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Serum free light chain and Hevylite analyses in the diagnosis, monitoring and prognosis of B cell disorders

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SUMMARY
The availability of automated serum free light chain (FLC) immunoassays has enabled routine and sensitive laboratory quantification of this important tumour marker. Heavy chain-light chain (HLC) assays have been developed which measure serum intact immunoglobulins of each light chain type [1]. These novel tests may provide a sensitive and quantitative way of diagnosing and monitoring patients with monoclonal gammopathies. In this review we summarise the utility of serum FLC and HLC assays in assessing monoclonal gammopathies and examine their potential applications in diseases with raised polyclonal levels of antibodies and FLCs.
Key words: heavy/light chain (HLC), free light chain (FLC), monoclonal gammopathies.

SOUHRN
Legg A., Harding S., Hughes R. G., Levoguer A. M., Bradwell A. R.: Volné lehké řetězce imunoglobulinů v séru a Hevylite analýza v diagnostice, monitorování a prognóze monoklonálních gamapatií
Dostupnost automatizované metody na stanovení volných lehkých řetězců (FLC) umožnila rutinní, citlivé a kvantitativní stanovení tohoto důležitého nádorového markeru. Nové soupravy na současné stanovení těžkých a lehkých řetězců (HLC) umožňují kvantitativní stanovení intaktních imunoglobulinů v séru pro každý typ lehkých řetězců zvlášť. Tento test poskytuje novou, citlivou a kvantitativní alternativu při diagnostice a monitorování nemocných s monoklonální gamapatií. V tomto článku shrnujeme možnosti využití souprav na stanovení FLC a HLC při hodnocení monoklonálních gamapatií a také jejich potenciální aplikaci u chorob se zvýšenými hodnotami polyklonálních protilátek a polyklonálních FLC.
Klíčová slova: heavy/light chain (HLC), volné lehké řetězce (FLC), monoklonální gamapatie.

Introduction

In monoclonal gammopathies, the monoclonal proteins produced can be detected and quantified by a variety of electrophoretic and nephelometric/turbidimetric techniques. Monoclonal immunoglobulins are traditionally quantified using serum protein electrophoresis (SPE) however this can be of limited use when there are low levels of monoclonal protein or the M-spike co-migrates with other serum proteins. Immunofixation (IFE) resolves some of these issues, but is qualitative and so cannot be used to accurately monitor patient responses. Polyclonal antisera have been developed which recognise conformational, junctional epitopes between the heavy and light chains of the immunoglobulin molecule (Fig. 1). These assays can distinguish between different light chain types of each immunoglobulin class (eg. IgGκ versus IgGλ). By measuring these immunoglobulins as pairs and expressing the results as a ratio (IgGκ/ IgGλ), the HLC tests can be used to type and quantify monoclonal immunoglobulin production [1].

Monoclonal FLCs tend to be produced in low concentrations. They can be detected using either urine protein electrophoresis (UPE) with IFE or the FLC assay. However, UPE techniques can be negative when there are detectable levels of monoclonal FLCs in the serum. One possible explanation for this may be that the primary function of the kidneys is to prevent loss of proteins into the urine through reabsorption within the proximal tubules.

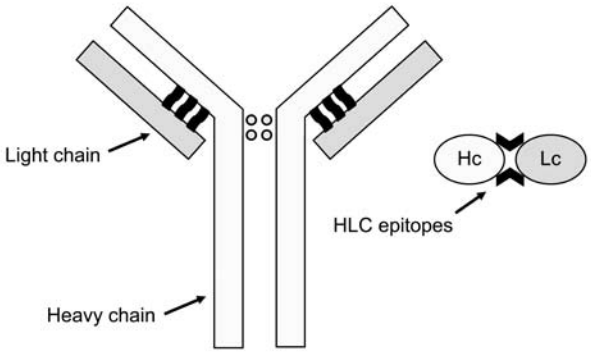

Fig. 1. Schematic representation and cross section of HLC targeted epitopes which span the junction between the heavy chain and light chain of the immunoglobulin molecule
Hc = heavy chain and Lc = light chain

The FLC and HLC assays identify the presence of monoclonal proteins through abnormal κ/λ ratios. In patients with monoclonal gammopathies, the κ/λ ratio is outside of a defined normal range [1, 2]. When abnormally high, this identifies monoclonal kappa immunoglobulins and conversely lambda paraproteins when abnormally low. For the FLC assay, the ability to cal-

culate a κ/λ ratio is known to give additional sensitivity because it takes into account immunoparesis. Patients with subtle underlying diseases, such as AL amyloidosis and light chain deposition disease (LCDD), can often have normal absolute levels of FLCs but still have an abnormal ratio due to suppression of the uninvolved, polyclonal FLC (eg. κ 16 mg/L, λ 1 mg/L, κ/λ ratio 16).

