Canadian Agency for Drugs and Technologies in Health

RAPID RESPONSE REPORT:
SUMMARY WITH CRITICAL APPRAISAL

TITLE: Protein Testing in Patients with Multiple Myeloma: A Review of Clinical Effectiveness and Guidelines

DATE: 09 January 2015

CONTEXT AND POLICY ISSUES

Multiple myeloma (MM) is a hematological cancer that involves the clonal expansion of malignant plasma cells.[1] MM is the most common malignant plasma cell tumour (of all plasma cell dyscrasias [PCD]) and the second most common hematologic malignancy in the United States (US).[1] The US age-adjusted incidence rate is 5.5 cases per 100,000,[1] and the annual incidence reaches approximately 6 to 7 per 100,000 in the United Kingdom.[2]

MM is a heterogeneous disease characterized by protein manifestations and molecular and genetic alterations.[3] Two staging systems are currently in use for the diagnosis of MM; that of Durie and Salmon and the International Staging System (ISS). The Durie and Salmon staging system involves features that assess tumour cells mass, elevated serum immunoglobulin (Ig)G levels, end-organ damage, and osteolytic bone lesions.[3] The more recent ISS places more emphasis on the disease burden based on β_2-microglobulin levels and serum albumin levels.[3] In addition, the International Myeloma Working Group (IMWG) has also proposed a new classification system that takes into account both molecular and cytogenetic abnormalities.[3]

The natural history of MM is variable between patients.[4] Survival can range anywhere from several months to many years[4] thereby increasing the need for effective monitoring and testing. In addition, many patients are diagnosed with a range of different PCDs prior to progression to symptomatic MM. These include monoclonal gammopathy of undetermined significance (MGUS),[1] asymptomatic MM (AMM),[5] and smoldering MM (SMM).[6] These PCDs require surveillance at specified intervals in order to ascertain when patients have entered the symptomatic stage of MM.[2]

Protein manifestations characteristic of MM include increases of monoclonal (M)-protein concentrations (IgG, IgA, IgA, IgD), light chain concentrations (including kappa [κ] and lambda[λ]), abnormal β_2-microglobulin, serum albumin, creatinine, and hemoglobin levels, and findings of bone marrow plasma cells (of greater than or equal to 5%).[7,8] Measurement of the protein manifestations produced by patients can be achieved by numerous methods. Traditional

tests that measure M-proteins are the 24-hour urine collection test, urine protein electrophoresis (UPEP), serum protein electrophoresis (SPEP), and immunofixation electrophoresis (IFE). One newer test (developed in 2001) is the serum free light chain (sFLC) assay.[9] The frequency with which to test using any of the tests along with the clinical utility of the newer tests remains in question. It is for this reason that a review of the clinical effectiveness, clinical utility, and guidelines of repeat testing for protein abnormalities in patients with MM was undertaken.

RESEARCH QUESTIONS

1. What is the evidence for the clinical effectiveness and clinical utility of repeat testing for protein abnormalities in patients with MM?

2. What is the evidence for the clinical effectiveness and clinical utility of testing for protein abnormalities in patients with MM?

3. What are the evidence-based guidelines regarding the frequency and clinical utility of testing for protein abnormalities in patients with MM?

KEY FINDINGS

There remains a paucity of information on the clinical effectiveness and clinical utility of repeat testing for protein abnormalities in patients with multiple myeloma. In addition, there is a lack of good quality evidence regarding the clinical utility of the serum free light chain assay and no evidence on the clinical utility of serum protein electrophoresis and urine protein electrophoresis published in the last five years. Studies identified regarding the clinical utility of the serum and urine protein electrophoresis, and the serum free light chain test were heterogeneous in their populations, interventions, treatment regimens, and outcomes. In addition, results may be confounded by the small sample sizes and non-comparative nature of the studies. Therefore, caution should be heeded when interpreting these results.

British Society for Haematology guidelines recommend the use of the serum free light chain assay to monitor response to therapy in all patients with oligosecretory disease, non-secretory disease, and light chain only multiple myeloma. In addition, monitoring of patients with asymptomatic multiple myeloma should take place at regular three months intervals to assess for any emergence of myeloma-related organ and tissue impairment using the serum and urine M-protein electrophoresis tests and the serum free light chain assay when indicated.

METHODS

Literature Search Methods

A limited literature search was conducted on key resources including PubMed, The Cochrane Library (2014, Issue 12), University of York Centre for Reviews and Dissemination (CRD) databases, Canadian and major international health technology agencies, as well as a focused Internet search. No filters were applied to limit the retrieval by study type. Where possible, retrieval was limited to the human population. The search was also limited to English language documents published between January 1, 2009 and December 4, 2014.

Rapid Response reports are organized so that the evidence for each research question is presented separately.

Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

Table 1: Selection Criteria

Population	Any person diagnosed with multiple myeloma
Intervention	• Serum protein electrophoresis • Urine protein electrophoresis • Serum free light chains • 24-hour urine protein
Comparator	None
Outcomes	Q1: • Benefits or harms associated with frequency of testing • Change in treatment of patients based on test results Q2: • Change in treatment of patients based on test results • Prognostic value of test results • Changes in protein parameters that are considered clinically significant Q2: • Guidelines on the frequency of testing • What changes would be considered clinically significant for treatment considerations • How treatment can be changed based on test results
Study Designs	Health technology assessments, systematic reviews, meta-analyses, randomized controlled trials, non-randomized studies, evidence-based guidelines

Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 1, they were duplicate publications, or were published prior to 2009. Guidelines were excluded if they lacked appropriate reporting of methodology or were not evidence-based.

Critical Appraisal of Individual Studies

Key methodological aspects relevant to each study design were appraised and summarized narratively. The AMSTAR tool[10] was used to guide the critical appraisal of the methodological quality of the systematic review (SR) included in this report. Emphasis was placed upon the methods used to conduct the literature search, study selection, quality assessment, data extraction, and conflict of interest declaration. Using the Downs and Black Checklist[11] to guide the critical appraisal, an assessment of the study design, reporting, representativeness of populations, and sample size was included for the randomized and non-randomized studies. The AGREE II instrument[12] was used to guide the critical appraisal of the evidence-based guidelines and focused on the following domains: scope and purpose, stakeholder involvement, rigor of development, clarity and presentation, applicability, and editorial independence.

SUMMARY OF EVIDENCE

Quantity of Research Available

A total of 259 citations were identified in the literature search. Following screening of titles and abstracts, 231 citations were excluded and 28 potentially relevant reports from the electronic search were retrieved for full-text review. Five potentially relevant publications were retrieved from the grey literature search. Of these potentially relevant articles, 21 publications were excluded for various reasons, while 12 publications met the inclusion criteria and were included in this report. Appendix 1 describes the PRISMA flowchart of the study selection.

Additional references of potential interest are provided in Appendix 2.

Summary of Study Characteristics

One SR,[1] one RCT,[13] nine non-randomized studies,[5-8,13-17] and one evidence-based guideline were included in this review. Detailed study characteristics are provided in Appendix 3, Table 2.

