,000 serum samples were collected from the blood donors ≥40 years who had presented themselves at the Santa Casa of São Paulo Blood Bank. The sera was analysed with κ & λ Freelite kit on a SPA_{PLUS} (The Binding Site, UK). *Results.* Among the remaining donors, 36.85% are between 40 and 45 years, 21.12% are between 46 and 50, 15.29% are between 51 and 55, 8.25% are between 56 and 60 and 4.47% are between 61 and 65 years. The men predominated being 58.10% of the donors. We have analyzed 224 samples and in 6 of them, monoclonal production was identified by Freelite, 5 κ (above 1.65) and 1 λ (below 0.26). We will perform the results from 1,000 blood donors. *Conclusions.* The present study is the first one performed to evaluate the Freelite test in one cohort of Brazilian blood donors.

H125 - IgM HEVYLITE® ASSAY AS A BIOMARKER OF COLD AGGLUTININ DISEASE

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Background. Chronic cold agglutinin disease is a rare disorder affecting 15% of patients with autoimmune hemolytic anemia. It is characterized by the presence of a circulating monoclonal antibody, usually IgM, directed against I/i antigens expressed at the surface of erythrocytes. The IgM monoclonal component is a result of either by a mild excess of plasma cells (MGUS) or an underlying lymphoproliferative disease such as lymphoma, chronic lymphoid leukemia, infection or Waldenström's macroglobulinemia. In both cases serum concentration of the monoclonal IgM is usually weak and may be not find using routinely used methods. *Objectives.* To investigate the interest of the IgM Hevylite® assay in cold agglutinin disease. *Methods.* We retrospectively analysed sera collected at diagnosis of 35 patients (median age 78 years (37–93)) with cold agglutinin disease. Samples were subjected to immunofixation (IFE), serum protein electrophoresis (SPE), Hevylite® (on SPA-Plus Turbidimeter) assay and cold agglutinins were titered. *Results.* All 35 patients presented positive cold agglutinin titer values. While combination of SPE and IFE detected 16 sera with monoclonal IgM, the Hevylite® IgMκ/IgMλ ratio (IgMκ (3.185-721.86); IgMλ (0.050-1.1786) was abnormal in 22 samples (17 IgMκ and 5 IgMλ). Indeed, in 4 cases, Hevylite® allowed the detection and quantification of an increased level of IgMλ which were not detectable using standard SPE and IFE. Only 8 monoclonal protein were quantified with SPE (8 peak non measurable or <2g/l and 19 negative SPE). No monoclonal protein could be found in 13/35 patients (37%). *Conclusions.* The IgM hevylite® ratio allows detection of IgM even when IFE and SPE are negative due to lower sensitivity. This new test allow the quantification and follow up of monoclonal IgM level even when <2g/l. It may be useful to follow patients with cold agglutinin disease and should be evaluated in a larger series with follow up samples.

H126 - FREE LIGHT CHAIN MEASUREMENTS IN PLEURAL FLUID OF MULTIPLE MYELOMA PATIENTS

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Background. Pleural effusions are relatively uncommon (6%) in multiple myeloma (MM) and are often transudative. Malignant pleural effusions (MPEs) present in 1-2% of MM patients. Although the gold standard for MPE is cytology, the free light chain (FLC) assay presents a possible method for more rapid and quantitative diagnosis. *Objectives.* To determine the predictive value of FLCs in pleural effusion. *Methods.* FLC was validated using pleural fluid from 8 control patients. FLC concentrations and ratios (FLCr) were measured before and after pleural fluid was spiked with serum containing high FLCs. Thoracentesis was performed in 6 MM patients as part of routine clinical care. Ten samples were evaluated for the presence of FLC using a SPAplus analyzer. *Results.* FLCrs in pleural fluid were comparable to corresponding serum controls. Serum FLC from myeloma patients were recoverable from spiked pleural fluids. Relatively high concentrations of involved FLCs were found in 8 of 10 samples, however, only 6 were MPEs, 5 from the same individual. Of 3 patients with negative cytology, all had pleural FLCr comparable to those found in serum, suggesting passive movement of FLCs. To distinguish between FLC leakage into the pleural space and active secretion, we explored a pleural fluid-to involved sFLC ratio (PS-iFLCR). A ratio of 1 was seen with non-malignant effusions. In contrast, all 6 MPEs had a PS-iFLCR of >2 suggesting direct secretion of FLC. In a chemo-refractory MM patient with a pleurex catheter, serum and pleural FLC varied during the course of treatment, while the PS-iFLCR remained relatively constant. *Conclusions.* FLCs can be accurately measured in pleural fluid.

Analogous to the Light's criteria, a cut-off of >2 for the PS-iFLCR identified 6 of 7 MPEs but none of the 3 non-malignant effusions.

H127 - RELATIONSHIP BETWEEN CIRCULATING SYNDECAN-1 (CD138S) LEVELS AND SERUM FREE LIGHT CHAINS IN MONOCLONAL GAMMOPATHIES

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Background. Monoclonal gammopathies represent an increasingly growing global issue as they account for an elevated amount of cancers. For this purpose, free light chains (FLC) quantification has been widely accepted and incorporated into international guidelines as valid tool. Nevertheless, early and effective management of such patients is still cumbersome, and growing attention has focused on the role of tumor microenvironment on cancer. In this context, Syndecan-1 (CD-138) constitutes an attractive candidate due to its function and antigenic stability. CD-138 is a heparan-sulfate proteoglycan that is highly expressed and shed by myeloma plasma-cells. Shed CD138s circuits into serum and accumulates in other tissues, enhancing expression and bioavailability of signaling molecules and conditioning tumor microenvironment. Elevated serum levels of CD138s are specifically associated to malignancies and correlate with poor outcome in myeloma patients. *Objectives.* The aim of our study was to compare CD-138 levels and serum FLCs in patients affected by intact immunoglobulin multiple myeloma (IIMM) or light chain myeloma (LCMM) in order to assess their utility as complementary tools in this clinical setting. *Methods.* 84 patients affected by IIMM and LCMM (at the time of first diagnosis, undergoing no therapy, without renal failure) were recruited along with 40 healthy donors. CD-138 and FLCs were quantified for each sample according to the manufacturer's instructions. Data was analyzed by StatGraph and Prism4. *Results.* Significantly higher mean CD138 values were observed among IIMM patients as opposed to LCMM patients. Regression analysis of CD138/FLC values show opposite trends for the two groups. Extrapolated data from ROC curves show 95% specificity of CD-138 already at values above 15,50 ng/mL for IIMM and 10,00 ng/mL for LCMM (Figure 1). *Conclusions.* Our observations highlight a differential relationship between CD-138 shedding and FLC production in myeloma patients, and may offer novel approaches for a more effective management of the myeloma patient.

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H128 - IgG SUBCLASS DISTRIBUTION IN PATIENTS WITH MONOCLONAL GAMMOPATHY

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Background. Monoclonal gammopathies are characterized by uncontrolled proliferation of plasma cells. To date exact prediction of progression onset and thus start of individual therapy is not possible. Further risk factors and potential classification criteria must be identified. *Objectives.* We determined IgG subclasses in patients with multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS) and other underlying conditions. *Methods.* IgG subclasses were determined with the turbidimetric SPAPlus™ analyzer in 43 MM patients, 28 MGUS patients and 38 patients with other underlying conditions with distinct monoclonal IgG fraction as established by immunofixation. Additionally, in 93 of the samples, IgG heavy and light chain detection was performed with Hevylite™. *Results.* In 34 samples (23.53% MGUS, 26.47% MM, 50% other underlying conditions), subclass distribution was within the reference range. IgG1 was most often increased and IgG3 was most often decreased. The MGUS group revealed a significant difference between subclasses (p <.001). IgG1 was most often increased, while IgG3 and IgG4 were

most often decreased. In the MM group a significant difference in subclasses (p=.001) was found, while IgG1 showed a significant increase compared to IgG2, IgG3 and IgG4 (p=.008, p=.002, p=.004). In samples from other underlying conditions, no significant difference in subclasses was found (p=.070). In contrast to the MGUS/Other samples (12%/ 6.45%), 45.94% MM patients showed an increased heavy and light chain ratio. Comparison of pathological *vs.* non-pathological Hevylite™ quotients revealed a significant difference in IgG1 subclass (p <.001). Samples with non-pathological Hevylite™ quotients had on average a higher IgG1 subclass level than samples with pathological Hevylite™ quotients (p=.04). *Conclusions.* This study provides first important findings regarding IgG subclass distribution and heavy and light chain ratio in monoclonal gammopathies.

H129 - EVALUATION OF FREE LIGHT-CHAIN ABNORMALITIES AS PROGNOSTIC MARKER IN BINET A STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. We utilized the large, multicenter CLL1 study, to determine the prognostic value of FLC abnormalities in early stage CLL. *Methods.* The phase III CLL1 trial was conducted to assess if use of fludarabine prolongs progression free survival (PFS) in early stage CLL pts with a high risk (HR) profile. HR was defined based on thymidine kinase (TK), β-2-microglobulin (B2MG), lymphocyte doubling time, and bone marrow infiltration pattern. FLC κ and λ were measured in serum samples of a representative subset (n=169, 27%) of Binet stage A pts randomized to the watch & wait strategy in both the low (N=521) and high risk (N=96) arm of the CLL1 study protocol. *Results.* Median age of the 169 pts at study entry was 61 years (range 35-75), whereof 97 (57%) were male, and 24 (14%) were HR according to the CLL1 study protocol. Median FLC κ and λ were 12.2 mg/l (range 2.1-59.3) and 12.1 mg/l (range 2.0-142.0), respectively. Normal FLC were observed in 123 pts (73%), while in 26 (15%) an absolute FLC elevation (κ or λ) was present. FLC ratio was abnormal in 32 (19%) pts. Subgroups of FLC abnormalities included: 14 (8%) monoclonal, 18 (11%) ratio-only, 14 (8%) polyclonal. FLC abnormalities were significantly associated with IgVH status and CLL1 high risk profile and FLC levels were significantly correlated with B2MG (summated FLC κ and λ, Spearman r=0.38, p<0.0001). Progression was observed in 84 pts (50%), 21 (12%) died. In univariate analysis, FLC ratio-only and monoclonal changes were significantly associated with PFS (hazard ratio 2.40, 95%-CI 1.33-4.34 and 2.22, 95%-CI 1.13-4.37, respectively). No significant association was found in multivariate analysis. *Conclusions.* In line with previous reports, FLC abnormalities were associated with established prognostic markers. However, we did not observe an independent prognostic value with regard to PFS or OS.

H130 - SUSPECTED MULTIPLE MYELOMA REVEALED AS SJÖGREN'S SYNDROME

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Introduction. Multiple Myeloma as well as rheumatic disorders can be associated with high serum levels of Immunoglobulines. In addition a distinct but not well characterised correlation between monoclonal gammopathies and rheumatic disorders is known. In order not to initiate the wrong treatment it is essentially to distinguish between these diseases and one tool can be the Heavylite® assay. *Case report.* A 51-year old female patient presented at a referring hospital with history of recurrent varicella-zoster infections. Investigation of blood and bone marrow was performed showing monoclonal gammopathy type IgG κ with elevated IgG (50 g/l) and plasma cell percentage of 8% in bone marrow biopsy without fulfilled CRAB criteria. Diagnosis of smouldering multiple myeloma was made and she was admitted to our center for second opinion regarding the recurrent infections and high level of IgG. *Results.* Based on external findings our interdisciplinary myeloma board recommended initiation of anti-myeloma therapy including high dose melphalan with autologous stem cell transplantation due to the recurrent zoster infections and high IgG level. In further investigations in our center before start of treatment immunofixation of serum and urine showed no monoclonal bands and a more precisely view on the serum protein electrophoresis revealed no typical M-Spike but a broad increase in the gamma globuline fraction. Suspecting a polyclonal proliferation we performed a Heavylite® assay confirming our hypothesis with elevation of both IgG κ and IgG λ but normal ratio. We forwarded the patient to our rheumatologist who made the diagnosis of sjogrens syndrome with high levels of RF, ANA and SSA-antibodies. *Conclusions.* Rheumatic disorders can be associated with high levels of immunoglobulines due to chronic B cell stimulation. Differentiation of clonality is crucial to separate from monoclonal plasma cell disorders requiring intensive chemotherapy and therefore Heavylite® is a helpful tool.