Diagnosis of monoclonal gammopathies

Studies have demonstrated the high diagnostic sensitivity of FLC assays in different diseases. At presentation, the FLC κ/λ ratio has been shown to identify up to 100% of patients with light chain only multiple myeloma (LCMM) [3]. Approximately 70% of NSMM display an abnormal FLC ratio and therefore are secreting monoclonal FLCs below the detection threshold of IFE techniques [4, 5]. It has been proposed that NSMM with abnormal FLC κ/λ ratios should be reclassified as 'cryptosecretory'. Finally, in AL amyloidosis and LCDD, FLC tests achieve higher overall sensitivity for monoclonal FLCs than urine IFE [6].

It is accepted that the FLC assay can replace urine electrophoresis in a myeloma screening panel. Numerous screening studies have analyzed the combination of SPE plus FLC assays or urine electrophoresis (reviewed in [7]). The serum only panel consistently achieved higher overall sensitivity with no additional malignancies detected through the inclusion of urine electrophoresis. In a study of 428 patients positive by urine IFE, a combination of SPE, serum IFE and FLC tests identified all except two patients; one was false positive and the remaining FLC-MGUS required no clinical intervention [8].

Rare lambda FLC AL amyloidosis patients have been reported with disparate urine IFE and FLC results [9]. The reduced sensitivity of the FLC assays for these patients may be explained by the presence of amyloid deposits causing renal impairment. The kidneys are frequently affected in AL amyloidosis and this could have several effects: the reduced glomerular filtration rate may lead to increased polyclonal levels of both FLCs, masking any immunoparesis and reducing the sensitivity of the ratio. Furthermore, the loss of the preferential filtration of kappa leads to an increased κ/λ ratio (discussed in next section), which would reduce the sensitivity of the κ/λ ratio for lambda monoclonal FLCs. Glomerular damage and saturation of the protein reabsorption by albumin and other serum proteins could also explain the increased sensitivity of urine IFE for monoclonal FLCs.

To clarify the optimal combination of assays in a primary screening panel, Katzmann et al. analysed 1877 patients with a variety of B cell disorders. All patients had been previously assessed by SPE, UPE, serum IFE, urine IFE and FLC tests [10]. In combination, SPE and FLC tests provided 100% sensitivity for large tumour burden diseases, such as multiple myeloma and Waldenström's macroglobulinemia. For AL amyloidosis, SPE and FLC tests showed 96% diagnostic sensitivity. This increased to 98% by including serum and urine IFE. Use of SPE and FLC assays did result in a reduced sensitivity for monoclonal gammopathy of undetermined significance (MGUS). The normal FLC κ/λ ratios and negative SPEs of these patients allowed the interpretation that these MGUS patients were amongst the lowest at risk for progression to malignancy [11]. The authors concluded that SPE and FLC tests provide a simple and efficient initial diagnostic screen, reducing the need for urine studies and serum IFE [10].

Consideration of the cumulative retrospective and prospective data resulted in International guidelines recommending the use of the FLC assay plus serum electrophoresis as the primary screening panel for monoclonal gammopathies. Furthermore, it was concluded that FLC tests can replace urine IFE in all circumstances except when AL amyloidosis is suspected. Here a urine IFE should also be carried out to ensure maximum diagnostic sensitivity [12].

Renal reference range for the FLC κ/λ ratio

In patients with renal impairment, borderline high κ/λ ratios have been observed. The normal range for the FLC assay was derived using 282 healthy donor sera [2]. Although a healthy bone marrow will typically have twice as many kappa than lambda producing plasma cells, the monomeric kappa FLCs are filtered faster by the kidneys than the larger, lambda dimers. This preferential removal results in a median FLC κ/λ ratio of 0.6, with a 100% range of 0.26–1.65 [2]. When renal function decreases, clearance of FLCs by the reticuloendothelial system becomes increasingly important. This mechanism demonstrates no preference for the molecular weight of the FLC and so the effect of preferential removal of kappa is gradually lost. Therefore, the κ/λ ratio increases progressively reflecting the underlying production rates, potentially leading to a raised, false positive κ/λ ratio [13].