Systematic Review

The 2012 SR[1] included in this review originated in the United States by the Agency for Healthcare Research and Quality (AHRQ). This SR focused on the comparative effectiveness of the serum free light chain (sFLC) assay with traditional tests (any type of testing, i.e. SPEP, UPEP, IFE, serum M-protein sizing and typing, bone marrow biopsy, skeletal lesion detection tests) for the diagnosis, management, and prognosis of PCDs. A total of 15 studies were included, of which eight studies examined whether the sFLC assay was a better indicator of response to treatment when performed in parallel with traditional tests in patients diagnosed with MM. The search timeframe included studies published between 2000 and 2012 because the sFLC assay was approved by the United States Food and Drug Administration in 2001. In addition, one other study was also included that examined whether the sFLC assay reduces the need for other traditional tests in patients with an existing diagnosis of MM.

Primary Studies

One randomized controlled trial (RCT)[13] and nine non-randomized studies[5-8,14-18] were included in this review. Three of these studies originated in the United States[6,17,18] with the other studies originating in China,[15] Denmark,[7] Greece,[5] Japan,[14] Serbia,[16] Spain (the RCT),[13] and South Korea.[8] Sample sizes in the primary studies ranged from 14 to 586 patients with median ages ranging from 58 to 72 years.[5-8,13,14,16-18] Four of the included non-randomized studies were retrospective analyses,[6,8,14,17] four studies were prospective in nature,[5,7,15,16] while one study[18] analyzed test results from patients included in clinical trials. Two of the non-randomized studies attempted to assess the predictability of progression to symptomatic MM in patients classified as either having AMM[5] or SMM[6] at diagnosis while the other studies included patients classified as having MM.[7,8,14,16-18] Most studies focused on either the sFLC assay results,[13,15,16,18] or free light chain kappa/lambda (FLC κ/λ) ratios,[5,6,14,16] while the rest of the studies focused on involved free light chains (iFLC),[7] Bence-Jones protein (BJP) excretion,[17] or the detection of abnormal protein bands by SPEP or UPEP.[8] Outcomes of interest included prognostic impact of stringent complete response (CR),[13] iFLC measurements to determine efficacy of treatment,[7] sFLC results for response evaluation,[7] significance of abnormal protein bands (APB),[8] sFLC κ/λ

ratios on prognosis[14,15] or progression,[5,6,16] sFLC for monitoring,[18] or reliability of 24-hour urine collection for assessing BJP.[17]

Evidence-Based Guidelines

One evidence-based guideline[2] by the British Society for Haematology (BSH) was included in this review and represents a major revision of their earlier 2006 guidelines. The population of interest included patients suspected of having MM, patients with MGUS, patients with AMM, and patients with symptomatic MM (henceforth referred to as MM).The focus of the guidelines ranged from prognostic factors and staging, to measuring response to therapy, and to other areas not of interest to this review (including diagnosis).

Summary of Critical Appraisal

Details of the critical appraisal are provided in Appendix 4, Table 3 (systematic review and primary studies) and Table 4 (evidence-based guidelines).

Systematic Review and Primary Studies

The AHRQ comparative effectiveness SR[1] reported rigorous methodology that included clearly described *a priori* inclusion criteria, comprehensive literature searches, and duplicate data selection. Due to the heterogeneity of the included studies, a meta-analysis was not performed; however, this was planned had the studies been similar. While 15 studies were identified, there was a paucity of comparative data between the sFLC assay and the traditional tests; rather there was only evidence identified that provided results on tests run in parallel. For this reason (along with the heterogeneity of the studies), the evidence was deemed insufficient to make recommendations on its use. Lists of included and excluded studies were not provided in the report and there was an absence of any declaration of conflicts of interest. In addition, the included studies lacked sufficient power for comparisons of different plasma cell dyscrasias. There was also the potential in the AHRQ review, though thorough in its methodology, for some publication and selection bias as the search was limited to English only studies.

Most of the primary studies had clearly defined objectives,[5-8,13-15,17,18] interventions,[5-7,13-15,18] and outcomes,[5-8,13-15,18] and only three studies[16-18] did not report baseline patient characteristics. The RCT by Paiva et al.[13] included appropriate blinding with regard to the assessment of the test results but was an open label trial with regards to the treatments patients were randomized to receive. Small sample sizes were evident in a number of the included studies[5,7,13-18] and the study populations were heterogeneous in a number of factors such as treatment regimens (including chemotherapy treatment, stem cell transplantation), types of MM at baseline (e.g. AMM, SMM, or MM), myeloma subtype (IgG, IgA, IgD), isotype (kappa or lambda), and disease stage. In addition, most studies used traditional tests concurrently[5-8,14-18] rather than using these to directly compare with sFLC assay results or FLC κ/λ ratios. While conflicts of interest were declared in most primary studies, there were a few studies that lacked this declaration.[7,16,18]

Evidence-Based Guidelines

The BSH guidelines[2] were developed by examining and grading the literature along with incorporating the expertise of professionals and involving patient advocacy groups. Methods for recommendation formulation were provided and the recommendations themselves were clearly presented and described. The recommendations were graded according to the levels of

evidence available (Appendix 5, Table 5). Additionally, the guidelines and recommendations were externally reviewed and potential conflicts of interest were described. Concerns associated with these guidelines include the lack of clarity surrounding whether the literature was systematically searched, a lack of clarity between the recommendations and the supporting evidence, and a lack of a clearly described objective. If the literature was not systematically searched there remains the possibility that the information included is not complete. Also, by not clearly linking the recommendations with the supporting evidence, one cannot be certain as to where the information in the recommendations actually came from and the trustworthiness of the information sources. In addition, since these are guidelines from Britain and there is not list of included and excluded studies, there remains the possibility that these results cannot be generalized to Canadian patients with MM.

Summary of Findings

Detailed findings from individual studies are provided in Appendix 6, Table 6.

What is the evidence for the clinical effectiveness and clinical utility of repeat testing for protein abnormalities in patients with MM?

No evidence was identified that examined the clinical effectiveness and clinical utility of repeat testing; therefore no summary can be provided.

What is the evidence for the clinical effectiveness and clinical utility of testing for protein abnormalities in patients with MM?

The AHRQ comparative effectiveness review[1] set out to ascertain whether sFLC assay results would lead to a difference in treatment decisions, would be better than traditional tests is determining treatment response, and whether it would lead to a reduction of other tests in patients with an existing diagnosis of MM. Evidence on the use of the sFLC assay results to promote treatment change in duration, timing, or type when compared to traditional tests was deemed insufficient as no studies were identified. In addition, the evidence was also deemed insufficient for the use of the sFLC assay as a better indicator of treatment response and of outcomes when compared with traditional tests. Most of the identified evidence was heterogeneous with regard to populations, treatment regimens, adjustments for confounding, and interventions. In addition, the sFLC assay was not formally compared directly to the traditional tests but rather run in parallel. There was also insufficient evidence to indicate that the sFLC assay reduces the need for other tests, as only one study was identified and the authors concluded that bone marrow biopsy was still necessary in patients with MM who had negative serum and urine immunofixation electrophoresis.