H131 - IMMUNOGLOBULIN QUANTITATIVE MEASUREMENTS BY HEVYLITE IN CHRONIC LYMPHOCYTIC LEUKEMIA REVEAL FREQUENTLY ABNORMAL RATIOS RELATED TO A SHORTER TIME TO TREATMENT

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CLL is a frequent and usually indolent disease that may require only follow-up; however, about 1/3 of patients are symptomatic, have a worse outcome and need to be treated. It was shown that serum free light chains (sFLC) are the most commonly detected paraprotein in CLL. Here, we investigated whether Ig quatitative measurements by HEVYLITE™ could provide additive informations. *Patients and methods.* 100 CLL patients were studied of whom 65%, 22% and 13% were Binet staged A, B and C respectively. 55% never required treatment while 45% required treatment either at the time of diagnosis or during their follow-up. The median follow-up time of patients was 56,5 months and the median time to first treatment (TFT) 53 months. Ig quantification was made by both classical nephelometry and the new Hevylite™ technique. The second method measures the Ig fractions bound to either κ or λ light chains thus enabling to determine the ratio of Ig-κ/λ of each Ig class (IgG-HLCR, IgA-HLCR, IgM-HLCR). Statistical analysis was performed conventionally. *Results.* Frank paraprteinemia was present in 3 patients, however abnormal HLCR concerning IgG-HLCR, IgA HLCR, IgM-HLCR were present in 14%, 7% and 34% of patients respectively. HLCR abnormality could concern all 3 Ig classes (2%), 2 (19%) or 1 (35%) in the same patient. 53 patients with at least one abnormal HLCR had a shorter TFT (p=0,035); presence of abnormal HLCR correlated with Binet stage (p=0,02) and anaemia. In addition IgM hypogammaglobulinaemia as measured by the two methods was an adverse marker for TFT (p=0,017 by conventional nephelometry and 0,005 with hevylite). *Conclusions.* The presence of abnormal HLCRs and reduced IgM serum levels are related to shorter time to first treatment in CLL patients. IVgH clonotypic analyses should be performed to determine whether these abnormal HLCRs represent additional disease subclones.

H132 - POLYCLONAL IMMUNOGLOBULIN FREE LIGHT CHAINS IN PATIENTS WITH MYASTHENIA GRAVIS

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Background and Objectives. Previous studies have found increased systemic free light chains (FLC) concentration in patients with various chronic inflammatory conditions such as rheumatoid arthritis and upper and lower airway diseases. In this study we evaluated the serum free light chains in patients with myasthenia gravis, to investigate their clinical significance in this autoimmune disease. *Methods.* The study included 30 patients with myasthenia gravis (age:19-64 years; 18 men, 12 woman) and 30 matched normal contontrols. All patients had elevated acetylcholine receptor antibodies and did not have monoclonal gammopathy, kidney diseases or hypergammaglobulinemia. Serum κ and λ isotype levels and their combined level were measured using an immunoturbidimetric assay on the Roche Modular P analyzer (Freelite kit, The Binding Site Group Ltd, Birmingham, UK). *Results.* Of 30 patients with myasthenia gravis, 14 (46,6%) had elevated FLC concentration (11 κ and 3 lamba) compared with only one of the controls who had increased κ FLC concentration (p<0,001); 2 patients had very high k FLC concentration (293 and 102,9 mg/L respectively, with an abnormal increased κ/λ ratio); also the combined FLC level was higher in patients with myastenia gravis (median 41,3 mg/L) that in healthy controls (median 21,57 mg/L). *Discussion and Conclusions.* Our results show a significant increase of κ or λ FLC in about half of the patients with myasthenia gravis in whom monoclonal gammopathy or reduced glomerular filtration rate were excluded. Therefore, the increased concentration of κ or λ component in patients with myasthenia gravis may reflect the policlonal B lymphocyte activation in this disease, as observed in other autoimmune diseases (rheumatoid arthritis, Sjogrens syndrome and SLE).

H133 - THE USE OF FREELITE ASSAYS IN UNCOMMON PLASMA CELL NEOPLASMAS: BI-CLONAL PLASMA CELL MYELOMA AND SCHNITZLER SYNDROME

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Background. Freelite assays have been used for years in multiple myeloma and other plasmacytic or lymphoplasmacytic neoplasms, and have been included in guidelines for the diagnosis and monitoring of plasma cell myelomas that are monoclonal. However, its use in uncommon plasma cell neoplasms such as bi-clonal plasma cell myeloma has not been described. In addition, the use of Freelite assays in treatment monitoring of a rare disorder called Schnitzler syndrome, which is characterized by fever, bone and/or joint pain, lymphadenopathy, chronic urticarial and monoclonal IgM gammopathy, has not been reported. *Objectives.* To access the clinical utility of Freelite assays in bi-clonal plasma cell neoplasms and Schnitzler syndrome. *Methods.* Identify and review cases of bi-clonal plasma cell neoplasms and Schnitzler syndrome, for which Freelite assays were performed. *Results.* Four cases of bi-clonal plasma cell neoplasms were identified: (1) IgG-λ myeloma and κ light chain myeloma, with elevat-

ed serum free κ/λ (sκ/λ) ratio; (2) initially IgG-λ myeloma, followed by concomitant κ light chain myeloma detected by elevation of sκ/λ ratios; (3) concomitant IgG-κ and IgG-λ myelomas with persistent elevation of sκ/λ ratios; (4) initially IgG-λ myeloma with normal sκ/λ ratios, followed by concomitant IgG-λ and IgA-λ myelomas and decreased sκ/λ ratios of 0.03-0.08. In addition, one case of Schnitzler syndrome with IgM-κ paraprotein and elevated sκ/λ ratios was identified; after successful treatment, the sκ/λ ratio returned to normal. *Conclusions.* The Freelite assays can help detect concomitant or emerging bi-clonal plasma cell myelomas; however, its use in treatment monitoring could be problematic because the two neoplastic clones may produce different light chains and the production is often imbalanced. Freelite assays could help diagnose and monitor patient with Schnitzler syndrome.

H134 - HEVYLITE ASSAYS HELP DISCRIMINATE RHEUMATOID ARTHRITIS FROM PLASMA CELL DISORDERS

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Background. Hevylite is an immunoassay that allows measurement of clonality in intact immunoglobulin pairs such as IgA-κ and IgA-λ. When diagnosing a plasma cell disorder (PCD) standard tests such as serum protein electrophoresis (SPEP), immunofixation electrophoresis and serum free immunoglobulin light chains are used. Nevertheless in some patients results of these tests do not always allow discrimination of PCD from other immunological and inflammatory disorders such as rheumatoid arthritis or lupus. The role of Hevylite testing in these patients is not fully known. *Objectives.* Determine if Hevylite can add value in the discrimination of PCD patients from other disorders that have elevated polyclonal immunoglobins when other standard tests do not provide a clear result. *Case Report.* A 30 year old woman with rheumatoid arthritis was evaluated in the community with unexplained anemia–Hb was 9gm/dL, SPEP revealed an M spike of 6.1mg/dL (quantitative IgG 5900mg/dL) and bone marrow revealed 5-10% plasmacytosis. Both κ and λ light chains were mildly elevated. She was referred to our center for myeloma therapy. Repeat testing confirmed the M spike on SPEP, but Heavylite testing revealed IgG κ 4450mg/dL and IgG λ 1890mg/dL, resulting in a normal heavy chain ratio of 2.35. It was determined that the abnormalities, including the "M" spike were therefore polyclonal and consistent with rheumatoid arthritis and not a PCD. The anemia was later explained by iron deficiency. *Conclusions.* Hevylite assays provided additional clinically relevant information when assessing PCD in patients with inflammatory or immunological disorders, and in this case critically determined the patient did not have myeloma and did not require therapy. Heavylite testing should be considered in similar contexts.

H135 - CLINICO-PROGNOSTIC CONTRIBUTION OF FREELITE AND HEVYLITE IN EARLY CHRONIC LYMPHOCYTIC LEUKEMIA

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We analyzed a cohort accounting for 60 CLL Binet stage A patients in whom FLC measurements were performed at the diagnosis using a commercially available immunoassay (The Binding Site Group Ltd, Birmingham, UK). Using the cut-off proposed by Pratt *et al.* for the polyclonal serum FLC (*i.e.*, 50 mg/L) we found that patients whose levels were below this threshold had a shorter time to first treatment (TTFT) (HR, 2.08; 95% CI, 1.00-5.53; P=0.03) (Figure 1A). Patients with lower and higher polyclonal serum FLC levels (*i.e.*, lower and higher than 50 mg/L) were alike with respect to age (P=0.24), gender (P=0.57), ß2-microglobulin (P=0.98), LDH (P=0.67), mutational status of IGHV (P=0.54), ZAP-70 (P=0.64) and CD38-expression (P=0.98). In contrast, patients with lower FLC serum levels had more frequently Rai stage 0 (P=0.006) and lower thymidine-kinase activity (P=0.02) (Figure 1B). The Hevylite assay which enabled us to accurately measure each isotype-specific heavy and light chain (HLC) (that is, IgGk, IgGλ, IgAk, IgAλ, IgMk and IgMλ) was available in 45 out of 60 patients and revealed that 37 (82.2%) had at least one Ig measurement below the normal value. In detail, HCL abnormalities in terms of low Ig serum concentrations, ranged from 69% (IgMλ) to 4.2% (IgGλ). Patients with FLC lower and higher than 50 mg/L had a similar likelihood to experience HCL decrease: IgMk (P=0.75), IgMλ (P=0.54), IgGK (P=1.00),IgGλ (P=1.00), IgAk (P=0.16),IgAλ (P=0.08). Increases of Ig serum concentrations were unfrequent (*i.e.*, IgGλ, 4.7%; IgAκ, 4.7%; IgGκ, 2.3%). IgGκ/IgGλ ratios were always in the normal range, while one patient (2.3%) had abnormal IgAκ/IgAλ ratio and 3 (7.1%) patients had abnormal IgMκ/IgMλ ratio. In conclusion, this external validation analysis confirms the prognostic value of FCL measurement in CLL. The Hevylite assay demonstrates that the degree of immunoparesis was higher for IgMλ. Clinical implications of these results should be explored in larger CLL series.

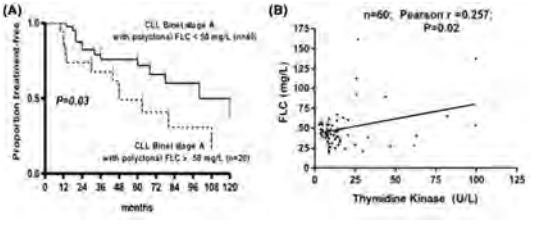

Figure 1.

H136 - EVALUATION OF THE BEHAVIOR OF MONOCLONAL AND POLYCLONAL LIGHT CHAINS IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background. Kappa/Lambda (κ/λ) ratio is a prognostic factor in MGUS. We observed a significant correlation between abnormal κ/λ ratio and immunoparesis in 171 patients: κ/λ ratio is abnormal in 62% of patients with immunoparesis and in 38% of patients without immunoparesis (p=0,0016). *Aims.* To investigate separately the behavior of monoclonal and polyclonal light chains. *Methods.* To evaluate polyclonal component, we stratified the patients in two groups (value below or above the mean value of the normal range). Then, we evaluated patients with monoclonal light chain value beyond the upper limit of normal range. *Results and Discussion.* Polyclonal chain levels are low in 76% of patients with immunoparesis and 55% without immunoparesis (p=0,03). They are low in 67% of patients with an abnormal κ/λ ratio and in 31% with a normal one (p<0,0001). Monoclonal light chain levels are increased in 92% of patients with abnormal κ/λ ratio and in 37% with a normal one (p<0,0001). Alteration of κ/λ ratio could thus be related both to a polyclonal light chains decrease and to a monoclonal light chain increase. Finally, patients were stratified according to the time since the diagnosis. The alteration of κ/λ ratio is more frequent in patients with a diagnosis of more than four years (68 *vs* 45%, p=0,01) and they have a marked reduction of polyclonal light chain in 81% (p<0,05), while there isn't increase of patients with monoclonal light chain levels beyond the upper normal limit. The first data could be due to a gradual exhaustion of immune response, while the lack of a monoclonal component increase could be related to a selection of patients with less tendency to evolve in Myeloma. We need to monitor patients to clarify the prognostic role of the alteration of different light chain levels and the importance of their changes during the follow-up.