It is recommended that FLC results are interpreted in the context of clinical findings and renal function. In patients with renal impairment, a 100% renal reference range for the κ/λ ratio (0.37–3.1) can be applied to remove false positive results. In an audit of patients with newly diagnosed, dialysis dependent acute renal failure, applying the renal reference range improved the diagnostic specificity from 93% to 99%, without compromising the 100% diagnostic sensitivity [14].

Monitoring of monoclonal gammopathies

AL amyloidosis and LCDD: AL amyloidosis patients tend to have a low tumour burden and often produce monoclonal proteins at levels only detectable by IFE. The serum FLC assay can provide a sensitive, quantitative measurement of the amyloidogenic light chain. Reduction of the amyloidogenic FLC is associated with disease response and regression of the amyloid deposits on serum amyloid P scans, leading to improved survival [6]. The assay is therefore recommended for monitoring AL amyloidosis in National and International guidelines [12, 15].

LCDD shares many characteristics with AL amyloidosis but due to the rarity of the disease, there is limited data to make formal guideline recommenda-

tions. In a single case report of a patient with biopsy proven LCDD, FLC tests provided a baseline value of κ 526 mg/L, λ 64.6 mg/L (κ/λ ratio 8.1) despite normal SPE and UPE results [16]. Following chemotherapy, an improvement in renal function was associated with normalisation of the FLC ratio, indicating elimination of the nephrotoxic FLC. This illustrates the utility of the serum FLC assay in monitoring this patient group [12].

Light chain only multiple myeloma: The concentrations of serum or urine monoclonal FLC are directly related to kidney function. This can depend on the reabsorption within the proximal tubules or the degree of renal impairment. LCMM patients can have significant levels of monoclonal serum FLCs but non-measureable disease in the urine. In patients with glomerular damage, the leakage of serum proteins into the urine can result in large quantities of urine FLCs but lower levels of monoclonal FLCs in the serum. The variability in renal function across a group of patients likely accounts for the poor correlation observed between serum and urine FLC measurements. Due to this poor correlation, current guidelines recommend FLC tests for monitoring oligosecretory myeloma, with UPE remaining the gold standard for patients with measureable disease [12]. Preliminary evidence indicates that UPE measurements, unlike FLC tests, are highly susceptible to analytical error. One report suggests up to 19% of samples may show aberrant increases in the urine M protein levels [17]. Further studies comparing UPE and FLCs tests are now warranted to further define their relative benefits for monitoring LCMM.

Intact immunoglobulin multiple myeloma (IIMM) and clonal change: During disease course, the relative production rates of monoclonal intact immunoglobulin and serum FLCs can change. Terminology used to describe this relapse includes Bence Jones or light chain escape (LCE). This form of clonal change is defined by rising monoclonal FLC production at relapse without an increase in the original monoclonal intact immunoglobulin. Two studies put the overall incidence of this relapse at 2.46 and 8% [18, 19]. Recent data suggests the occurrence is isotype specific, with LCE occurring in 5% of IgG and 15% of IgA IIMM [20].

LCE may be preceded by the growth of clones producing FLCs only in an IIMM patient. This hypothesis is supported by the presence of multiple tumour clones within the bone marrow; which have been identified using dual staining techniques [21]. Consistent with this dual clone theory, a recent analysis of the Myeloma VII trial observed intact immunoglobulin escape [18]. Renal impairment is a frequent complication of LCE due to production of nephrotoxic monoclonal FLCs [19]. To enable optimal detection of LCE, latest guidelines recommend using either urine electrophoresis or FLC tests alongside SPE during IIMM monitoring [12]. This is due to the insensitivity of SPE for monoclonal FLCs and the potential for aggressive disease course [21].