Of the primary studies identified, three studies focused on prognosis,[8,13,14] two studies focused on the predictive ability of certain tests or markers,[7,15] one study focused on test results as risk factors for both progression and prognosis,[16] two studies examined the risk of progression to symptomatic MM from either AMM[5] or SMM,[6] one study focused on the utility of 24-urine collections for BJP results,[17] and one study examined tests results in serial monitoring in patients with relapsed or refractory MM.[18]

In one study, it was determined that normalization of the FLC κ/λ ratio (regardless of baseline FLC concentrations) after various treatment regimens was an independent prognostic factor for overall survival (three-year median overall survival of 96.3%).[14] In another study comparing

conventional clinical response (CR: defined as less than 5% of bone marrow plasma cells along with negative immunofixation) with stringent CR (CR plus normal FLC κ/λ ratio) and immunophenotypic response (IR), it was determined that attaining the IR led to longer progression free survival and time to progression.[13] In addition, sustained disease control was observed upon maximal responses of both stringent CR plus IR.[13] Finally, the study that assessed abnormal protein band occurrence (measured using SPEP or UPEP and IFE) in the follow-up period determined that the APB was independently associated with prolonged overall survival.[8]

In one study that assessed predictive ability, it was determined that involved free light chain (iFLC) changes early in treatment did not predict ultimate response as an iFLC reduction of more than 20% within the first eight days of treatment occurred in all patients regardless of whether these patients were responding to treatment.[7] However, in patients with baseline iFLC greater than 75 mg/l, achievement of at least very good partial response (VGPR) was strongly associated with iFLC reductions of greater than 80% three weeks after beginning treatment.[7] Another study reported that baseline sFLC levels and FLC κ/λ ratios were successful in predicting overall survival in patients with newly diagnosed MM (see Table 6, Appendix 6 for 1-year and 3- year overall survival results for those with low versus high sFLC levels and FLC κ/λ ratios).[15]

One study aimed to evaluate whether FLC κ/λ ratios were a significant factor in the prognosis for remission, for disease progression, and for survival.[16] The authors reported that patients with highly abnormal FLC κ/λ ratios had a median survival of 22 months, compared with patients with intermediate, low, and normal FLC κ/λ ratios (30 months, 39 months, and 51 months, respectively).[16]

One study that examined the risk of progression from AMM to symptomatic MM reported that patients with extensive bone marrow plasma cell infiltration of greater than or equal to 60% and/or a highly abnormal FLC κ/λ ratio of greater than or equal to 100 had a substantial risk of developing symptomatic MM within two years of their AMM diagnosis.[5] In addition, the authors also recommended an initial FLC assessment along with 24-hour urine collections for UPEP performed at three to six month intervals once diagnosed with AMM.[5] In agreement with the aforementioned study,[5] the authors of a different study examining the risk of progression from SMM to symptomatic MM reported that patients with a high FLC κ/λ ratio were at a high risk of symptomatic MM development or a related disorder within two years of SMM diagnosis.[6]

The remaining studies reported on various other interventions. One study examined the reliability of the 24-hour urine collection (measured by protein electrophoresis) for the assessment of BJP.[17] To ascertain the reliability of the 24-urine collection itself, they also measured 24-hour creatinine. They observed that coefficients of variation ranged from 12-30% in creatinine collections and there was a strong correlation between BJP differences (maximum differences ranged from -1588 mg/dl to 2315 mg/dl) and differences in 24-hour creatinine values.[17] Hence, they concluded that 24-hour urine collections appeared to be inconsistent and thereby may lead to quantification mistakes in BJP.[17] Another study focused on comparing sFLC assay measurements and electrophoretic M-spikes when monitoring disease status.[18] The authors concluded that sFLC measurements did not show changes earlier that those of M-spikes in patients with relapsed or refractory MM.[18]

What are the evidence-based guidelines regarding the frequency and clinical utility of testing for protein abnormalities in patients with MM?

Detailed recommendations are provided in Appendix 6, Table 7.

The BSH guidelines[2] provided guidance on both the frequency of testing in patients with AMM and in measuring response to therapy for those with MM. For patients diagnosed with AMM, the recommendations stated that assessment for the myeloma-related organ and tissue impairment (characteristics necessary for the diagnosis of symptomatic MM) should typically be performed at three month regular intervals. Recommended tests for monitoring include serum and urine M-protein electrophoresis and the sFLC assay when indicated.[2] The society also recommends using the sFLC assay to assess response to therapy in patients oligosecretory disease, non-secretory disease, and light chain only diseease.[2]

Limitations

The AHRQ review[1] attempted to examine the comparative effectiveness of the serum free light chain assay with traditional tests for management and prognosis purposes. One main limitation of the included studies was the lack of actual comparative studies, with most studies simply performing the traditional tests in parallel to that of the sFLC assay. While results were provided from both the sFLC assay and other tests, nor formal comparison was performed, thus making it difficult for the AHRQ authors to ascertain comparative effectiveness. In addition to this, new comparators to traditional monitoring (i.e. positron emission tomography) were not examined.

Similar to the AHRQ review, the studies included in this report did not actively compare the tests of interest (SPEP, UPEP, SFLC assay, or 24-urine collections) and were heterogeneous in their populations, interventions, methodology, and outcomes. A large number of the non-randomized studies were retrospective analyses and many did not contain baseline patient characteristics, which potentially confounds the results.

The Paiva et al. RCT[13] was a study that did not assess the actual tests per se but, instead, assessed the value of achieving either IR, CR, or stringent CR when treated with two different treatment regimens. Using these results, the authors concluded that obtaining IR or IR plus stringent CR has a genuinely good and long impact on patient outcomes. Only through indirect inference can one then conclude that the sFLC assay should be performed in order to ascertain the stringent CR designation. This RCT was not explicit in stating this.

Another important consideration was the study sample size. While the RCT and a few of the non-randomized studies had sample sizes larger than 150, four of the nine non-randomized studies contained small sample sizes (14 to 36 patients) and four more with 126 or less. The lack of statistical power to detect clinically meaningful changes limits the interpretation of these results.

While there appeared to be some evidence on the clinical utility of the new sFLC assay, in the search timeframe for this review, there was no evidence identified on the clinical utility of SPEP and UPEP. This may have been due to the fact that these are already established tests used currently for the diagnosis and prognosis of MM and also for evaluating response to therapy. In addition, there was no evidence identified regarding the frequency of using these tests for disease monitoring except from guidelines which were not clearly evidence-based.[4,9,19,20] Results from these guidelines may not reflect the true capabilities of the tests.

CONCLUSIONS AND IMPLICATIONS FOR DECISION OR POLICY MAKING

There remains a paucity of information on the clinical effectiveness and clinical utility of repeat testing for protein abnormalities in patients with multiple myeloma. In addition, there is a lack of good quality evidence regarding the clinical utility of the sFLC assay and no current evidence on the clinical utility of SPEP and UPEP in the last five years. The AHRQ SR[1] did not identify any evidence on the effectiveness of the sFLC assay compared with other traditional tests for the diagnosis, prognosis, and management of MM. Only studies that contained results from tests that were performed in parallel to the sFLC assay were identified and these were heterogeneous in their populations, interventions, treatment regimens, and outcomes. For these reasons, AHRQ concluded that the evidence was insufficient to support the use of the sFLC assay in the diagnosis, prognosis, and management of MM. Many of these same issues were encountered in the primary studies that were identified for this review. The randomized controlled trial and non-randomized studies were heterogeneous in their populations, interventions, and outcomes, generally tended to include small sample sizes, lacked sufficient power to detect changes in PCDs, and many were retrospective in nature. Therefore caution is required when interpreting the results.