H137 - A PILOT STUDY TO ASSESS SERUM FREE LIGHT CHAIN AS A POTENTIAL BIOMARKER FOR CHRONIC GRAFT-*VERSUS*-HOST DISEASE

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Background. Graft-*versus*-host disease (GVHD) is a debilitating and potentially fatal complication of allogeneic hematopoietic cell transplantation (HCT). Acute GVHD is mediated by donor T cells. The etiology of chronic GVHD (cGVHD) is less certain. Donor B cells may cause cGVHD. Rituximab, a B cell depleting drug, can induce a clinical response in some patients with cGVHD. B cell activating factor (BAFF) may be elevated in patients with cGVHD. Miklos *et al.* (2005) has shown human Y chromosome-encoded (H-Y) proteins in female-to-male HCT patients as a cGVHD biomarker. *Objectives.* Because elevated serum free light chain (SFLC) is a biomarker in autoimmune disorders, we sought to determine whether patients with cGVHD demonstrate abnormal SFLC patterns. *Methods.* Twenty patients were chosen from Stanford's HCT database. Myeloma or amyloidosis patients were excluded. Eleven patients developed cGVHD and nine patients did not. Cryopreserved peripheral plasma samples at pre-transplant and six post-transplant time points were assayed. SFLC were measured using Freelite Human κ and λ reagents (The Binding Site, Birmingham, UK). Total IgG levels were measured using the Prospec nephelometer (Siemens Medical Solutions Diagnostics, Tarrytown, NY). *Results.* Sufficient plasma was available in 117 out of 140 specimens. The mean free κ and free λ levels at each time point were not different between the cGVHD and non-GVHD populations (Figure 1). In five cGVHD patients, SFLC levels were compared with levels of H-Y allo-antibodies. Only one patient, who experienced an Epstein-Barr reactivation, did the levels correlate. *Conclusions.* This pilot study did not show a significant difference in SFLC in cGVHD. Most patient samples were hypogammaglobulinemic, which likely affected our ability to detect differences in SFLC levels.

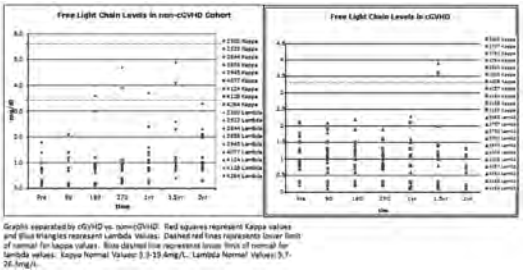

Figure 1. Free light chain levels.

H138 - ELEVATED SERUM FREE LIGHT CHAINS INDEPENDENTLY IDENTIFY STAGE A CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS WITH AGGRESSIVE DISEASE

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Background. Chronic lymphocytic leukaemia (CLL) is a heterogeneous disease and identifying early stage patients who are likely to progress remains difficult. *Objectives.* To investigate the usefulness of polyclonal serum free light chains (cFLC) as a tool to identify aggressive disease in untreated stage A patients. *Methods.* 167 Binet

stage A, untreated CLL patients were retrospectively analysed. The levels of FLC and β 2-microglobulin (b_2M) were assessed using nephelometric immunoassays. Comparison to previously recorded measurements for clinical markers (age (<or >65 years), sex, CD38$^{+/-}$, ZAP-70$^{+/-}$, lymphocyte doubling time (LDT<or >12 months) and *IGVH* mutational status (<or >98% homology)) was made. *Results.* Elevated cFLC (>50mg/L) were significantly associated with a shorter time to first treatment (TTFT, median 115 months v 175 months respectively; p=0.043) and overall survival (75% survival 101 v 170 months respectively, p=0.005). Univariate analysis identified elevated cFLC as being independently associated with poor outcome in this population. Multivariate analysis indicated that LDT<12 months (HR 4.1, p<0.0001), ZAP-70^{+ve} (HR 2.3, p=0.004) and cFLC>50mg/L (HR 2.0, p=0.024) were independently associated with shorter TTFT. A two tiered risk model using cFLC>50mg/L and ZAP-70^{+ve} identified patients with aggressive disease and was significantly associated with TTFT. Patients with 1 or 2 risk factors (FLC>50mg/L and / or ZAP-70^{+ve}) had a much shorter TTFT than patients with 0 risk factors (FLC<50mg/L and ZAP-70^{-ve}; median TTFT 113 v 218 months respectively, p<0.0001). *Conclusions.* Elevated cFLC>50mg/L aids in the identification of untreated CLL stage A patients with aggressive disease.

H139 - SOLUBLE BCMA BINDS BAFF RESULTING IN IMMUNE DEFICIENCY IN MULTIPLE MYELOMA PATIENTS

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Background. B-cell maturation antigen (BCMA) is a receptor expressed on B-lymphocytes and plasma cells. When it is bound by its ligand BAFF, it drives late B-cell development and antibody production. We have identified BCMA in the serum of MM patients. *Objectives.* We hypothesized that circulating BCMA binds BAFF, a B-cell mitogen, and blocks antibody production in MM patients. *Methods.* BCMA-Fc and control Ig-Fc were obtained from R&D Systems. Human BCMA and mouse BAFF, IgM and IgA levels were measured using ELISA. Human IgA and IgG levels were determined using nephelometry (Immage 800, Beckman Coulter). Hevylite® Assays (Binding Site) were used to quantify heavy-light chain isoform pairs. *Results.* rhBCMA was injected into mice. Plates were coated with an anti-BAFF capture Ab, plasma added and an anti-human-BCMA detection Ab added. rhBCMA-mBAFF complexes formed, whereas none were found in control mice. Furthermore, decreases in IgA and IgM levels were observed when compared to baseline on days 4 and 6 (P=0.0031, P=0.0064; P=0.0087, P=0.0221, respectively), and untreated (P=0.0001) and Ig-Fc (P=0.0088) control groups. BCMA/BAFF complexes were found in serum from MM patients. BCMA levels inversely correlated with uninvolved IgG in IgA MM and uninvolved IgA in IgG MM (P <0.0001). Using the Hevylite Assay, similar results were observed for the levels of BCMA compared to uninvolved IgG isoforms in both pts with involved IgGλ (n=62, P=0.0006) and IgGκ (n=117, P <0.0001) MM. *Conclusions.* We now demonstrate formation of BCMA-BAFF complexes, resulting in reductions in normal antibodies, and show that BCMA levels inversely correlate with uninvolved Ig levels in MM pts. Thus, the lack of normal antibody production in MM pts results in part from circulating BCMA binding BAFF.

H140 - INTACT IMMUNOGLOBULIN AND FREE LIGHT CHAIN GAMMOPATHIES IN HEART TRANSPLANT RECIPIENTS. A PILOT STUDY

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Background. Solid organ transplant recipients are at increased risk of developing malignant tumours, estimated to be 2x higher, when compared with nontransplant population. Posttransplant lymphoproliferative diseases (PTLD) are one of the most frequent complications in OTR with 40-70% mortality, and recent data suggest that gammopathies may be one of the underlying pathogenic mechanisms for PTLD. *Objectives.* To analyse the development and timelines of intact immunoglobulin gammopathies post-transplantation in a cohort of heart transplantation patients. *Methods.* We performed a pilot study on 80 patients which underwent heart transplantation (HTX) in years 2010-2012. Four sequential serum samples were obtained from each patient at defined time points (pre HTX and 9th, 18th and 24th month post HTX). Patient medical history served as a source of relevant clinical data. *Results.* We evaluated data from 80 patients (67 men, 13 women) after HTX aged 21-68 years (median 52 years). Results are shown in Table 1.

Table 1. Gammopathies post HTX.

	Totally (number of cases)	**Specific groups** (number of cases)
SPE/IFE	14	• 6 transient • 5 stable • 3 progressive
Free light chains concentration and ratio	38	• 32 with pathology in concentration • 21 with pathology in K/L ratio • 37 with time development
Heavy/light chain pairs (intact immunoglobulins) concentration and ratio	56	• 48 with pathology in concentration • 29 with pathology in K/L ratio • 40 with time development

All patients had negative serum protein electrophoresis (SPE) and immunofixation (IFE) pre HTX. There were no patients in our cohort with PTLD during 2 year follow up. *Conclusions.* During a 2 year follow up period we identified a remarkable number of monoclonal and polyclonal gammopathies in HTX patients using SPE/IFE, heavy/light chain pairs and free light chain analysis, which were multiple in occurence and heterogeneous in nature. This pilot study requires further investigation to understand the underlying mechanisms responsible for the pathological gammopathies.

FREE LIGHT CHAINS IN MULTIPLE SCLEROSIS

I141 - PREDICTIVE VALUE OF FREE κ CHAINS IN CEREBROSPINAL FLUID IN THE CONVERSION FROM CLINICALLY ISOLATED SYNDROME TO MULTIPLE SCLEROSIS

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Background. Multiple sclerosis (MS) initiates with a first attack or clinically isolated syndrome (CIS). The importance of an early treatment in MS leads to the search for novel biomarkers. At the moment, there is no diagnostic marker for MS who predicts conversion from CIS to MS. *Objectives.* Assay the level of free κ chains (FKC) in cerebrospinal fluid (CSF) which predicts conversion to MS in patients with CIS. *Methods.* We quantified CSF FKC from 70 patients with non-inflammatory neurological diseases [41 with normal pressure hydrocephalus (NPH)], 77 with CIS, and 29 with relapsing-remitting MS. FKC level was quantified by nephelometry using the Freelite® Human κ Free kit on the Siemens® BN II analyzer. We used Chi square or Fisher test for statistical analysis. *Results.* We calculated the CSF FKC values of NPH patients (control group). They were 0.18±0.10 mg/l [mean±SD (standard deviation)]. We calculated a cut-off value of 0.38 mg/l as the mean plus 2SD of control group values. We classified CIS patients according to this cut-off value. Group 1 included 32 patients with FKC below 0.38 mg/l and group 2 was integrated by 45 patients with FKC value above 0.38 mg/l. A higher number of patients from group 2 fulfilled Barkhof-Tintore criteria for dissemination of lesions in space and showed oligoclonal IgG bands in CSF. Almost 80% of patients from group 2 converted to MS, while 87% from those from group 1 remained as CIS. *Conclusions.* The cut-off value established of 0.38 mg/l is lower to that found by other authors. We found statistically significant differences between the group 1 and 2 for CSF FKC fulfilling Barkhof-Tintore criteria and showing oligoclonal bands. Furthermore, the risk of conversion to MS in CIS patients is greater with high CSF FKC levels.

I142 - FREE LIGHT CHAINS TURBIDIMETRIC MEASUREMENT IN CEREBROSPINAL FLUID: ROLE IN MULTIPLE SCLEROSIS

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Background. Multiple Sclerosis is the most common inflammatory demyelinating disease affecting the Central Nervous System (CNS). Mc Donald criteria are the diagnostic reference standard and the analysis of cerebrospinal fluid (CSF) is of little help if compared to magnetic resonance or to clinical features. *Objectives.* Our aim was to verify whether κ and λ free light chains (kFLC and λFLC respectively) turbidimetric measurement in CSF and serum may represent a clinically useful marker, in comparison with the current analysis of oligloclonal bands. *Methods.* We measured kFLC and λFLC in serum and CSF of 59 patients diagnosed with Multiple Sclerosis (Group1) and of 31 patients presenting a non-inflammatory disease of the CNS (Group2) already tested for intrathecal immunoglobulin synthesis. FLC were performed on SPA Plus turbidimeter using Freelite kit (The Binding Site, Birmingham, UK). Oligoclonal bands were determined using SAS IgG IEF kit (Helena BioSciences, UK). *Results.* The median CSF kFLC concentration in Group1 was 2.53 mg/L (range 0.06-32.26) and 0.21 mg/L (range 0.06-1.27) in Group2. The median kFLC Index was 39.07 (range 2.78-1232) in Group1 and 1.92 (range 0.92-6.07) in Group2. Using hyperbolic curve [1] and the threshold line [2] the two populations are well separated (Figure 1). The Mann-Whitney test confirmed statistically significant differences between the two groups ($p<0,0001$). The median CSF λFLC was 0.51 mg/L (range 0.06-6.18) in Group1 and 0.15 mg/L (range 0.04-0.43) in Group 2. The median λFLC Index was 12.98 (range 2.89-503.5) in Group1 and 2.73 (range 1.51-23.4) in Group2 The areas under the ROC curves were 0.992, 0.941 and 0.925 for kFLC Index, CSF kFLC and oligloclonal bands respectively; lower values were obtained for λFLC Index (0.881) and CSF λFLC (0.868). *Conclusions.* Turbidimetric assay for kFLC is quantitative, objective and suitable to detect intrathecal immunoglobulin synthesis. λFLC measurement show inferior performance in our experience.

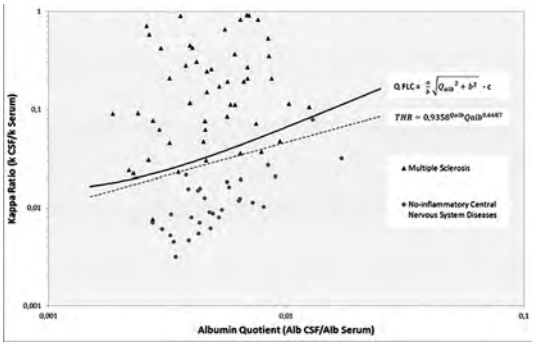

Figure 1. K Ratio *vs* Albumin Quotient (kFLC Index).