Myeloma kidney: Approximately 50% of myeloma patients have renal insufficiency at diagnosis with 10–20% presenting with acute renal failure. The main reason for renal failure is cast nephropathy (myeloma kidney), where the FLCs bind Tamm-Horsfall protein and form waxy casts. This process is reversible, although animal studies have shown that permanent damage occurs within one month of blockage. Currently, when treated with chemotherapy alone, 10–20% of patients will recover renal function sufficiently to become dialysis independent.

High cut-off haemodialysis can improve the rate of dialysis independence. In an analysis of 7 haemodialysis membranes, the Gambro HCO1100 membrane demonstrated highest efficiency at removing FLCs [22]. The pore sizes in standard high flux membranes are too small to allow efficient clearance of FLCs. However, the larger pores in the Gambro HCO1100 membrane enable proteins up to 50kDa to pass. This is sufficient to clear both kappa monomers and lambda FLC di-mers. Frequent HCO1100 haemodialysis sessions and successful chemotherapy were effective at reducing the nephrotoxic monoclonal FLC levels early in treatment [22]. In an analysis of 19 myeloma kidney patients, approximately 70% became dialysis independent at a median duration of 28 days [23]. To date, 66 patients have received HCO1100 extended haemodialysis treatment, with 41 (62%) achieving dialysis independence. Highest rates of renal recovery were seen in patients who achieved an early reduction in serum FLC concentrations [24]. This work has now been extended into a Phase II, international, randomised, controlled trial, termed EuLITE (European trial of free light chain removal by extended haemodialysis in cast nephropathy; overview in Figure 2). This study aims to determine if this HCO1100 dialysis treatment increases the rate of dialysis independence at 3 months compared to chemotherapy alone [25].

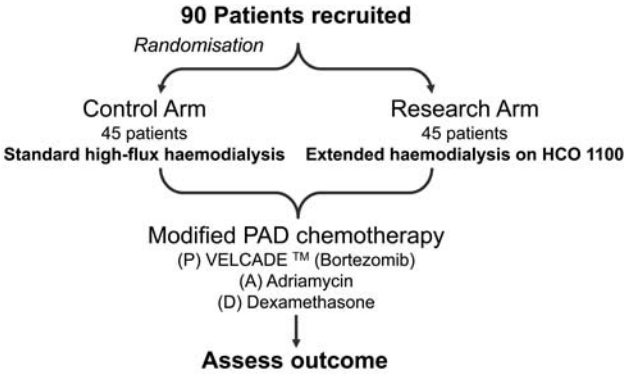

Fig. 2. Overview of the EuLITE trial
Patients are randomised into either the control arm (standard high flux haemodialysis) or the intervention arm (HCO1100 extended haemodialysis). All patients receive PAD chemotherapy and the primary aim is to determine the rate of dialysis independence at 3 months on both study arms.

Prognostic value of the serum FLC ratio

There is variation, from patient to patient, in the risk of MGUS progression to malignancy. An abnormal FLC ratio is a risk factor for progression and the relative risk

increases in relation to the extent of the abnormality [11]. This is independent of two other accepted prognostic markers, the size and type of the M-protein. A risk stratification model that uses these three risk factors has been shown to distinguish between high and low risk groups.

Analysis of two cohorts of myeloma patients with prediagnostic sera showed most patients had a preceding MGUS [26, 27]. In two thirds of patients, either the monoclonal intact immunoglobulin and/ or serum FLC result evolved prior to myeloma diagnosis. These 'evolving MGUS' situations may represent slowly growing myelomas from the beginning. In some LCMM or NSMM, an abnormal FLC ratio was the only abnormality prior to myeloma diagnosis. Therefore, FLC-MGUS may represent a clinically significant entity. Further work is required to determine if sequential FLC tests are warranted alongside serum electrophoresis during MGUS follow up.

The FLC ratio is also prognostic in other B cell disorders. An abnormal baseline κ/λ ratio identified subsets of solitary plasmacytoma of the bone and smouldering myeloma patients with increased risk of progression. An abnormal baseline ratio also determined AL amyloidosis and multiple myeloma patients with reduced survival prospects (reviewed in [7]). Due to its significant prognostic value in all these diseases, the 2009 International Myeloma Working Group guidelines recommend measuring FLCs at diagnosis [12].