One evidence-based guideline from the BSH was identified.[2] According to these guidelines, when measuring response to therapy, the sFLC assay is recommended in all patients with oligosecretory disease, non-secretory disease, and light chain only MM. In addition, they recommend that monitoring of patients with AMM should take place at regular three months intervals to assess for any emergence of myeloma-related organ and tissue impairment.[2] Measurements should be obtained through the use of SPEP and UPEP M-protein analysis and sFLC when indicated.[2] It should be noted that other guidelines are also available with regard to the management and monitoring of MM and that the BSH guidelines are in agreement with the guidelines, particularly from the frequently cited guidelines from IMWG.[9,20] The other identified guidelines, however, were not included in this review as there was uncertainty as to whether they were evidence-based. IMWG have general guidelines[9] for MM but also guidance specific to the sFLC analysis.[20] The general guidelines stipulate that disease monitoring should be performed using SPEP and UPEP monthly after the initiation of therapy (or more frequently if indicated) and then reduced to every two to three months during maintenance or follow-up.[9] The following tests (that fall into this review's interventions of interest) should be used for these assessments: M-protein quantification for IgA using electrophoresis or nephelometry and the sFLC assay for patients with oligosecretory and non-secretory MM. Changes in the sFLC κ/λ ratios alongside increases in iFLC is indicative of disease progression. In patients with light chain MM and measureable M-spikes, the 24-hour urine M-protein excretion should be included. Taken together, these guidelines[2,9,20] should be followed with caution due to the important inherent limitations previously mentioned.

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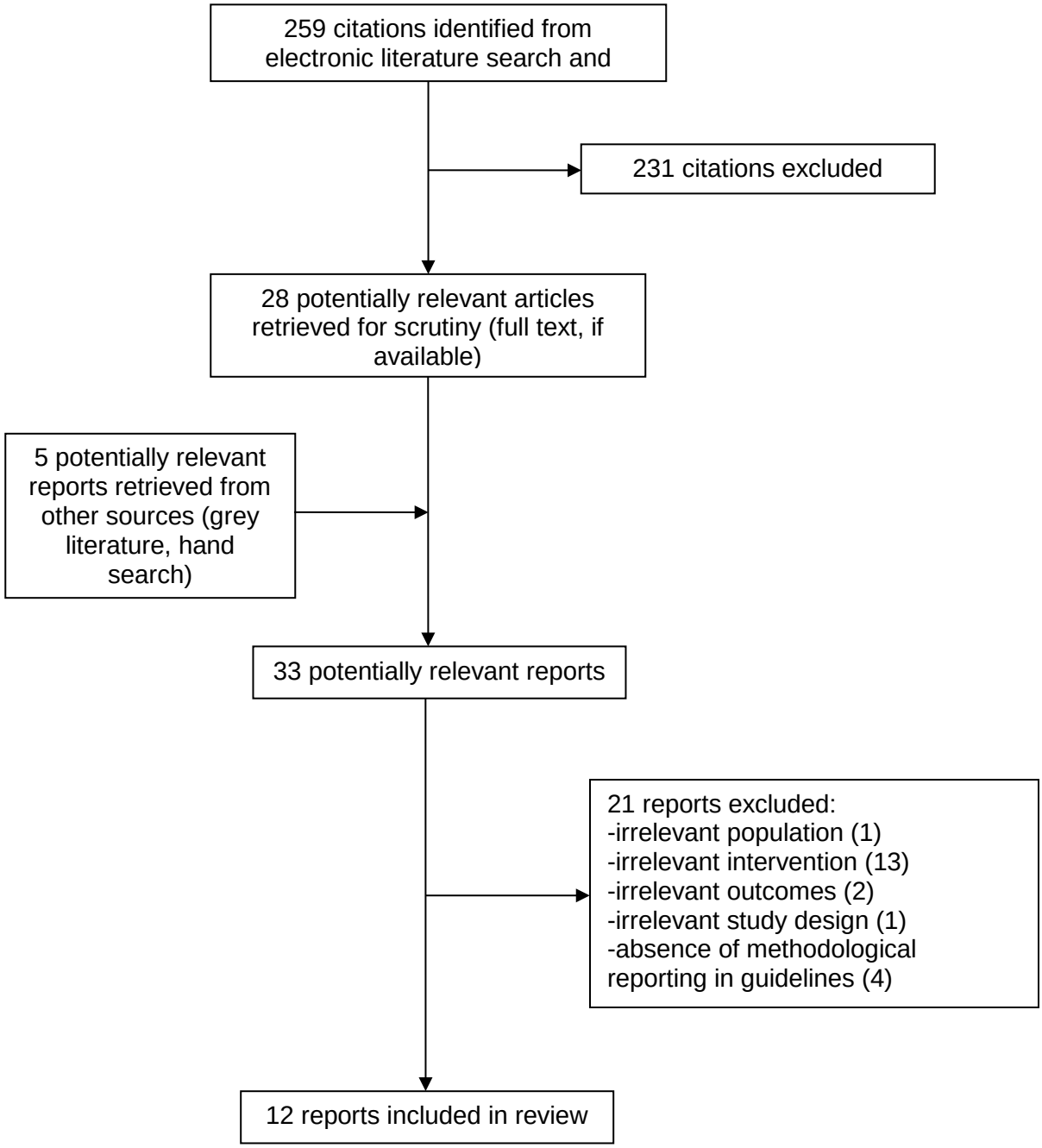
APPENDIX 1: Selection of Included Studies



APPENDIX 2: Additional References of Potential Interest

Non-Randomized Studies

Laboratory Specific Studies

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PubMed: PM19797521

Sample size n < 10

Dogaru M, Lazar V, Coriu D. Assessing the efficiency of free light chain assay in monitoring patients with multiple myeloma before and after autologous stem cell transplantation along with serum protein electrophoresis and serum protein immunofixation. Roum Arch Microbiol Immunol. 2011 Jan;70(1):15-22.
PubMed: PM21717807

Clinical Practice Guidelines – Methodology Uncertain/Not Provided

Multiple myeloma [Internet]. Edmonton: Alberta Health Services; 2013 Nov. [cited 2015 Jan 9]. (Clinical practice guideline LYHE-003 version 4). Available from: http://www.albertahealthservices.ca/hp/if-hp-cancer-guide-lyhe003-multi-myeloma.pdf
See: Follow-Up After Treatment, page 27
Disease Monitoring, pages 27-28

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See: Practical Recommendations for use of SPEP and UPEP, pages 17-18