I143 - IS THERE A ROLE FOR FREE LIGHT CHAIN ANALISIS IN THE DIAGNOSIS OF PATIENS WITH MULTIPLE SCLEROSIS?

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Introduction. Multiple sclerosis (MS) is a neurodegenerative disease affecting mainly young adults. Clinical manifestation starts with a first flare, usually called clinically isolated syndrome (CIS), which can evolve to increasingly debilitating states (clinically defined MS) or remain asymptomatic and stable. Early treatment initiation in MS patient is crucial to prevent permanent damage. However, early diagnosis is difficult to establish due to the poor specificity of the symptoms and of the presently available biomarkers. *Objectives.* Study the potential of free light chain analysis (FLC) in cerebrospinal fluid (CSF) as biomarker of MS. *Materials.* Cohort of 192 patients with available CSF and for which FLC were determined (SPAplus, TBS,UK). Serum and CSF Albumin, total IgG and IgM, and the presence of CSF oligoclonal bands (OCBs) were recovered from patients' records. *Results.* After reviewing patient's records, we identified 41 MS patients, 21 CIS, and 41 suitable controls (*i.e.* non-demyelinating conditions such as ischemia or dementia). CSF FLC-κ values progressively increased from control group, to the CIS group and to the MS group (Figure 1).
In fact, 63% of controls were below the sensitivity limit of 0.042mg/dL compared to 7% of MS patients. Dunn's multiple-comparison test showed that both

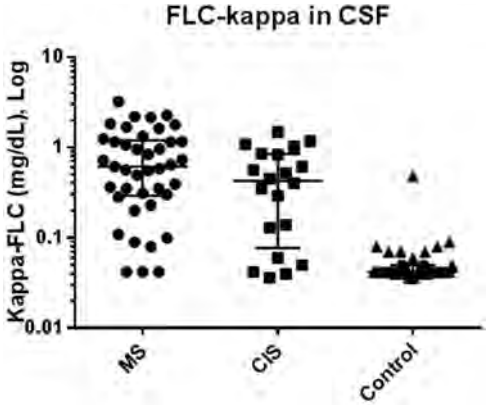

Figure 1. K free light chain values determined in CSF samples of three groups of patients. MS: Multiple Sclerosis group; CIS, clinically isolated syndrome group, and the Control group. The respective median values in mg/dL are 0.61, 0.42, and 0.05 (N: for the control group, only patients with measurable values were considered). The κ-FLC values are plotted in logarithmic scale.

References

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FLC-κ index and IgG-index are able to differentiate MS and CIS patients from the control group. Finally, using the cut-off value of 0.053mg/dL κ-FLC (Villar *et al.* 2012), we obtain a sensitivity of 93% and a specificity of 83%, compared to 81% and 93% for the OCBs, respectively. Using logistic regression, κ FLC predicts positivity for OCBs (OR 25.161, CI 3.184-198.811). *Conclusions*. Elevation of κ-FLC in CSF above 0.053mg/dL has confirmed to be a strong indicator of demyelinating CNS disease, both of MS and CIS. Follow-up studies are warranted to investigate the prognostic value of κ-FLC in the identification of CIS patients with high progression probability.

I144 - FREE LIGHT CHAIN ANALYSIS IN CEREBRO-SPINAL FLUID

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Background. Already 45 years ago, it has been reported that in the cerebro-spinal fluid (CSF) of patients with Multiple Sclerosis (MS) markedly higher κ/λ ratios of light chains are observed, this in contrast to findings in serum and in patients with other CNS diseases like neurosyphilis. Later work has confirmed this finding and extended it to free light chains. Furthermore, free κ light chains in CSF already appear in early (mono-symptomatic) MS before development of oligoclonal bands and their presence correlate well with changes in MRI. *Objectives.* The study reports on first experiences of introduction of free light chain analysis in CSF and serum as part of the routine CSF analysis in a general laboratory. All specimens referred to the lab for analysis of oligoclonal bands were subjected to free light chain analysis. The aims were to define cut-offs for free κ synthesis in CSF dependent on blood brain barrier (BBB) disturbance and to compare the synthesis rate with the occurrence of oligoclonal bands. *Methods.* K and λ free light chains were measured together with albumin, IgG, IgA, and IgM (Binding Site) in CSF and serum on a SPA_{PLUS} device. CSF/serum ratios, intrathecal synthesis rates and κ/λ ratios were calculated and results were included in a graphical CSF report in form of hyperbolic "Reiber"-type diagrams.

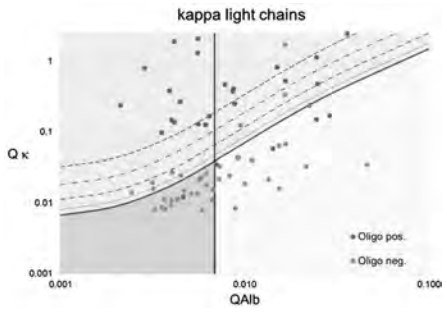

Figure 1.

Results. Based on BBB barrier measurements and oligoclonal band findings a hyperbolic cut-off could be defined that well separated cases with signs of intrathecal immunoglobulin synthesis and various degrees of BBB disturbance (Figure 1). Individual cases with special κ/λ signatures were compared with clinical findings. *Conclusions.* Free light chain analysis in CSF and serum is a valuable addition to the current analysis of albumin, immunoglobulins and oligoclonal bands and might be useful to detect early MS cases and to estimate their prognosis.

I145 - ELEVATED κ FREE LIGHT CHAINS IN THE CEREBRAL SPINAL FLUID ARE ASSOCIATED WITH POSITIVE OLIGOCLONAL BANDING IN PATIENT SAMPLES BEING EVALUATED FOR MULTIPLE SCLEROSIS

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Background. Multiple sclerosis (MS) is a difficult disease to diagnosis because the symptoms are typically nonspecific and hard to quantify. There is no single test which offers proof positive diagnosis; rather it is the preponderance of the clinical symptoms that leads to definitive diagnosis. *Objectives.* Due to the lack of definitive testing for MS and the fact that many other conditions can mimic the effects of MS, the development of additional biomarkers that can help rule out positive MS diagnosis would be beneficial. Intrathecal immunoglobulin synthesis has been observed in many studies of inflammatory disorders, including multiple sclerosis. In this study we aimed to correlate κ free light chain (FLC) production in the cerebral spinal fluid (CSF) of patients with positive oligoclonal banding (OCB). *Methods.* De-identified patient samples (n=62) routinely sent for evaluation of OCB for the screening were evaluated for intrathecal κ and λ free light production. FLC indices were determined using the following ratio: (CSF FLC/serum FLC)/(CSF albumin/serum albumin). In cases below the detection level (κ, <0.28 mg/L and λ <0.24 mg/L) we used the corresponding detection limit for analysis. *Results.* Of the patients evaluated (n=62), 18 patients had elevated κ FLCs using the KFLC index of (≥3.0), and 20 patients showed OCB. Sensitivity was 0.80, specificity was 0.95, positive predictive value of 0.88, and negative predictive value of 0.92. The mean KFLC index was 15.80 *versus* mean LFLC index of 2.23 in OCB positive samples (Figure 1).

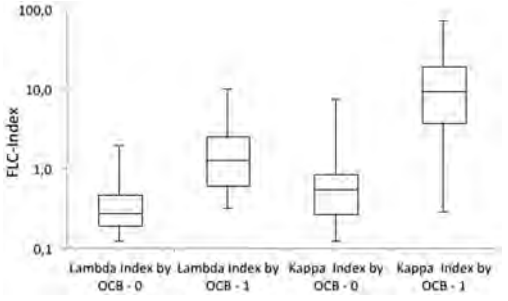

Figure 1. Box and whisker plot of κ and λ FLC indices with negative (OCB -0) or positive (OCB -1) oligoclonal banding.

Conclusions. There is a need for additional biomarkers in the diagnostic testing schema for MS. Our findings indicate that a KFLC index of 3.0 provides a high negative predictive value. Taken together with OCB findings these two basic laboratory tests can aid neurologists in ruling out MS in patients with neurological disorders.

I146 - FREE LIGHT CHAINS IN CEREBROSPINAL FLUID: DETERMINATION OF NORMAL VALUES OF CONCENTRATION, RATIO AND INDEX AND THEIR INTERESTS IN A 6 MONTHS PROSPECTIVE STUDY

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Background. The gold standard diagnosis marker for MS is oligoclonal banding whereas IgG index is not enough sensitive and have no value to predict future disease course. On the contrary, intrathecal IgM synthesis predicts a more severe disease. If FLC κ CSF exploration is a high sensitive marker for MS, contradictory results have been reported for the predictive interest of CSF FLCs. Moreover, different assay methodologies and different calculations have been reported in previous publications and normal values are still lacking. *Objectives.* New FLCs kits are available for the SPAplus system. We thus perform a prospective study in order to establish normal values for FLCs concentrations, serum/CSF FLCs quotient and the ratio between the FLCs quotient and albumin quotient. *Methods.* Matched serum and CSF samples were prospectively collected from 600 patients who underwent routine exploration for CNS or PNS, non inflammatory or inflammatory diseases. albumine, IgG, IgM, IgA, FLC κ and λ concentrations were quantified on SPAplus analyzer. Oligoclonal IgG banding were searched for all patients. Electrophoresis studies for IgM and IgM were performed when the corresponding index was positive. *Results.* Each normal value was calculated from 200 cases selected on following criteria: absence of oligoclonal banding, normal albumine ratio and absence of inflammatory CNS disease. We then confirmed that the κ index (κ quotient/Alb quotient) is a highly sensitive test for the diagnosis of inflammatory CNS diseases. Moreover, we observed several cases with highly altered κ/λ CSF ratio. Some patient suffered from inflammatory CNS diseases, but unexpectedly FLCs exploration allowed the discovery of infiltrative lymphoma of the CNS. *Conclusions.* Normal values are now available for each calculation and FLCs CSF exploration is useful for the diagnosis of inflammatory CNS diseases and the detection of some B cell CNS lymphoma.

I147 - UTILITY OF FREE LIGHT CHAINS IN CEREBROSPINAL FLUID FOR THE STUDY OF MULTIPLE SCLEROSIS

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Background. Multiple sclerosis (MS) is one of the most common demyelinating CNS diseases in young and middle-aged adults. The etiology and pathogenesis of MS is multifactorial and not well known. Moreover, the diversity of symptoms and signs provides almost unlimited possibilities for misdiagnosis. *Objectives.* To evaluate the diagnostic value of free light chains (FLC) determinations in CSF in patients with MS: comparison to traditional tests that demonstrate an intrathecal immunoglobulin synthesis. *Methods.* Parallel serum and cerebrospinal fluid samples from 77 neurological patients for whom oligoclonal bands (OB) assessment was requested were included. The OB were determined by isoelectric focusing using IgG EIF kit Helena BioScience. K- and L-FLC levels were determined on a BNII using Freelite reagents. Total proteins, IgG and albumin were measured to calculate the IgG index. Based on the medical history, patients were classified into two groups: group1 - definite+probable MS; group2 - suspected disease but unable to confirm the symptoms' cause. 23 patients without neurological damage were used as control. *Results.* 85% of patients from group1 were positive for OB, whereas in all cases the K-CSF levels and IgG Index were significantly higher than those found in the control group (Table 1). Also, a highly significant correlation between K-CSF levels and the presence of OB and IgG Index ($r=0.550$; $p<0.005$) was observed in patients from group1 but not from group2. K-CSF and IgG levels in CSF, and IgG Index showed statistically significant differences in groups 1 and 2 with respect to controls. *Conclusions.* Our results confirm recent data from the literature that support the high value of K-CSF in the diagnosis of MS. The strong correlation observed between the K-CSF and intrathecal IgG synthesis suggests that these seem to have a diagnostic meaning similar to that of OB.

Table 1. Median values and ranges of the different parameters determined for the theere studied groups.