Abnormal κ/λ ratios were also detected in chronic lymphocytic leukaemia (CLL) [28] and non-Hodgkin's lymphoma (NHL). Abnormal FLC ratios have been detected in 38% of patients up to 9.8 years prior to the development of CLL [29]. In addition, an abnormal ratio before treatment has been associated with significantly reduced survival and was independent of other known prognostic factors [28]. In patients with hepatitis C, abnormal baseline FLC ratios correlated with virological response to treatment and was consistently associated with the presence of mixed cryoglobulinemia vasculitis and NHL [30].

Polyclonal gammopathies: the future for the serum FLC test?

Polyclonal FLC concentrations can be increased due to renal impairment, autoimmune disease and the normal immune response to infection. Therefore, significantly raised absolute concentrations of kappa and lambda FLCs, with a normal κ/λ ratio can indicate a significant, underlying pathology.

The role of FLCs in chronic kidney disease (CKD) is of considerable interest. In patients with CKD, the reduced glomerular filtration rate results in fewer FLCs being cleared from the serum. The 95% normal ranges for the absolute levels of kappa and lambda are 3.3–19.4 mg/L and 5.7–26.3 mg/L respectively [2]. In patients with severe renal impairment, serum FLC concentrations can increase to 100mg/L or higher [14]. As the number of nephrons decrease, the FLC concentrations rise further, increasing the FLC load across the remaining, functional nephrons. This in itself may lead to further and progressive kidney damage. Monoclonal FLCs are known to induce pro-inflammatory signals within proximal tubular cells and raised polyclonal FLCs may induce similar damage. In type II diabetic patients, polyclonally raised serum and urinary FLCs can be detected prior to the onset of overt kidney disease [31]. The identification of increased polyclonal serum and/or urinary FLCs may identify patients at increased risk of progressive renal damage and provide a useful tool for the management of early diabetic kidney disease.

Polyclonally raised FLCs display significant prognostic value in the development of B cell malignancies. In HIV positive individuals, polyclonally elevated serum FLCs strongly predicted the development of NHL and the risk increased in relation to the FLC concentrations [32]. Similarly, 16% of CLL patients had polyclonally increased levels of FLCs years prior to the development of disease thus indicating a role of chronic immune stimulation in development of CLL [29]. The routine use of FLC measurements in these diseases has not yet been clinically validated and further research is required.

The heavy chain-light chain assay

Development of the HLC assays now enables κ/λ ratios to be calculated for intact immunoglobulins. Calculating a HLC ratio gives several benefits as it may compensate for changes in IgG half life, plasma volume and haematocrit [1]. In addition, a HLC ratio gives additional sensitivity as it measures isotype specific immunoparesis. This could be particularly important for the assessment of residual disease and early relapse in patients that are SPE/ serum IFE negative.

There is a particular need for alternative ways of quantifying monoclonal proteins that co-migrate with other serum proteins in the α or β regions of SPE gels. In an analysis of 2007 monoclonal gammopathies, 54/866 (6.2%) of IgG and 245/425 (57.6%) of IgA M-spikes migrated within the β region [33]. This highlights the difficulty in quantifying monoclonal IgA (and to a lesser extent IgG) proteins. HLC tests may provide accurate, quantitative values for these hidden monoclonal proteins [1]. In combination with FLC tests, these tests provide complementary values for sequentially monitoring all potential tumour clones within the bone marrow [1, 21]. Studies on the relative merits of monitoring monoclonal gammopathies with HLC assays compared to SPE/ IFE will be of particular interest.

Similar to the FLC test, the HLC ratio has significant prognostic value. The International Staging System (ISS) uses two independent risk factors, β2M and albumin, to stratify multiple myeloma patients. In a recent IFM 2005 trial, the HLC ratio strongly predicted progression free survival and was independent of both β2M and albumin [34]. Incorporating the HLC assays into the ISS may improve the risk stratification for multiple myeloma. In IgG MGUS, isotype specific suppression of the opposing HLC pair (eg. IgGλ suppression in an IgGκ MGUS) was associated with progression to myeloma [35]. This did not occur in IgA MGUS patients; this suggests a 'niche effect' specific to IgG MGUS tumours. The abi-

lity of the HLC ratio to account for immunoparesis may be fundamental to its prognostic value in monoclonal gammopathies. Further work is required to determine if the HLC ratio is independent of other known MGUS risk factors and whether this can be used to improve risk stratification and management of these patients.