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See: Follow-up, page v157

APPENDIX 3: Characteristics of Included Publications

Table 2: Characteristics of Included Primary and Secondary Studies

First Author, Publication Year, Country	Study Design and Patient Characteristics	Interventions	Comparators	Clinical Outcomes
Systematic Review (Comparative Effectiveness)				
Rao, 2012,[1] AHRQ US	• 15 studies included in this comparative effectiveness review ***Inclusion criteria:*** • No restrictions on study designs • Study designed to address comparative effectiveness of sFLC assay (compared with predefined tests including: SPEP, UPEP IFE, and other tests specific for PCD diagnosis, i.e. BMPC, skeletal surveys) • *Population:* o Patients with existing PCD diagnosis (MM, NSMM, of AL amyloidosis) o With or without disease measured by traditional testing	• sFLC assays along with FLC κ/λ ratio	• Any type of traditional testing (i.e. SPEP, UPEP, IFE [by urine or serum], serum M-protein sizing and typing, bone marrow biopsy, skeletal lesion detection tests)	• Timing, duration, and type of tmt • OS, PFS, response to tmt or remission (PR, CR, stringent CR [defined by decrease in M-protein or FLCs], light chain escape, or QoL • Clinic visits, BMPC results, skeletal surveys
Randomized Controlled Trial				
Paiva, 2011,[13] Spain	***Study Design and Characteristics:*** • Prospective RCT • Randomly assigned to receive 6 cycles of either VMP or VTP • N=260 pts randomized • N=102 focus of study[a] • Blinded for results obtained with each technique used to assess MM (IR, sFLC, MFC)	• VMP (induction) followed by bortezomib/thalidomide (maintenance) for up to 3 years	• VTP (induction) followed by bortezomib/prednisone (maintenance) for up to 3 years	• Prognostic impact of attaining CR, versus CR plus normal sFLC ratio (stringent CR), versus IR

Table 2: Characteristics of Included Primary and Secondary Studies				
First Author, Publication Year, Country	**Study Design and Patient Characteristics**	**Interventions**	**Comparators**	**Clinical Outcomes**
	Patient Characteristics: • Median age=72 (range 65-84) • *Myeloma subtype, n (%)* o IgG=54 (56) o IgA=37 (33) o Light chain only=11 (11) • *ISS Disease Stage, n (%):* o I=29 (29) o II=39 (38) o III=34 (33)			
Non-Randomized Studies				
Hansen, 2014,[7] Denmark	***Study Design and Characteristics:*** • Prospective observational study of patients between 2002-2012 • N=36 ***Patient Characteristics:*** • Pts with symptomatic MM • Mean age=67.2 (range 48-84) • iFLC above upper reference limit (κ: 3.3-19.4 mg/l; λ: 5.7-26.3 mg/l) and abnormal FLC κ/λ ratio (0.26-1.65) prior to treatment • iFLC >75 mg/l in n=28 • *Tmt regimens, n (%):* o Bortezomib containing=26 (72) o Thalidomide containing=6 (17) o Revlimid containing=1 (3) o Alkylator based=3 (8) • *Line of treatment, n (%)* o First=28 (78) o Second=8 (22)	• iFLC (achieved by using sFLC assay)	• M-protein	• Measurements of iFLC to determine efficacy of treatment (faster and more reliably) compared to M-protein in serum and urine • Using sFLC measurements for response evaluation

Table 2: Characteristics of Included Primary and Secondary Studies				
First Author, Publication Year, Country	**Study Design and Patient Characteristics**	**Interventions**	**Comparators**	**Clinical Outcomes**
	• *Myeloma subtype, n (%)* o IgG=22 (61) o IgA=7 (19.5) o Light chain only=7 (19.5) • *Free Light Chain, n (%)* o Kappa=25 (69) o Lambda=11 (31)			
Jo, 2014,[8] South Korea	***Study Design and Characteristics:*** • Retrospective analysis • N=377 • Parallel testing of IFE or SPEP/UPEP ***Patient Characteristics:*** • Patients with MM • Patients treated with wither standard chemotherapy or high dose chemotherapy followed by ASCT • Median age=61 (range 30-89) • *ECOG performance status, n:* o 0/1=302 (80.1% o 2-4=75 (19.9%) • *Paraprotein type, n:* o IgG=176 (46.7%) o IgA=88 (23.4%) o IgD=19 (5.0%) o Free κ light chain=45 (11.9%) o Free λ light chain=49 (13.0%) • *International staging system, n:* o I=71 (18.8%) o II=149 (39.5%) o III=145 (38.5%)	• Detection of APB by either/or SPEP/UPEP	• None	• Clinical significance and frequency of APB during MM treatment • Description of APB patterns during treatment

Table 2: Characteristics of Included Primary and Secondary Studies				
First Author, Publication Year, Country	**Study Design and Patient Characteristics**	**Interventions**	**Comparators**	**Clinical Outcomes**
	• *ASCT, n:* o Yes=148 (39.3%) o No=229 (60.7%) • *APB, n:* o Yes=37 (9.0%) o No=343 (91.0%)			
Iwama, 2013,[14] Japan	***Study Design and Characteristics:*** • Retrospective analysis between 2004-2012 • N=126 • Tmt according to IMWG criteria • Tmt response evaluated at time of maximal response during course of tmt according to IMWG • Parallel testing of hemoglobin, β2-microglobulin, albumin, LDH, IFE, BMPCs; in addition identified disease stage ***Patient Characteristics:*** • Newly diagnosed MM pts • Pts received at least 1 cycle of tmt • Pts treated heterogeneously[b] • Median age=71 yrs • *IMWG response, n:* o CR=34 o VGPR=37 o PR=39 o SD or less=16 • *Myeloma subtype, n* o IgG=66 o IgA=18	• sFLCκ/λ[c] (normalization ratio of sFLCκ/λ ratio required 2 consecutive determinations of normal ratio ≥ 4 weeks apart)	• None	• Relevance of sFLCκ/λ ratio normalization after tmt to prognosis of pts with newly diagnosed MM

Table 2: Characteristics of Included Primary and Secondary Studies

First Author, Publication Year, Country	Study Design and Patient Characteristics	Interventions	Comparators	Clinical Outcomes
	○ IgD=1 ○ Light chain only=31 • *Isotype, n* ○ Kappa=71 ○ Lambda=55 • *ISS Disease Stage, n:* ○ I=21 ○ II=36 ○ III=69 ○ SCT=22			
Kastritis, 2013[5] Greece	***Study Design and Characteristics:*** • Prospective observational study with minimum follow-up of 18 months • N=96 • Attempting to identify pts at high risk of progressing to symptomatic MM • Parallel testing using standardized tests ***Patient Characteristics:*** • Pts with AMM • Median age=63 years • Median serum M-protein=1.65 g/dl • M-protein levels ≥ 3g/dl in 12% pts	• Features[d] to aid in the identification of very high-risk AMM pts[e] ○ FLC ratio ○ Ig type of MM ○ M-peak	• None	• Progression to symptomatic MM
Larsen 2013[6] US	***Study Design and Characteristics:*** • Retrospective analysis • N=586 • Time frame 1970-2010 • Attempting to assess the predictive value of sFLC values • Parallel testing using standardized tests	• sFLC ratio at SMM diagnosis	• None	• Ability of the sFLC ratio at SMM diagnosis to predict progression to symptomatic MM within 2 yrs of diagnosis