	Group 1	Group 2	Control Group
Patients number	29	48	23
K-CSF (mg/L)	5.8 ± 1.32	0.14 ± 0.03	0.12 ± 0.01
Range (mg/L)	0.76 – 31.8	0.05 – 0.78	0.07 – 0.44
L-CSF (mg/L)	0.8 ± 0.4	0.13 ± 0.03	0.09 ± 0.05
Range (mg/L)	0.05 – 7.54	0.05 – 1.1	0.05 – 0.38
IgG Index	0.8 ± 0.05	0.51 ± 0.01	ND
range	0.6 – 1.56	0.36 – 0.88	

I148 - HIGH LEVELS OF IMMUNOGLOBULIN FREE κ LIGHT CHAINS IN CEREBROSPINAL FLUID AS INDICATORS OF MULTIPLE SCLEROSIS

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Introduction. Multiple Sclerosis (MS) is an autoimmune and demyelinating disease of the central nervous system (CNS) affecting mainly young adults (20-40). Although magnetic resonance (MRI) and IgG oligoclonal bands (OCB) represent a help for MS diagnosis, final decision is frequently done by ruling out other conditions whilst no test is still able to reliable differentiate patients with benign clinical isolated syndrome (CIS) from those who will progress to chronic or relapse/remitting MS. Our aim was to show the value of cerebrospinal fluid (CSF) κ-free light chain (FLC) determination sin the diagnosis of clinically defined MS. *Materials and Methods.* CSF samples collected from 254 consecutive patients who underwent a lumbar puncture, of which 89 were assessed for their κ-FLC levels by nephelometry using FreeLite®. Albumin and IgG were quantified by nephelometry in CSF and by turbidimetry in serum, while IgG Oligoclonal bands (OCBs) were done by iso-electro-focusing. *Results.* After reviewing patients' records, we identified 39 MS cases, 4 possible MS, and the remaining had other CNS inflammatory diseases (OID). FLC determinations were available for 37 MS patients, 4 possible MS and 48 cases of OID. Median κ-FLC levels (Figure 1) were significantly higher for MS patients than for OID patients (4.36mg/L *vs* 0.47mg/L, p<0.0001). One MS patient showed zero OCBs, however the k-FLC levels (2.8 mg/L) were 6-fold the median levels of the OID group. Conversely, 26 (12%) of the non-MS patients had 1 or more OCBs, however their median κ-FLC levels (0.47 mg/L) were still significantly lower than the MS group (p<0.0001). *Conclusions.* The significantly higher κ-FLC values of the MS group compared to the non-MS show that this test is useful for the diagnosis of MS, particularly in patients presenting unclear on single OCBs patterns. In our cohort, 12% of the non-MS patients presented 1 or more OCBs. Therefore, including CSF κ-FLC analysis in the study of suspected MS patients should significantly increase the accuracy of the diagnosis.

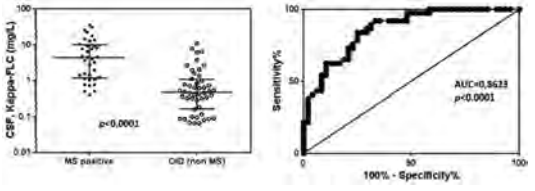

Figure 1. K-FLC CSF values for the MS patients and for the patients with other inflammatory diseases (OID), and respective ROC analysis. Mann-Whitney test shows that the difference between the medians of both groups is statistically significant. Horizontal lines represent the median and interquartile range of each group. The right panel shows the ROC analysis of the FLC levels using the OID group as control.

I149 - CEREBROSPINAL FLUID FREE LIGHT CHAINS ARE ELEVATED IN CLINICALLY ISOLATED SYNDROME AND MULTIPLE SCLEROSIS AND CORRELATE WITH LESION LOAD AND CORTICAL THINNING

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Introduction. Cerebrospinal fluid (CSF) oligoclonal bands (OCB) are a well established tool to support the diagnosis of multiple sclerosis (MS) and predict the conversion of clinically isolated syndrome (CIS) to MS. However, up to now only scarce information exists on correlates of FLC with conventional and non-conventional magnetic resonance imaging (MRI) metrics, in particular with measures of brain regions with direct contact to CSF. *Objectives.* To compare FLC κ (KFLC) and FLC λ (LFLC) levels in CSF and serum between MS patients and controls, and to investigate their relation to MRI based measures of cortical thinning and periventricular lesion load. *Methods.* FLC in CSF and serum were measured by nephelometry in 61 MS patients and 60 controls. Routine laboratory work up included analysis of serum and CSF levels of IgG, IgM, IgA, albumin, and CSF cell count. MS patients underwent MRI at 3T to determine the extent of cortical thinning and periventricular lesion load. *Results.* In MS we found increased CSF KFLC and LFLC levels, quotient and indices compared to controls (all p<0.001). CSF KFLC and LFLC correlated with CSF cell count (r=0.579, p<0.001 and r=0.301, p=0.018, respectively), and FLC indices with the IgG index (r=0.714, p<0.001 and r=0.543, p<0.001) in MS. Regarding MRI we found CSF LFLC to be correlated with the percentage of periventricular lesion load (r=-0.356, p=0.005). Furthermore, the KFLC index correlated with the mean cortical thickness (r=0.274, p=0.036). *Conclusions.* Our study demonstrates increased intrathecal synthesis of KFLC and LFLC in MS supporting the notion of an altered B-cell response. The correlation of KFLC and LFLC with MRI based measures of cortical and periventricular tissue destruction suggests these markers to be involved in the pathology of MS. Further studies with longitudinal clinical and MRI data are necessary to confirm our findings.

I150 - COMPARISON OF CEREBROSPINAL FLUID FREELITE• SPECIFICITY AND SENSITIVITY WITH OLIGOCLONAL BANDING, IN PATIENTS WITH SUSPECT MULTIPLE SCLEROSIS/CLINICALLY ISOLATED SYNDROME

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Background. Multiple Sclerosis (MS) diagnostic work-up includes Oligoclonal banding (OCB) and IgG index.

However, OCB is time consuming and the interpretation requires a skilled operator; IgG index is less sensitive than OCB. Immunoglobulin Free light Chains (FLC) are secreted by central nervous system B cells and may better reflect intrathecal synthesis of IgGs, providing further support to MS diagnosis. *Objectives.* Comparison of CSF Freelite® specificity and sensitivity with OCB, in patients with suspect MS/CIS. *Methods.* We collected coupled serum-CSF samples from 122 patients, who were divided into 3 groups: 1) 76 patients, 66 OCB negative and 10 with mirror serum-CSF pattern; 2) 19 OCB positive patients (1- 5 OCB bands); 3) 27 patients with >5 OCBs. Freelite K index (cut-off 12) were compared to OCB (Hydragel 9 CSF Isofocusing) and IgG Link index (cut-off 0.7) (BN II). *Results.* Group 1 - OCB negative: K Index and Link correlate with OCB in 97% and 92% of cases respectively; group 2: K index correlates with OCB in 53% of cases, whereas Link correlates in 11% of cases. Group 3: K index correlates with OCB in 99.7% of cases, Link index in 78%. Taking OCB as the Gold Standard, K index sensitivity is 0.89 (89%) and specificity is 0.87 (87%), with Positive Predictive Value 0.78 and Negative predictive value 0.93 (Table 1). *Conclusions.* These preliminary results suggest K index has greater correlation with OCB than Link index, and K index could be supportive in dubious cases of MS diagnosis.

Table 1.

		LINK		*K index*		*TOTAL*
		<0.7	*>0.7*	*<12*	*>12*	
Group 1	No OCB	61	5	64	2	66
	mirror OCB	10	0	7	3	10
Group 2	1-5 OCBs	17	2	9	10	19
Group 3	>OCBs	6	21	1	26	27
TOTAL		94	28	81	41	122

I151 - CEREBROSPINAL FLUID FREE IMMUNOGLOBULIN LIGHT CHAIN ANALYSIS IS A MORE SENSITIVE MARKER OF INTRATHECAL IMMUNOGLOBULIN PRODUCTION THAN OLIGOCLONAL BANDS IN A PEDIATRIC POPULATION WITH INFLAMMATORY CENTRAL NERVOUS SYSTEM DISORDERS

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Background. Inflammatory and autoimmune disorders of the central nervous system (CNS) include a diverse group of diseases for which there are no definitive diagnostic tests. Typical investigations include oligoclonal bands (OCB) in cerebrospinal fluid (CSF) and CSF IgG:protein albumin index. Both are limited by poor sensitivity and/or specificity. *Objectives.* To investigate the utility of CSF free light chain analysis in a paediatric population with CNS disorders. *Patients and Methods.* Paediatric CSF samples were analysed and results correlated with diagnoses determined by retrospective review and classified according to inflammatory or non-inflammatory pathogenesis. *Laboratory analysis.* CSF OCB was performed by agarose gel isoelectric focussing/immunoblotting. CSF free κ and λ light chain analysis was performed using the Freelite assay by nephelometery on both the Immage 800 and the BN II. *Results.* Of 72 samples analysed 31 (44%) had an inflammatory diagnosis, 24 (34%) had proven/ presumed genetic non-inflammatory aetiology, 3 (4%) had neurodevelopmental delay and in 13 (18%) no definite diagnosis was made. OCB was detected in 4 patients; 2 with an inflammatory and 2 with a genetic disease. Thirteen patients had elevated κ or λ light chains as detected on the Immage 800, defined as greater than the detection limit of the assay (0.600 mg/L for CSF free κ, and 0.490 mg/L for CSF free λ). Of these 12 (92%) had an inflammatory disease. On the BN II using optimal cut-offs of 0.29 mg/L and 0.11 mg/L for CSF free κ and λ respectively, 24 patients had elevated results, of which 21 (88%) had an inflammatory disease. *Conclusions.* The results of this study support the use of CSF free light chain analysis for the detection of intrathecal immunoglobulin synthesis in the diagnosis of paediatric CNS inflammatory conditions.

POLYCLONAL FREE LIGHT CHAINS

J152 - ELEVATED COMBINED FREE LIGHT CHAIN CONCENTRATIONS ARE INDEPENDENTLY ASSOCIATED WITH MORTALITY IN EARLY CHRONIC KIDNEY DISEASE

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Background. A major component of increased mortality risk in people with chronic kidney disease (CKD) is associated with non-traditional cardiovascular risk factors including markers of inflammation. Combined free light chains (cFLC) are a novel marker of systemic inflammation that have previously been associated with mortality in CKD patients recruited within the secondary care environment. *Objectives.* We investigated whether cFLC is an independent risk factor for all-cause mortality (ACM) in people with early stage 3 CKD. *Methods.* In a prospective community-based cohort, 1695 participants with stage 3 CKD had cFLC concentrations measured. cFLC levels were determined using the summation of the Freelite® κ and λ assays. All other bio-clinical variables were collected at the time of sample collection. Kaplan-Meier plots and Cox proportional hazards analysis were used to assess the relationship between high cFLC levels (>43.3 mg/L) and mortality. *Results.* At recruitment, cFLC were elevated (>43.3 mg/L) in 34% of patients (median 36.3 mg/L, IQR 28.6-47.9 mg/L). By the point of analysis, there were 167 deaths (10%) after a median of 1375 days. cFLC concentrations were higher in participants who'd died compared with those still alive; median 46.5 mg/L (IQR: 36.1-65.4 mg/L) and 35.4 mg/L (28.1-46.6 mg/L) respectively, p<0.001. Kaplan-Meier survival analysis demonstrated that participants with cFLC>43.3 mg/L levels had a shorter overall survival than individuals with cFLC within the normal range (p<0.001). Elevated concentrations of cFLC were independently associated with ACM (Hazard ratio: 1.50; 95% confidence interval: 1.04-2.16; p=0.03). Other independent risk factors for ACM were: older age, male gender, previous cardiovascular event, lower eGFR and higher high sensitivity C-reactive protein. *Conclusions.* Elevated cFLC levels predict reduced survival in people with stage 3 CKD, independently of established risk factors and other markers of inflammation. Further work is now required to assess whether the prospective measurement of cFLC in CKD can improve patient risk stratification.

J153 - ELEVATED COMBINED SERUM FREE LIGHT CHAINS ARE ASSOCIATED WITH ACTIVE DISEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background. There is a paucity of biomarkers for the hyper-stimulated B cell conditions characteristically found in patients with systemic lupus erythematosus (SLE) and those available do not always reflect disease activity. Polyclonal combined serum free light chains (cFLC), markers of immune status and renal function) are elevated in SLE. *Objectives.* This study compared cFLC levels with both conventional SLE disease activity biomarkers and the British Isles Lupus Assessment Group Index (BILAG) for disease activity in a prospectively collected SLE cohort. *Methods.* Sera from 62 SLE patients (revised ACR classification criteria positive) were assayed for cFLC (Combylite®), IgG, IgA, IgM and cystatin C and the results compared to anti-dsDNA antibodies, C3, lymphocyte count, and erythrocyte sedimentation rate (ESR). Serologically active disease was defined as patients with elevated anti-dsDNA antibody levels (>50 IU/ml) and reduced C3 (<0.9 g/L). Clinically active disease was defined as a BILAG score of A or B score in any organ system. Statistical analysis was performed using SPSS v21. *Results.* The median age was 43 years (range: 21-86); 90% were female and 50% were Caucasian. The median cFLC concentration in all SLE patients was 31.86 mg/L (IQR: 22.27-58.17; median concentration in a healthy population: 20 mg/L). cFLC was elevated in serologically active disease (N=19, 32%, median cFLC active=58.1 mg/L (34.67-80.9) *vs* inactive disease=28.9 mg/L (20.56-45.09), p=0.02) and clinically active disease (N=26, 42%) (median cFLC active=39.6 mg/L (IQR: 25.3-80.9) *vs* inactive=29.7 mg/L (IQR: 19.3-49.3), p=0.05). Anti-dsDNA antibody levels were also elevated: 258 IU/ml (16-644.5) *vs* 45 IU/ml (10-181), p=0.04). No other markers were significantly different in active disease. *Conclusions.* Elevated cFLC levels correlated with disease activity, supporting the hypothesis that cFLC reflect B cell activity and represent attractive markers in SLE. However further work is required to determine how cFLC could be applied to monitor disease progression.