Conclusions

The FLC assay and its κ/λ ratio can provide important clinical information for the diagnosis, monitoring and prognosis of patients with monoclonal gammopathies. The absolute concentrations of both FLCs also provide significant information. There is considerable interest in the role of polyclonal FLCs in the progression of CKD, management of early type II diabetic kidney disease as well as the prognosis of patients with HIV and hepatitis C. Preliminary data suggests HLC measurements have greater sensitivity than IFE and can aid patient monitoring. Furthermore, there is a strong association between the degree of HLC ratio abnormality and the risk of reduced progression free survival. Accurate monitoring of myeloma patients with these assays may provide clinicians with therapeutic opportunities not currently available using standard methods.

References

1. **Bradwell, A. R., Harding, S. J., Fourrier, N. J. et al.** Assessment of monoclonal gammopathies by nephelometric measurement of individual immunoglobulin kappa/lambda ratios. *Clin. Chem.*, 2009, 55, 9, p. 1646–1655.
2. **Katzmann, J. A., Clark, R. J., Abraham, R. S. et al.** Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin. Chem.*, 2002, 48, 9, p. 1437–1444.
3. **Bradwell, A. R., Carr-Smith, H. D., Mead, G. P. et al.** Serum test for assessment of patients with Bence Jones myeloma. *Lancet*, 2003, 361, 9356, p. 489–491.
4. **Drayson, M., Tang, L. X., Drew, R. et al.** Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. *Blood*, 2001, 97, 9, p. 2900–2902.
5. **Katzmann, J. A., Abraham, R. S., Dispenzieri, A. et al.** Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. *Clin. Chem.*, 2005, 51, 5, p. 878–881.
6. **Lachmann, H. J., Gallimore, R., Gillmore, J. D. et al.** Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. *Br. J. Haematol.*, 2003, 122, 1, p. 78–84.
7. **Jagannath, S.** Value of serum free light chain testing for the diagnosis and monitoring of monoclonal gammopathies in hematology. *Clin. Lymphoma. Myeloma*, 2007, 7, 8, p. 518–523.
8. **Katzmann, J. A., Dispenzieri, A., Kyle, R. A. et al.** Elimination of the need for urine studies in the screening algorithm for monoclonal gammopathies by using serum immunofixation and free light chain assays. *Mayo Clin. Proc.*, 2006, 81, 12, p. 1575–1578.
9. **Palladini, G., Russo, P., Bosoni, T. et al.** Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. *Clin. Chem.*, 2009, 55, 3, p. 499–504.
10. **Katzmann, J. A., Kyle, R. A., Benson, J. et al.** Screening panels for detection of monoclonal gammopathies. *Clin. Chem.*, 2009, 55, 8, p. 1517–1522.
11. **Rajkumar, S. V., Kyle, R. A., Therneau, T. M. et al.** Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. *Blood*, 2005, 106, 3, p. 812–817.
12. **Dispenzieri, A., Kyle, R., Merlini, G. et al.** International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia*, 2009, 23, 2, p. 215–224.
13. **Hutchison, C. A., Harding, S., Hewins, P. et al.** Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. *Clin. J. Am. Soc. Nephrol.*, 2008, 3, 6, p. 1684–1690.
14. **Hutchison, C. A., Plant, T., Drayson, M. et al.** Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. *BMC Nephrol.*, 2008, 9, 1, p. 11.
15. **Bird, J. M., Cavenagh, J., Samson, D. et al.** Guidelines on the diagnosis and management of AL amyloidosis. *Br. J. Haematol.*, 2004, 125, 6, p. 681–700.
16. **Brockhurst, I., Harris, K. P., Chapman, C. S.** Diagnosis and monitoring a case of light-chain deposition disease in the kidney using a new, sensitive immunoassay. *Nephrol. Dial. Transplant.*, 2005, 20, 6, p. 1251–1253.
17. **Siegel, D. S., McBride, L., Bilotti, E. et al.** Inaccuracies in 24-hour urine testing for monoclonal gammopathies. *Lab. Med.*, 2009, 40, 6, p. 341–344.
18. **Hobbs, J. A., Drayson, M. T., Sharp, K. et al.** Frequency of altered monoclonal protein production at relapse of multiple myeloma. *Br. J. Haematol.*, 2009.
19. **Kuhnemund, A., Liebisch, P., Bauchmuller, K. et al.** Light-chain escape-multiple myeloma' – an escape phenomenon from plateau phase: report of the largest patient series using LC-monitoring. *J. Cancer Res. Clin. Oncol.*, 2009, 135, 3, p. 477–484.
20. **Drayson, M. T., Morgan, G. J., Jackson, G. H. et al.** Prospective study of serum FLC and other M-protein assays: when and how to measure response? *Clin. Lymphoma Myeloma*, 2009, 9, p. A346a.
21. **Ayliffe, M. J., Davies, F. E., de Castro, D. et al.** Demonstration of changes in plasma cell subsets in multiple myeloma. *Haematologica*, 2007, 92, 8, p. 1135–1138.
22. **Hutchison, C. A., Cockwell, P., Reid, S. et al.** Efficient removal of immunoglobulin free light chains by hemodialysis for multiple myeloma: in vitro and in vivo studies. *J. Am. Soc. Nephrol.*, 2007, 18, 3, p. 886–895.
23. **Hutchison, C. A., Bradwell, A. R., Cook, M. et al.** Treatment of acute renal failure secondary to multiple myeloma with chemotherapy and extended high cut-off hemodialysis. *Clin. J. Am. Soc. Nephrol.*, 2009, 4, 4, p. 745–754.
24. **Hutchison, C. A., Airia, P., Cook, M. et al.** Improving outcomes in patients with renal failure secondary to multiple myeloma: results from ChAR2M2. *Presented at ASH 2009*, 2009, p. 1875a.
25. **Hutchison, C. A., Cook, M., Heyne, N. et al.** European trial of free light chain removal by extended haemodialysis in cast nephropathy (EuLITE): a randomised control trial. *Trials*, 2008, 9, 1, p. 55.
26. **Landgren, O., Kyle, R. A., Pfeiffer, R. M. et al.** Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood*, 2009, 113, 22, p. 5412–5417.