Table 2: Characteristics of Included Primary and Secondary Studies				
First Author, Publication Year, Country	**Study Design and Patient Characteristics**	**Interventions**	**Comparators**	**Clinical Outcomes**
	Patient Characteristics: • Pts newly diagnosed with SMM *Baseline:* • Mean age=64 • *Serum M-protein* o < 3 g/dl=67% o ≥ 3 g/dl=33% • Pts with light chain only SMM, n=23 (4%) • *Involved light chain* o Kappa=63% o Lamba=37% • *Median light chain [conc], mg/dl* o Kappa=3.02 (0.007-761) o Lambda=1.26 (0.04-1715) • *BPMCs* o 10-60%=95% o >60%=5% • Mean difference in involved and uninvolved FLC, mg/dl (range) o 6.9 (0.03-1714)			
Xu, 2013,[15] China	***Study Design and Characteristics:*** • Prospective observational study • N=122 • Attempting to assess sFLC as tumour markers and prognostic value of baseline sFLC and FLCκ/λ ratio • sFLC in healthy controls (aged 20-85 years) performed	• sFLC in patients with MM	• None	*Primary endpoint:* • OS *Other endpoints:* • Correlation with other clinical factors • Prognostic value of sFLC and FLCκ/λ ratio

Table 2: Characteristics of Included Primary and Secondary Studies				
First Author, Publication Year, Country	**Study Design and Patient Characteristics**	**Interventions**	**Comparators**	**Clinical Outcomes**
	Patient Characteristics: • Median age= 58 years (range 30-83) • *Tmt regimens:* o Conventional chemotherapy (received VAD or TAD) or o Bortezomib (PAD or BCD) o After at least 4 cycles (if pts had partial remission or better), consolidation therapy of either ASCT or chemotherapy (original regimen) o Maintenance therapy = thalidomide for 1 year • *Myeloma subtype, n (%)* o IgG=65 (53.3) o IgA=29 (23.8) o IgM=2 (1.6) o Light chain=24 (19.7) o Non-secretory=2 (1.6) • *Durie-Salmon stage, n (%)* o I=7 (5.8) o II=21 (17.2) o IIIA=77 (63.1) o IIIB=17 (13.9) • *ISS stage, n (%)* o I=24 (19.7) o II=64 (52.4) o III=34 (27.9)			
Radovic, 2012,[16] Serbia	***Study Design and Characteristics:*** • Prospective study, 7 year period • N=101 (total) • β_2-microglobulin and serum albumin measured in parallel	• FLCs and FLC κ/λ ratios	• None	• Risk factors (FLC κ/λ ratios)for disease progression • Median time to disease

Table 2: Characteristics of Included Primary and Secondary Studies				
First Author, Publication Year, Country	**Study Design and Patient Characteristics**	**Interventions**	**Comparators**	**Clinical Outcomes**
	Patient Characteristics: • Pts with MM (n=95) • Pts with NSMM (n=3) • Pts with primary amyloidosis (n=3 • At diagnosis, 93.7% pts had FLC [conc] deviating from reference interval (κ=3.3-19.4; λ=5.71-26.3) • 12.6% pts had FLC κ/λ ratios with reference interval (0.26-1.65) • *Tmt regimens:* o MP in pts >65 years, not ASCT candidates o MPT in pts ≤ 65 years, not ASCT candidates o VAD and CTD in pts <65 years, ASCT candidates o Interferon and thalidomide as maintenance after ASCT			progression
Kaplan, 2011,[17] US	***Study Design and Characteristics:*** • Retrospective analysis between 2003-2008 • N=14 • Quality assurance/improvement effort ***Patient Characteristics:*** • Pts with BJP who had ≥4 24-hour urine collections analyzed	• 24-hour BJP excretion • 24-hour creatinine excretion	• None	• Reliability of 24-hour urine collections for assessing amount of BJP
Uljon, 2011,[18] US	***Study Design and Characteristics:*** • Analysis of test results from pts from Phase I clinical trial, up to 63.4 months • N=17	• sFLC • SPEP • IgGA • M-spike	• None	• Assess the additional benefit of serial sFLC monitoring (in addition to both SPEP and IgGA) to

Table 2: Characteristics of Included Primary and Secondary Studies				
First Author, Publication Year, Country	**Study Design and Patient Characteristics**	**Interventions**	**Comparators**	**Clinical Outcomes**
	• Pts treated with RVD ***Patient Characteristics:*** • Pts with relapsed/refractory MM • *Myeloma subtype, n* o IgG=16 o Free lambda=1 • *Involved light chain* o Kappa=13 o Lamba=4			patient management

AHRQ = Agency for Healthcare Research and Quality; APB = abnormal protein band; ASCT = autologous stem cell transplant; BCD = bortezomib/cyclophosphamide/dexamethasone; BPMC = bone marrow plasma cell; BJP = Bence-Jones proteinuria; [conc] = concentration; CD = cyclophosphamide/dexamethasone; CR = complete response; CTD = cyclophosphamide/thalidomide/dexamethasone; FLC = free light chain; FLCκ/λ = free light chain kappa to lambda; ECOG = Eastern Cooperative Oncology Group; IFE = immunofixation electrophoresis; iFLC = involved free kappa and lambda light chains; Ig = immunoglobulin; IgGA = individual Ig heavy chain assessment by nephelometry; IMWG = International Myeloma Working Group; IR = immunophenotypic response; ISS = International Staging System; LDH = lactate dehydrogenase; M = monoclonal; MFC = multiparameter flow cytometry; MM = multiple myeloma; MP = melphalan/prednisone; MPT = melphalan/prednisone/thalidomide; NSMM = non-secretory multiple myeloma; NRS = non-randomized study; OS = overall survival; PAD = bortezomib/adriamycin/dexamethasone; PCD = plasma cell dyscrasias; PFS = progression free survival; PR = partial response; pts = patients; QoL = quality of life; RCT = randomized controlled trial; RVD = lenalidomide/bortezomib/dexamethasone; SCT = stem cell transplantation; SD = stable disease; sFLC = serum free light chain; sFLCκ/λ = serum free light chain kappa/lambda; SMM = smoldering multiple myeloma; SPEP = serum protein electrophoresis; TAD = thalidomide/adriamycin/dexamethasone; tmt = treatment; UPEP = urine protein electrophoresis; VAD = vincristine/adriamycin/dexamethasone; VGPR = very good partial response; VMP = bortezomib/melphalan/prednisone; VTP = bortezomib/thalidomide-/prednisone.

[a] "…serum samples for investigating response bsed on sFLC; hence, this sample was focus of study."

[b] All patients received chemotherapy regimens containing at least one novel agent (thalidomide, bortezomib, or lenalidomide).

[c] sFLCκ/λ classified as normal when values were 0.26 to 1.65 and abnormal when values were <0.26 or >1.65.

[d] Of interest to this particular report (e.g. not including analyses of BM infiltration, etc.)

[e] Those patients at very high-risk of progressing to symptomatic multiple myeloma.