J154 - THE ASSOCIATION OF COMBINED SERUM FREE LIGHT CHAINS WITH MORTALITY IN THE SETTING OF EMERGENCY CARE

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Background. A large population based study (Dispenzieri, Mayo Clinic Proceedings, 2012) and subsequently two disease focused studies (Anandram, J.

Clin Pathol, 2012; Hutchison, Mayo Clinic Proceedings, 2014) have shown elevated polyclonal combined free light chains (cFLC) are associated with shorter overall survival. Here we report on the utility of this novel observation in patients attending a medical admissions unit (MAU). *Objectives.* To determine cFLC levels in patients attending MAU and to assess their association with all-cause mortality (ACM). *Methods.* 3063 patients were prospectively recruited between January and August 2013 (New Cross Hospital, Wolverhampton). Patients were followed-up for 12 months and cause of death was recorded. C-reactive protein (CRP), albumin and cFLC (Combylite®) were analysed using admission sera processed within the first 24 hours. The Vitalpac early warning Score (ViEWS) was calculated at presentation and patients were scored as: ≤5, 6-10 or >10. Renal function was determined using estimated glomerular filtration rate (eGFR). Statistical analysis, including multivariable logistic regression, was performed using STATA. *Results.* Median age of the population was 68yrs (IQR: 49-80); 53% were male. The median (IQR) or mean [SD] of the laboratory variables were: cFLC=33.5 mg/L (22.2-55.5), CRP=12 mg/L (4-50) and albumin=37.8 g/L [6.4]. N=853 (28%) patients had an eGFR<60 ml/min/1.73m^2 and N=200 (7%) patients had a ViEWS>5. There were weak to moderate correlations between cFLC and: CRP (r=0.38), age (r=0.40), and albumin (r=-0.46). cFLC concentrations were significantly elevated in patients who died (N=597, 20%) compared to the surviving population median: 52.8 mg/L (34.1-84.3) *vs* 30.2 mg/L (20.9-48.5) respectively, p<0.001. Following multivariable analysis, cFLC remained an independent predictor of mortality; age, CRP, albumin and ViEWS were also independently associated (Table 1). *Conclusions.* This is the first study to determine cFLC concentrations in MAU. cFLC was elevated and independently associated with ACM. Further work is required to assess the utility of cFLC measurements in this setting.

Table 1.

	Variable	*Odds ratio*	*95% CI*	*P value*
Multivariable	cFLC (per 10mg/L)	1.04	1.02-1.07	0.001
analysis	Age (Years)	1.05	1.04-1.05	<0.001
	(CRP/100)-0.5 (mg/L)	0.88	0.83-0.93	<0.001
	Albumin (g/L)	0.91	0.89-0.93	<0.001
	ViEWS≤5	1.00	-	-
	ViEWS >5 and ≤10	2.39	1.67-3.42	<0.001
	ViEWS>10	5.33	1.18-24.03	0.03
	eGFR>60 ml/min/1.73m^2	1.00	-	-
	eGFR 30-59 ml/min.1.73m^2	1.09	0.84-1.41	0.52
	eGFR 15-29 ml/min/1.73m^2	1.57	1.00-2.45	0.05
	eGFR<15 ml/min/1.73m^2	0.68	0.35-1.30	0.24

J155 - FREE LIGHT CHAIN AND OTHERS IMMUNOLOGICAL PARAMETERS CHANGES UNDER IVIg TREATMENT IN INFLAMMATORY NEUROMUSCULAR DISORDERS

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Background. Predictive biomarker for IVIG efficacy in neuromuscular diseases is lacking. It has been asserted that kinetic of IgG levels correlated with the treatment response in GBS and CIDP and that efficacy depends of inflammatory status before treatment. Polyclonal free light chains (FLCs) represent relevant candidates, never been tested in this context, allowing both the evaluation of endogenous IgG synthesis and B cell inflammatory status. *Objectives.* We will thus perform a preliminary study measuring polyclonal FLCs before and after IVIg infusion and correlate the values with others biological markers. *Methods.* We analyzed 20 patients suffering from inflammatory neuropathies, myasthenia gravis and myositis. Serum concentrations of polyclonal FLCs were measured before and after IVIg (Day 2) using the Freelite™ assay (Binding Site) on the BN ProSpec system (Siemens). We measured others parameters in parallel: protidemia, haemoglobin, haptoglobin, reticulocytes, hematocrit, high-sensitivity c-reactive protein, MDRD equation for renal function evaluation. *Results.* We observed an expected increase of IgG levels (delta 16.1 Gr/L; range 9.7, 24.3 Gr/L) but without any correlation with the injected dose. IgA and IgM levels significantly decreased (p <0,001 for both markers) probably explained by hemodilution induced by the increase of protidemia. We observed a moderate increase of FLCs levels in 18 patients (median: 4 mg/L; range -6, +23 mg/L). This increase is not explained by renal dysfunction or exogenous supply but more probably by B cell proliferation and/or inflammation because we observed an increase of CRP level in 80% of patient. *Conclusions.* IVIg did not reduce endogenous IgG production but may induce B cell activation. This preliminary observation thus justifies a larger study of FLCs measurement in patient treated with IVIg with a 1 month follow-up up and additional B cell analysis.

J156 - HIGH LEVELS OF POLYCLONAL FREE LIGHT CHAINS ARE ASSOCIATED WITH ASYMPTOMATIC CORONARY CALCIFICATION AND CORONARY EVENTS IN FEMALES

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Background. We analyzed polyclonal free light-chain (FLC) elevation in the prediction of coronary events utilizing 10-year follow-up data of an ongoing epidemiologic study in Germany. *Methods.* The Heinz Nixdorf Recall study comprises 4,814 men and women from 3 large adjacent cities in Germany. Subjects were randomly selected from statutory lists of residence. Myocardial infarction based on symptoms, electrocardiographic signs, cardiac enzymes, and necropsy during follow-up was considered a coronary event. Coronary artery calcification (CAC) was measured by nonenhanced EBCT scans (GE Imatron, South San Francisco, California) and quantified by the Agatston score. FLC concentrations were measured by a nephelometric immunoassay (FreeLite, The Binding Site, UK). Study participants with known coronary heart disease, a pathologic FLC κ:λ ratio, or documented lymphoproliferative disease were excluded. Summated FLC (tFLC) were used as a measure of polyclonal FLC elevation and categorized into quintiles. Crude and adjusted hazard ratios (HR) and corresponding 95% confidence intervals (95%-CI) were calculated stratified by sex. The fully-adjusted models included age, Framingham Risk Score variables, estimated glomerular filtration rate (MDRD formula), high-sensitivity C-reactive protein (hsCRP) and CAC. *Results.* Complete data was available for 3,772 study participants (52.6% female, mean age 59.2±7.7 years). During a median follow-up time of 9.3 years 142 coronary events occurred (101 in males, 41 in females). The proportion of study participants with CAC scores ≥400 increased with increasing tFLC quintile especially in females (lowest quintile 2.3%, upper quintile 7.8%, p-trend=0.0002). High tFLC were associated with known cardiovascular risk factors. In the fully-adjusted multivariable Cox regression models tFLC upper quintile *vs.* lower quintiles remained independently associated with coronary events in females (HR 2.45, 95%-CI 1.27-4.69). *Conclusions.* High levels of polyclonal FLC are associated with several known cardiovascular risk factors including asymptomatic coronary calcification and independently predict coronary events in females.

J157 - POLYCLONAL IMMUNOGLOBULIN FREE LIGHT CHAINS IN PATIENTS WITH MYOTONIC DYSTROPHY WITH HIGH CATABOLIC RATE OF IgG AND RESPIRATORY DYSFUNCTION

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Background. An accelerated breakdown of IgG in myotonic dystrophy (Dystrophia Myotonica, DM) has been known since 1966. Intracellular trafficking dynamics, protein kinase families and FcRn-mediated recycling of IgG are involved in this process, but the entire mechanism still remains unclear. We hypothesize that serum concentrations of polyclonal free light chains (sFLCs) in DM patients could possibly reflect immunoglobulin production rate better than immunoglobulin itself. *Methods.* All patients (40 in total) that were genetically identified as myotonic dystrophy type 1 (DM1, 24 patients) or myotonic dystrophy type 2 (DM2, 16 patients) had reduced aeration. Serum FLC concentrations were measured using the Freelite® assay (The Binding Site) performed on a Siemens BNII nephelometer. Other laboratory tests were done to assess their inflammatory state, kidney function, metabolic syndrome, and oxygen levels. *Results.* None of the patients had signs of renal insufficiency (normal eGFR and microalbuminuria). Increased polyclonal κFLCs were found in 12 patients (30%). The concentration of κFLCs in all 40 patients was higher than median of the reference interval ($p<0.0001$). An increased value of λFLCs was found in one patient. The mean value of the κ/λ ratio was 1.10 (all results of the ratio were above the median of the reference value). Concentrations of IgG were below the reference value (7 g/L) in 18 patients (45 %). In 36 patients (90 %), the IgG concentration was below the medium reference value (10 g/L). *Conclusions.* As expected, IgG catabolism is enhanced in DM patients, but the production of FLCs seems normal. Surprisingly, we found a slower clearance of κFLCs than that of λFLCs. To our knowledge, similar results were found only in patients with Sjögren's syndrome. The underlying causes may be different but one of the possible explanations includes a water countercurrent due to enhanced carbonic anhydrase activity in reduced oxygen levels.

J158 - POLYCLONAL FREE LIGHT CHAINS: A POTENTIAL BIOMARKER FOR IMMUNE ACTIVATION AND PREDICTOR OF MORTALITY IN ALPHA-1-ANTITRYPSIN DEFICIENCY RELATED AND USUAL CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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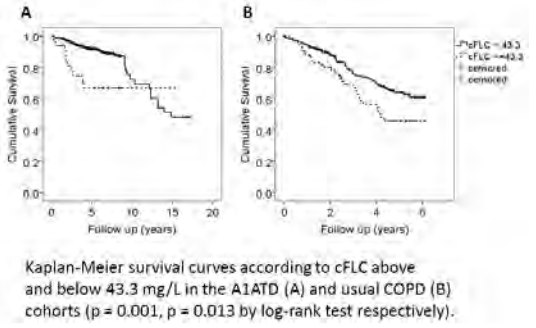
Background. Immunoglobulin free light chains (FLCs) are produced by B cells as a by-product of antibody synthesis. A polyclonal increase in FLCs has been observed in a number of chronic inflammatory and autoimmune conditions and FLCs have recently been implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). *Objectives.* To investigate the utility of serum & plasma FLCs as a marker of disease severity and predictor of mortality in patients with disease COPD. *Methods.* We measured FLC levels in 547 patients with severe α-1-antitrypsin deficiency (A1ATD) and 327 patients with 'usual' (non A1ATD related) COPD using the Freelite® assay. 18 patients with an abnormal κ/λ ratio were then excluded from the analysis. We compared combined (κ & λ) FLC levels (cFLC) in different subgroups and performed a cross sectional analysis to look for correlations with lung function parameters. *Results.* Partial correlations controlling for age and renal function revealed no strong correlations with

lung function parameters in either cohort. However, in both cohorts, patients who had subsequently died (A1ATD: n=69, usual COPD: n=91) had significantly higher cFLC levels compared to those still alive (A1ATD: median (IQR) cFLC dead 29.2mg/L (22.7-39.9) *versus* alive 25.2mg/L (21.0-31.0), p=0.001; usual COPD: median cFLC dead 36.4mg/L (26.0-51.5) *versus* alive 31.5mg/L(23.3-41.3), p=0.014; median in a healthy population is 22.1mg/L (17.7-25.7). Multivariable cox regression analyses revealed that a cFLC above the upper limit of normal (43.3mg/L) was associated with an increased risk of death in both groups: A1ATD (hazard ratio (HR) 2.89, p=0.002; usual COPD HR 1.80, p=0.009). Significant differences in survival curves were also seen (Figure 1). *Conclusions.* In patients with severe A1ATD and usual COPD, raised polyclonal cFLC are associated with an increased risk of mortality. Further evaluation is needed to establish if cFLC could serve as a useful clinical biomarker for phenotyping or risk-stratification in COPD.