27. **Weiss, B. M., Abadie, J., Verma, P. et al.** A monoclonal gammopathy precedes multiple myeloma in most patients. *Blood*, 2009, 113, 22, p. 5418–5422.
28. **Pratt, G., Harding, S., Holder, R. et al.** Abnormal serum free light chain ratios are associated with poor survival and may reflect biological subgroups in patients with chronic lymphocytic leukaemia. *Br. J. Haematol.*, 2009, 144, 2, p. 217–222.
29. **Tsai, H.-T., Caporaso, N. E., Kyle, R. A. et al.** Evidence of serum immunoglobulin abnormalities up to 9.8 years prior to diagnosis of chronic lymphocytic leukemia: a prospective study. *Blood*, 2009, p. blood-2009-08--237651.
30. **Terrier, B., Sene, D., Saadoun, D. et al.** Serum-free light chain assessment in hepatitis C virus-related lymphoproliferative disorders. *Ann. Rheum. Dis.*, 2009, 68, 1, p. 89–93.
31. **Hutchison, C. A., Cockwell, P., Harding, S. et al.** Quantitative assessment of serum and urinary polyclonal free light chains in patients with type II diabetes: an early marker of diabetic kidney disease? *Expert. Opin. Ther. Targets*, 2008, 12, 6, p. 667–676.
32. **Landgren, O., Goedert, J. J., Rabkin, C. S. et al.** Circulating serum free light chains as predictive markers of AIDS-related lymphoma. *J. Clin. Oncol.*, 2010, 28, 5, p. 773–779.
33. **Wang, H., Gao, C. F., Xu, L. L. et al.** Laboratory characterizations on 2007 cases of monoclonal gammopathies in East china. *Cell Mol. Immunol.*, 2008, 5, 4, p. 293–298.
34. **Avet-Loiseau, H., Harousseau, J. L., Moreau, P. et al.** Heavy/light chain specific immunoglobulin ratios at presentation are prognostic for progression free survival in the IFM 2005-01 myeloma trial. *Presented at ASH 2009*, 2009, p. 1818a.
35. **Katzmann, J., Clark, R., Dispenzieri, A. et al.** Isotype-specific heavy/light chain (HLC) suppression as a predictor of myeloma development in monoclonal gammopathy of undetermined significance (MGUS). *Presented at ASH 2009*, 2009, p. 1788a.

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