APPENDIX 4: Critical Appraisal of Included Publications

Table 3: Strengths and Limitations of Included Primary and Secondary Studies[a]

Strengths	Limitations
Systematic Review (Comparative Effectiveness)	
Rao, 2012,[1] AHRQ	
• Obtained input from clinical experts during the topic refinement phase. • Clear and decisive research questions and inclusion criteria established *a priori.* • Comprehensive literature search (with one issue: see limitations) and rigorous study selection. • Double author independent study selection.	• Potential for selection bias as only English language studies were searched. • Meta-analysis not performed; however, this was due to the heterogeneity of the included studies. • List of included and excluded studies not provided. • As per the author`s discussion (more of a limitation to the evidence they acquired), few studies were available to test the comparative effectiveness of sFLC to other standardized tests. In addition, newer advances were not looked at as a comparator, e.g. positron emission tomography, and studies were not powered for comparisons with PCD subgroups (e.g. NSMM). • No declaration of conflicts of interest.
Randomized Controlled Trial	
Paiva, 2011[13]	
• Prospective RCT blinding during test result assessments. • Clearly stated objective, intervention, and outcomes. • Patient characteristics were provided. • Conflicts of interest declared.	• Open label • Out of the 260 enrolled, only 102 patients had their serum samples based on sFLC results available; hence, smaller sample size then originally intended. • Patients with suboptimal response to either treatment arm were not referred for investigation; therefore, potentially not representative of full elderly population with MM. • Patients lost to follow-up not documented.
Non-Randomized Studies	
Hansen, 2014,[7]	
• Clearly stated objective, intervention, and outcomes. • In depth patient characteristics were provided. • Used standardized and accepted test (M-protein assessed in urine and serum) alongside that of the sFLC test.	• Small sample size (n=36). • Short study duration (6 weeks). • Many different variations of treatment regimens with the small sample sizes; therefore, potential to underestimate or overestimate the effect of the treatment regimens.

Table 3: Strengths and Limitations of Included Primary and Secondary Studies[a]	
Strengths	**Limitations**
• Missing data were accounted for using linear interpolation.	• No declaration of conflicts of interest.
Jo, 2014[8]	
• Clearly stated objective and outcome. • In depth patient characteristics provided. • Used standardized and accepted test (IFE) alongside that of the SPEP/UPEP test to confirm presence of APB. • Conflicts of interest declared.	• Not a study on the test per se; rather the presence of APB (obtained using either/or SPEP or UPEP). • Chemotherapy regimens not thoroughly described. • As per the authors, patient characteristics were not identical between subgroups.
Iwama, 2013[14]	
• Clearly stated objective, intervention, and outcomes. • Patient characteristics were provided. • Other tests performed concurrently along with sFLC test. • Conflicts of interest declared.	• Retrospective analysis (computerized medical record system). • Relatively small sample size (n=126). • Patients underwent a variety of different treatment regimens (with some patients not treated with the newer novel agents).
Kastritis, 2013[5]	
• Prospective study design. • Clearly stated objective, intervention, and outcome. • Patient baseline characteristics provided • Other tests performed concurrently to verify analysis. • Conflicts of interest declared.	• Not specifically looking at the tests per se; but rather what aberrant FLC ratios and increases in M proteins mean in AMM patients at high risk of progression to symptomatic MM. • Small sample size (n=96).
Larsen, 2013[6]	
• Clearly stated objective, interventions, and outcomes. • Patient baseline (time at SMM diagnosis) characteristics provided. • Patients identified as SMM according to IMWG definition; therefore, representative of those with SMM. • Conflicts of interest declared.	• Retrospective analysis (from computerized database). • Unclear of what happened to patients that were unaccounted for at different time points. • Not statistically powered to analyze cytogenetic or other risk factor subgroups.
Xu, 2013,[15]	
• Clearly stated objective, intervention, and outcomes.	• Small sample size (N=122). • Specific for newly diagnosed patients with MM

Table 3: Strengths and Limitations of Included Primary and Secondary Studies[a]

Strengths	Limitations
• In depth patient baseline characteristics provided. • Healthy volunteers used as controls for measurements. • Other tests performed concurrently to verify analysis. • Measurements of SPEP, serum and urine IFE, levels of serum and urine M-protein, BMPC, LDH, CRP, β2-microglobulin also performed. • Conflicts of interest declared.	and not representative of patients that had relapse/refractory MM. • FLC ratio cut-offs were wider than in other studies. • FLC κ/λ ratio cut-off of <0.04 or >25 was chosen arbitrarily in order to separate the cohort into groups of comparable sizes.
Radovic, 2012,[16]	
• Prospective study design. • Two other measurements were obtained (β2-microglobulin and serum albumin).	• Small sample size (n=101). • Objective not clearly stated. • No patient characteristics provided. • No declaration of conflicts of interest.
Kaplan, 2011[17]	
• Clearly stated objective. • Two measurements (creatinine and BJP) and the calculations (for BJP) were included to verify analysis. • SOP laboratory procedures, urine collection procedures, and instrumentation were consistent and instrumentation was used in accordance with manufacturer specifications. • Conflicts of interest declared	• Retrospective analysis (hospital laboratory database) • Small patient sample (n=14) with a total of 135 samples • No patient characteristics or demographics were provided
Uljon, 2011,[18]	
• Clearly stated objective, intervention, and outcome. • Used standardized and accepted tests alongside that of the sFLC test. • SOP laboratory procedures, urine collection procedures, and instrumentation were consistent and instrumentation was used in accordance with manufacturer specifications.	• Small sample size (N=17). • Only patients with relapsed/refractory MM were included; therefore, generalizability limited. • No control patients included; therefore, restricted to this population receiving this one type of intervention. • No patient characteristics provided, save the subtype of MM. • No declaration of conflicts of interest.

APB = abnormal protein band; AMM = symptomatic multiple myeloma; BJP = Bence-Jones protein/proteinuria; BMPC = bone marrow plasma cells; CRP = C-reactive protein; FLC = free light chain; FLC κ/λ = free light chain kappa and lambda; IFE = immunofixation electrophoresis; IMWG = International Myeloma Working Group; LDH = lactate dehydrogenase; MM = multiple myeloma; M-protein; monoclonal protein; NRS = non-randomized study; NSMM = non-secretory multiple myeloma; PCD = plasma cell dyscrasias; PFS = progression free survival; RCT = randomized controlled trial; sFLC = serum free light chain; SMM = smoldering multiple myeloma; SOP = standard operating procedure; SPE(P) = serum protein electrophoresis; electrophoresis; UPEP = urine protein electrophoresis.

[a] Amstar[10] was used to help guide the critical appraisal of the systematic comparative effectiveness review, while Downs and Black[11] was used to help guide the critical appraisal of both the randomized controlled trial and the non-randomized studies.

Table 4: Strengths and Limitations of Guidelines[a]	
Strengths	**Limitations**
BSH, 2010[2]	
• These guidelines represent a major revision to the 2006 version of the guidelines. • Those involved in the development of the guidelines included experts, professionals, and a patient advocacy group. • Methods for formulating recommendations and production of guidelines were provided. • Recommendations were clearly described and graded. • Guidelines and recommendations were externally reviewed. • Conflicts of interest declared.	• Objectives not clearly described. • Unclear if systematic review was used to identify the evidence. • List of included and excluded studies not provided. • Poor link between recommendations and supporting evidence.

BSH = British Society for Haematology.
[a] AGREE II[12] was used to guide the critical appraisal of the guidelines.