Kaplan-Meier survival curves according to cFLC above and below 43.3 mg/L in the A1ATD (A) and usual COPD (B) cohorts (p = 0.001, p = 0.013 by log-rank test respectively).

Figure 1.

J159 - COMBINED FREE LIGHT CHAINS ARE NOVEL PREDICTORS OF PROGNOSIS IN HEART FAILURE

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Background. Inflammatory pathways are recognized in the pathogenesis and progression of heart failure (HF). Free light chain (FLC) elevation is conventionally associated with monoclonal gammopathies, including multiple myeloma. Polyclonal increases in both kappa and lambda FLC occur in autoimmune and other chronic inflammatory conditions. There is therefore interest in whether FLC elevation might be clinically useful in risk stratification of HF patients. *Objectives.* To investigate the prevalence and potential incremental prognostic value of cFLC in patients recently hospitalized with decompensated HF. *Methods.* 628 patients recently hospitalised with decompensated HF were studied. Combined FLC (cFLC) were measured by turbidimetry using an immunoassay (Combylite). The incremental prognostic value of cFLC for mortality was evaluated using Cox proportional hazard models including 22 established predictors of outcome in HF. *Results.* Of 628 patients, 290 (46%) died during a mean (SD) follow-up of 3.2 (1.5) years. 270 patients (43%) had elevated cFLC (>45.7mg/L). There was a clear gradient in the risk of death according to cFLC quartile, with those in the top quartile having an unadjusted risk of mortality more than twice that of those in the lowest quartile (HR 2.38, p<0.0001). cFLC was also associated with mortality after additional stratification for levels of natriuretic peptide (Figure 1). Following multivariable analysis, cFLC remained an independent predictor of mortality, with an almost 50% higher adjusted risk for those in the top compared with bottom quartile. Older age, lower body mass index, NYHA III/IV, previous myocardial infarction, current smoking and BNP, bilirubin, hsCRP, glycated hemoglobin and lymphocyte concentrations were also independent predictors of mortality. *Conclusions.* cFLC are an independent predictor of mortality in patients recently hospitalised with decompensated HF. Further work is required to assess the effects of HF therapies on cFLC concentrations and whether or not directly targeting this marker of inflammation improves prognosis for patients with HF.

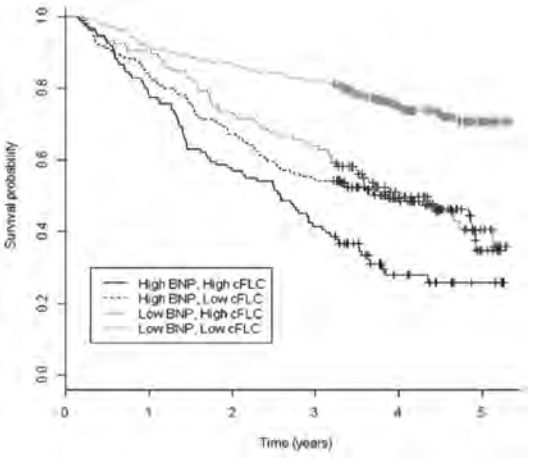

Figure 1.

J160 - THE IMPACT OF EXERCISE ON THE VARIATION OF SERUM FREE LIGHT CHAINS

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Background. Serum free light chain (sFLC) measurements play a key role in the diagnosis and monitoring of monoclonal gammopathies. More recently it was shown that polyclonal sFLC can act as a global marker of immune stimulation and can be linked to progression of immune disease, renal failure and overall survival. sFLC as such is regarded a biomarker of frailty. Since strenuous exercise is associated with immune stimulation, we hypothesized that exercise may affect sFLC concentrations. *Objectives.* Determine the effect of endurance exercise on sFLC concentrations. *Methods.* Serum κFLC, λFLC and the sum (Σ) FLC concentrations were longitudinally monitored in two exercise cohorts. Cohort A: 34 elderly volunteers who completed the Nijmegen Four-Days-Marches and walked 30 km at four consecutive days at a self-selected pace. Cohort B: 71 athletes who completed the Eindhoven marathon at a self-selected pace. *Results.* Volunteers in cohort A were 81±2 years old and walked 7h29min±1h04min per day at a speed of 4.2±0.7 km/h. At baseline, serum concentration κFLC was 19.2±9.0 mg/L, λFLC was 18.0±6.3 mg/L and ΣFLC was 37.2±14.0. Prolonged low intensity walking exercise did not result in significant sFLC changes (baseline ΣFLC 37.2 mg/L *versus* 38.8 mg/L after four days, p=0.26). A substantial ΣFLC increase of more than 20% was observed in five participants, which coincided with a doubled CRP concentration in all five participants. Volunteers in cohort B were 45±8 years old and ran at a speed of 11.5±1.4 km/h. At baseline, serum concentration κFLC was 13.6±7.5 mg/L, λFLC was 12.2±3.0 mg/L and ΣFLC was 25.8±9.6. Running a marathon did not result in significant sFLC changes (baseline ΣFLC 25.8 mg/L *versus* 26.0 mg/L after finishing the marathon, p=0.57). *Conclusions.* Both types of endurance exercise, walking and running, do not significantly affect sFLC concentrations. This finding emphasizes the stability of polyclonal sFLC as a biomarker.

J161 - SERUM FREE LIGHT CHAIN ANALYSIS AND THEIR ASSOCIATION WITH DISEASE ACTIVITY IN PRIMARY SJÖGREN'S SYNDROME

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Background. K and λ free light chains (FLC) are elevated in the serum of primary Sjögrens syndrome (pSS) patients, in comparison to healthy controls (HC). Consequently, FLC may represent potential biomarkers for disease activity in pSS. *Objectives.* To investigate the relationship between serum FLC and disease activity in the UK primary Sjögren's syndrome registry (UKPSSR), a cohort of clinically well-characterised pSS patients and HC. *Methods.* Sera from pSS patients (N=599) and HC (N=287) were analysed using Freelite® (κ and λ), BAFF, β2M, and Combylite®, a new assay for the determination of κ and λ FLC in a single test. Clinical data, including ESSDAI, ESSPRI and immunological markers, were retrieved from the UKPSSR database. Comparison of baseline pSS and HC FLC were analysed by ANOVA. Pearson correlations were calculated for patient characteristics and serum levels. Logistic regression analyses were performed to determine independent predictors of ESSDAI. *Results.* The Combylite measurement was comparable with the summated Freelite κ and λ assays: r=0.98 (p<0.001). K and λ FLC and cFLC, were significantly higher in pSS patients *vs* HC. In total, N=339 patients (57%) had abnormal FLC (high κ, high λ or abnormal κ:λ ratio). FLC levels correlated significantly with β2M, IgG and average Schirmer's but not with BAFF, age or disease duration. K FLC levels had higher correlations with clinical data than λ FLC in the majority of cases; cFLC falling between the two in most cases. Multivariate logistic regression indicated λ FLC was not associated with disease activity (p=0.312); however BAFF, β2M, κ FLC and cFLC were predictors of disease activity (all p<0.001). *Conclusions.* Serum FLC levels are increased in pSS patients in comparison to HC and correlate with several clinical factors. BAFF, β2M and FLC levels (κ or cFLC) were predictors of disease activity as determined by ESSDAI.

J162 - FREE LIGHT CHAINS AS POTENTIAL MARKERS OF INTESTINAL MUCOSA RECOVERY IN PATIENTS WITH CELIAC DISEASE

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Introduction. Celiac disease (CD) is a chronic immune-mediated small intestinal enteropathy, triggered by exposure to dietary gluten in genetically predisposed people. Previous reports shows increased serum free light chain levels (sFLC) in patients with auto-immune diseases including CD, which typical of conditions associated with increased B-cell activation. The existence of new reliable biomarkers could decrease the need for intestinal biopsy confirmation and improve response assessment after initiation of the gluten-free diet (GFD). Our goal is to assess the utility of sFLC levels as marker of intestinal mucosa recovery. *Materials and Methods.* Cohort of nineteen patients diagnosed with CD with serum samples obtained before GFD and six months after its initiation and 52 patients without CD(control). Serum biomarkers: antibodies IgA anti-transglutaminase and anti-endomysial (Menarini diagnostics) and FLC (Freelite®). *Results.* The median summated FLC levels (∑κ+λ) observed after six

months of GFD decreased significantly when compared to those observed at diagnosis (33.60 mg/L *vs* 19.34 mg/L, p=0.0012) (Figure 1). The average decrease is 1.7-fold, with only two patients (10%) showing no decrease. In fact, after GFD initiation, the median summated FLC values of these patients closely match does determined for the non-CD control group, with no statistical difference being observed (19.34 mg/L *vs* 17.96 mg/L, p=0.24). *Conclusions.* The statistical difference between the studied groups shows that summated FLC serum levels are a good indicator of disease response, probably reflecting the normalization of the intestinal mucosa. The only two patients that did not show a decrease in the FLC levels present initially with less altered values of the traditional biomarkers and less elevated FLC levels. Thus, they probably represent cases of early diagnosis with less involvement of intestinal mucosa. Since CD is typical of pediatric population, having a set of biomarkers that can reliably reflect recovery and avoid intestinal biopsy is of great importance.

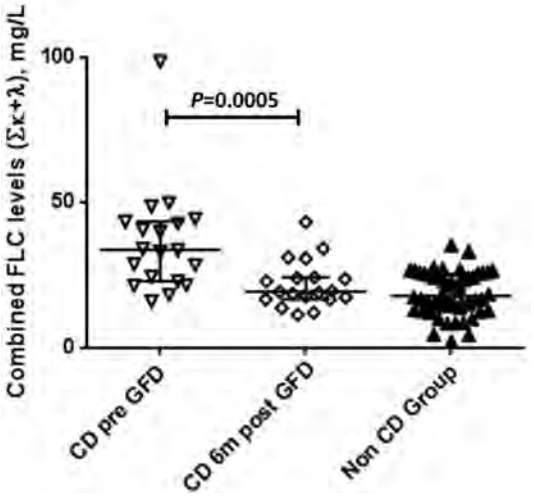

Figure 1. Summated serum free light chain values for 19 celiac disease (CD) patients before and after 6 months gluten-free diet (GFD) and for 52 patients without CD. The horizontal lines represent median values and interquartile ranges. Mann-Whitney statistical test.

J163 - UTILITY OF FREE LIGHT CHAIN DETERMINATIONS FOR MONITORING LUPIC PATIENTS: A CASE STUDY

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Systemic lupus erythematosus (SLE) is a multi-systemic disease in which antibody-mediated cellular and tissue damage occurs. A polyclonal hyperactivity is produced with antigen specific T- and B-cell response. The disease usually presents a chronic course in which flares alternate with periods of inactivity. The main problem in patient management is to diagnose and predict flares. Several publications using free light chains (FLC) assays suggest its use as a marker of SLE activity. We report a case in which FLC were very useful in monitoring an SLE patient. *Patients.* A 34-year old woman diagnosed with SLE in 2000 by arthritis, arthralgia, leukolinphopenia and anemia beginning treatment with steroids and azathioprine. Since 2003, due to renal involvement during flares, the treatment was cyclophosphamide alternating with mycophenolate. New flares occurred in 2005, 2010 and 2011, with renal impairment, proteinuria, leukolinphopenia. Monitoring was done with the usual markers (VGS, PCR, anti-dsDNA and C3, and C4 complement) plus FLC, since 2010. *Results* .During follow-up, no significant changes were observed in the concentrations of PCR levels, or VGS levels; C3 and C4 complement are always below normal levels (c3: 84-175mg/dL; C4: 10-40mg/dL) but a further decrease is observed during the flares (Figure1) as well as positive anti-dsDNA antibodies. Regarding the sum of FLC(κ+λ), a marked increase was observed during the flares when compared to the average levels outside the flare: 86.5mg/L and *Conclusions.* The FLC summated levels accurately reflected changes on the patient clinical state. Furthermore, upon flare ending, FLC levels seem to normalize more quickly than C3 and C4, possibly due to the short half-life of the FLC molecules. Further studies are needed to confirm these observations. However, the ease of use and the quick response time, make it a potentially interesting marker to include in panel of SLE study techniques.

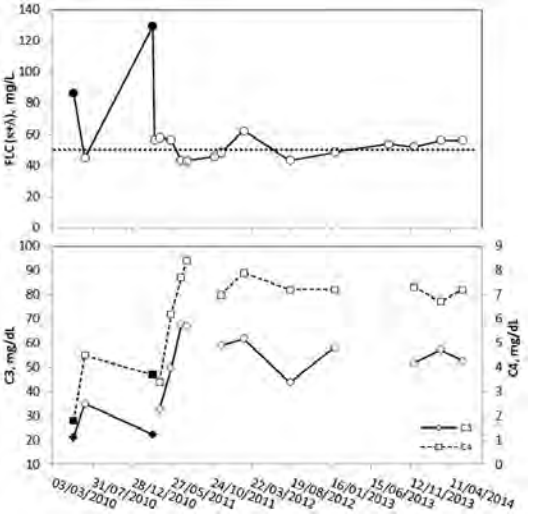

Figure 1. Patient monitoring with FLC (upper panel) and C3 and C4 complement (lower panel). Full symbols represent data obtained during a flare and the horizontal dotted line represents average FLC levels outside the flare (51.48mg/L).

J164 - ELEVATED SERUM FREE LIGHT CHAINS: A NOVEL MARKER OF INSULIN RESISTANCE?

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Background. Polyclonal immunoglobulin free light chains (cFLC, combined FLCk+FLCλ) are frequently elevated in inflammatory and autoimmune conditions and are associated with increased all-cause mortality and cardiovascular disease but their relevance in individuals with type 2 diabetes (T2D) is poorly understood. We examined the association between cFLC and other markers of inflammation and insulin resistance in a cohort of young individuals with T2D. *Objectives.* To determine whether serum cFLC is a marker of insulin resistance in young adults with T2D. *Methods.* We recruited a multi-ethnic cohort of 100 subjects with early onset T2D (<40 years). Detailed characteristics including anthropometry, co-morbidities and treatment were collected. Serum cFLC along with other biochemistry (HbA1c, Insulin, C-peptide, CRP) were measured in 88 of the 100 subjects. Variables were compared between subsets using Mann-Whitney U-test and correlation between the cFLC and the other variables was assessed using the Spearman's test. *Results.* Median cFLC were elevated in 13 of 88 patients with T2D. In these 13 patients there was a strong, positive correlation between cFLC and HOMA-IR, HOMA-β%, visceral fat rating and fasting insulin (Table 1). South Asian ethnicity (p=0.003), female gender (p=0.050), age diagnosis>25 years (p=0.003), acanthosis (p=0.018) and low SHBG (p=0.018) were significantly associated with raised cFLC, as were neuropathy (p=0.025) and cerebrovascular disease (p=0.029). On the other hand, there was no association between diabetic nephropathy (micro and macroalbuminuria) and high cFLC. *Conclusions.* cFLC concentrations were elevated in young adults with T2D compared with healthy subjects, especially in the South Asian ethnic group and women. There was significant association between raised cFLC and clinical/biochemical markers of insulin resistance. The role of cFLC as a novel marker of insulin resistance needs further investigation.

Table 1.

	cFLC (mg/L)	*HOMA-IR*	*HOMA-β%*	*Visceral Fat Rating*	*Fasting Insulin pmol/L*
Spearman's rho cFLC(mg/L)					
Rho	1.000	.687*	.581**	.644**	.621**
Sig. 2-tailed	.	.010	.037	.044	.024

*Correlation is significant at the 0.01 level (2-tailed); **Correlation is significant at the 0.05 level (2-tailed).

J165 - ELEVATED IMMUNOGLOBULIN SERUM FREE LIGHT CHAINS IN HEART FAILURE

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Background. Biomarkers reflecting the underlying pathology of heart failure (HF) are becoming increasingly important for patient assessment. Two important HF processes are inflammation and renal impairment, both of which can increase serum free light chain (FLC) concentrations. Elevated polyclonal FLC levels have been associated with increased overall mortality in a general population, and with poor cardiovascular outcomes in type 2 diabetes. *Objectives.* To determine FLC levels in patients with HF and compare to other clinical biomarkers. *Methods*. FLC κ and FLC λ (Freelite®, The Binding Site Group Ltd, UK) were assayed in stored serum samples from 66 Japanese patients with HF comprising 30 ischemic heart failure (IHF) and 36 dilated cardiomyopathy (DCM) subjects. FLC κ/λ ratio and summated FLC (ΣFLC=FLC κ+FLC λ) were calculated and compared to NTproBNP and high sensitivity CRP (hsCRP) measured at the time of sample collection. ΣFLC and FLC ratio were compared to 132 healthy Caucasian controls. *Results.* ΣFLC were elevated in HF patients compared with healthy controls; median (interquartile range) controls 22.1mg/L (17.7-25.7), HF 46.5mg/L (35.8-78.3, p<0.001, Figure 1) and were similar between disease groups; IHF 44.6mg/L (36.8-64.3), DCM 50.0mg/L (31.1-94.5), p=0.48. Median FLC ratio was significantly elevated in HF patients 1.12(0.94-1.26, p<0.001) compared to controls 0.94(0.85-1.05) but remained within the normal range (0.26-1.65). The FLC ratio was greater in IHF 1.19(1.00-1.44) than DCM 1.03(0.88-1.19), p=0.003. ΣFLC showed moderate correlations with hsCRP (Spearman r=0.51) and NTproBNP (r=0.44) in all HF subjects. However, the correlations were stronger in the DCM subgroup (hsCRP r=0.61, NTproBNP r=0.58) than in IHF subjects (hsCRP r=0.27, NTproBNP r=0.16). *Conclusions.* The ΣFLC concentration and ratio are elevated in HF. The moderate correlation of FLC with NTproBNP or hsCRP in DCM indicates FLC may provide additional information. The clinical significance of these observations requires further investigation.

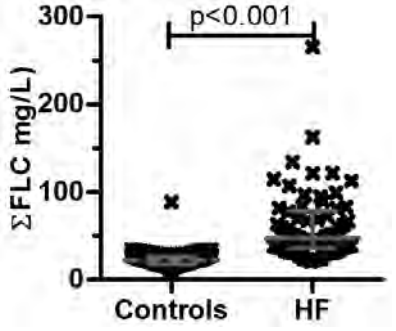

Figure 1.

J166 - ELEVATED POLYCLONAL FREE LIGHT CHAINS ARE ASSOCIATED WITH POOR PROGNOSIS IN CHRONIC KIDNEY DISEASE

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Background. Chronic kidney disease (CKD) is a progressive disease associated with poor outcomes and reduced survival. Elevated serum polyclonal combined immunoglobulin free light chains (cFLC), a marker of both immune status and kidney function, have previously been associated with reduced survival. *Objectives.* Here we evaluated a role for cFLC in predicting mortality and progression to end stage renal disease (ESRD) in individuals with stage 3-5 CKD. *Methods.* A cohort of 872 non-dialysis CKD patients recruited into the Chronic Renal Insufficiency Standards Implementation Study (CRISIS) were studied (median age 66 years; median estimated glomerular filtration rate (eGFR) 30 ml/min/1.73m^2; 32% had diabetes as a co-morbidity; 42% had a history of cardiovascular disease). cFLC were measured (κ+λFLC, Freelite®) and compared to other clinical parameters using Cox Regression analysis. Patients were followed-up for a median of 1259 days (interquartile range 862-2069 days). During follow-up, 202 patients progressed to ESRD (defined as dialysis, transplant or eGFR <9 ml/min/1.73m^2) and 287 patients died. *Results.* At recruitment cFLC levels above median (>68.9 mg/L) were independently associated with (i) all-cause mortality (ACM) (hazard ratio (HR)=1.93; 95% confidence interval (CI) 1.30-2.87, p=0.001) together with a history of cardiovascular disease and eGFR<30 ml/min/1.73m^2, and (ii) progression to ESRD (HR=3.73, 95%CI 2.02-6.88, p<0.001) alongside eGFR<30 ml/min/1.73m^2, urinary albumin-creatinine ratio>30 mg/mmol and serum phosphate>1.5 mmol/L. Using these variables, risk models were developed for ACM and ESRD. Compared to the reference group (0 risk factors (RF)), an increasing number of RF was positively associated with risk of ACM (1RF HR=2.71, 2RF HR=5.22, 3RF HR=7.92, all p<0.001) or progression to ESRD (1RF HR=4.28, 2RF HR=9.30, 3RF HR=18.76, 4RF HR=36.62, all p<0.001). *Conclusions.* An elevated serum cFLC concentration is an independent risk factor for ACM and progression to ESRD in patients with stages 3-5 CKD. Combining cFLC with other routinely measured biomarkers may support tailored patient management and thereby improve patient outcomes.

J167 - SERUM FREE LIGHT CHAINS: A POTENTIAL BIOMARKER IN PRE-ECLAMPSIA

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Background. Pre-eclampsia (PET) is a major cause of fetal & maternal morbidity and mortality worldwide. Early recognition of the disease could allow the opportunity for timely intervention and management to help minimise risk to mother and child. Thus a biomarker which could accurately predict women destined to develop PET or that helps confirm the diagnosis could substantially aid patient management. Serum free light chains (sFLC) are associated with systemic inflammation, reticulo-endothelial dysfunction and renal impairment which are all features of PET. *Objectives.* We tested for the first time whether sFLC could act as a suitable biomarker in PET. *Methods.* Following ethical approval and patient consent, serum samples were collected from pregnant women without known chronic illness admitted to hospital with PET (BP >140/90 and urine PCR>30mg/mmol) & healthy pregnant controls without PET. Samples were anonymised, stored at -20°C and subsequently analysed for sFLC using the Freelite® assay. Statistical analysis was performed using PRISM and multivariable analysis using SPSS. *Results.* Samples from 202 women (94 PET & 108 healthy controls) were analysed. Free κ and κ/λ ratio (both p<0.0001), but not λ light chain levels, were significantly higher in those with PET *versus* controls (Table 1). The association of κ and κ/λ ratio with PET remained significant after adjusting for renal function by linear regression. *Conclusions.* We identify for the first time elevated sFLC in women with PET which is independent of maternal renal function. Ongoing studies will identify whether sFLC predict those women destined to develop PET before the onset of clinical disease.

Table 1. sFLC levels in the PET and control group

Median values (with range)	*PET group (n=94)*	*Control group (n=108)*	*P value*
κ mg/l	15.39 (3.92-77.54)	11.30 (4.5-25.95)	<0.0001
λ mg/l	13.57 (5.6-37.5)	12.80 (6.57-29.25)	ns
κ/λ ratio	1.130 (0.49–3.83)	0.90 (0.49-1.89)	<0.0001

J168 - RISK FACTORS ASSOCIATED WITH ELEVATED PLASMA FREE LIGHT CHAIN LEVELS

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Background. Elevated serum polyclonal light chains (LC) are associated with increased risk of cardiovascular events in T2DM. *Objectives.* To explore factors associated with elevated plasma κ (κ), λ (λ) LC, and total free LC (κ+λ). *Methods.* Cross sectional study recruited 596 patients (age: 46.23±12.65, female: 52.4%; African Americans: 49.3%). Plasma free LC (pFLC) concentrations were measured on SPA$_{PLUS}$ protein analyzer (The Binding Site, Inc., and San Diego, CA) using Binding Site Freelite® immunoassays. Higher levels of pFLCs were defined based on clinical normal ranges, *i.e.* pκ>19.4, pλ>26.3 and pFLC>45.7 (mg/L). Multiple logistic regression models were used to examine risk factors associated with elevated pFLCs. *Results.* eGFR

[aOR=0.96; 95% CI; 0.96-0.99] but not albuminuria was associated with higher pκ Subjects without hypertension [aOR=0.39; 95% CI; 0.20-0.76]; who were Caucasian [aOR=0.31; 95% CI; 0.19-0.49], or had history of use of Angiotensin-converting enzyme inhibitor (Ace) and/or Angiotensin II receptor blocker (ARB) class of drugs [aOR=0.38; 95% CI; 0.18-0.84] were more likely to have lower pκ after adjusting for age, sex, waist hip ratio, T2DM, eGFR and albuminuria. In contrast, none of the above factors were associated with higher pλ except for albuminuria [aOR=1.013; 95% CI; 1.002-1.023]. For total pFLC [pκ+p λ], hypertension [aOR: 3.47; 95% CI; 1.45-8.32] was associated with higher levels; and use of Ace/ARB class of drugs was associated with lower levels [aOR: 0.28; 95% CI; 0.12-0.69] as compared with use of antihypertensive drugs other than Ace/ARB. *Conclusions.* eGFR was predictive of higher pκ level and history of hypertension was predictive of higher pκ or total pFLC levels. Use of Ace/ARB class of drugs is associated with lower levels of pκ and total pFLC levels. Longitudinal study is needed to assess if higher pFLC levels are predictive of progression of CKD.

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