APPENDIX 5: Levels of Evidence and Grading Schemes

Table 5: Levels of Evidence and Grading of Recommendations[a]	
Quality or Level of Evidence	**Grading/Strength of Recommendation**
BSH, 2010[2]	
Ia - Evidence obtained from meta-analysis of randomised controlled trials **Ib** - Evidence obtained from at least one randomised controlled trial **IIa** - Evidence obtained from at least one well-designed, non-randomised study, including phase II trials and case-control studies **IIb** - Evidence obtained from at least one other type of well-designed, quasi-experimental study, i.e. studies without planned intervention, including observational studies **III** - Evidence obtained from well-designed, non-experimental descriptive studies. Evidence obtained from meta-analysis or randomised controlled trials or phase II studies which is published only in abstract form **IV** - Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities	**Grade A Evidence level Ia, Ib** - Recommendation based on at least one randomised controlled trial of good quality and consistency addressing specific recommendation **Grade B Evidence level IIa, IIb, III –** Recommendation based on well conducted studies but no randomised controlled trials on the topic of recommendation **Grade C Evidence level IV –** Evidence from expert committee reports and/or clinical experiences of respected authorities

BSH = British Society for Haematology.
[a] Verbatim from the guidelines.

APPENDIX 6: Main Study Findings, Author's Conclusions and Recommendations

Table 6: Summary of Findings of Included Studies

Main Study Findings	Author's Conclusions
Systematic Review (Comparative Effectiveness)	
Rao, 2012,[1] AHRQ	
Insufficient evidence to determine if the sFLC assay result in differing treatment decisions: • No studies available that directly compared sFLC assay to other traditional tests ***Insufficient evidence to determine if the sFLC assay is a better indicator of response to treatment and outcomes when compared to traditional tests:*** • Clinical outcomes were evaluated when the sFLC assay and traditional tests were performed concurrently in prospective study (n=1), retrospective studies (n=10) , uncertain study design (n=1); Quality B (n=3), Quality C (n=8) o In the 8 studies examined patients with MM ▪ 5 of these examined the use of sFLC assay for assessment or prediction of treatment response ▪ Traditional tests were different depending on the study ▪ No evidence found that showed that sFLC response, when compared to traditional tests, was a better predictor of outcomes (i.e. survival) ***Insufficient evidence to determine if the sFLC assay reduces the need for other diagnostic tests in PCD:*** • One retrospective review examined this (Quality C) o Patients had a negative IFE of MM treatment and who had a concurrent bone marrow biopsy or aspiration ▪ Subset of patients had information on sFLC κ/λ ratio ▪ 14% patients with negative IFE had ≥ 5% BMPC; 10% with a normal sFLC κ/λ ratio also had ≥ 5% BMPC • sFLC can be used but bone marrow examination is still required for assessment of response to treatment	***In the determination of whether sFLC assay results in differing treatment decisions:*** • *"Because of the lack of directly applicable data, we rated the evidence as insufficient."* page 11 ***In the determination of whether sFLC assay was a better indicator of response to treatment and outcomes:*** • *",…there was no evidence to ascertain whether sFLC response was a better predictor of outcomes than traditional tests in MM. We rated the strength of evidence as insufficient for the sFLC response as a better predictor of survival in AL amyloidosis and insufficient for the sFLC response as a better predictor of survival in MM. "* page 11 ***In the determination of whether the sFLC assay reduces the need for other diagnostic tests:*** • *"The authors recommend that, even if the sFLC assay is used, bone marrow examination should not be eliminated for the assessment of response."* page 12

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
Randomized Controlled Trial	
Paiva, 2011[13]	
Response rates of 102 pts, n: • CR[a] = 44 (43%) • Stringent CR[b] = 31 (30%) • IR = 31 (30%) • Discordant results were observed between sFLC and IFE response criteria n = 22 (22%) ***PFS (in conventional CR by immunofixation [n=44]) comparison:*** *Normal sFLC ratio (stringent CR, n=31) vs abnormal sFLC ratio (n=13)* • 3 year PFS = 69% vs 64%, P = 0.4 • Similar results for TTP (P=0.20) and OS (P=0.9) • Positive immunofixation after induction (PR), trend for longer PFS = 46% vs 26% at 3 years, P = 0.2 o TTP, P = 0.1 o OS, P = 0.3 ***Multivariate Cox regression analysis for PFS:*** • IR was an independent prognostic factor (RR=4.1, 95% CI, 1.4-12.0; P=0.01) • CR and stringent CR not significant	• *"…On comparing the value of achieving stringent CR versus conventional CR after induction therapy, the former response category was associated with slightly better, although not significant, outcome. Even when we reclassified those patients with false positive (discordant) sFLC ratios and considered them as stringent CRs, their outcome was not superior to those in standard CR."* page 1631 • *"In summary, our results demonstrate the value of achieving an IR in elderly patients with MM (not candidates for transplantation) in the era of novel agents. Attaining an IR translates into longer PFS and TTP, which are clearly superior to those of patients in conventional CR or in stringent CR. In our series, achieving maximal responses after treatment (stringent CR plus IR) had a genuine impact on patients' outcome, which showed sustained disease control for up to 3 years. Therefore, an effort should be made to incorporate IR into the routine evaluation of patients with MM."* page 1632
Non-Randomized Studies	
Hansen, 2014,[7]	
Mean reductions or changes as a predictor of response, • *Day 3:* o *iFLC* ▪ ≥VGPR=52.3% ▪ PR=23.6%, (≥VGPR vs PR, P=0.021) ▪ <VGPR=28.6%, (≥VGPR and PR vs <VGPR, P=0.023) o *M-protein* ▪ ≥VGPR=11.3% ▪ PR=7.1%, (≥VGPR vs PR, P=0.631) ▪ <VGPR=4.4%, (≥VGPR and PR vs <VGPR, P=0.347)	• *"…, changes in sFLC are reliable and generally mimic changes in M-protein and intact immunoglobulin."* page 412 • *"…a significant early change in iFLC in the initial days of treatment is not predictive for ultimate response in patients with MM."* page 412 • *"…, a reduction of >80% in iFLC 3 weeks after start of treatment was strongly associated with the achievement of at least VGPR, provided that baseline*

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
• *Day 7:* o *iFLC* ▪ ≥VGPR=77.7% ▪ PR=47.5%, (≥VGPR vs PR, P=0.005) ▪ <VGPR=44.4%, (≥VGPR and PR vs <VGPR, P<0.001) o *M-protein* ▪ ≥VGPR=28.6% ▪ PR=19.0%, (≥VGPR vs PR, P=0.302) ▪ <VGPR=13.5%, (≥VGPR and PR vs <VGPR, P<0.064) ***Mean reductions or changes as a predictor of acquiring VGPR status:*** • *Day 14* o *iFLC* ▪ ≥VGPR=91.4% ▪ PR=69%, (≥VGPR vs PR, P<0.001) ▪ <VGPR=58.4%, (≥VGPR and PR vs <VGPR, P<0.001) o *M-protein* ▪ ≥VGPR=51.8% ▪ PR=34.5%, (≥VGPR vs PR, P=0.001) ▪ <VGPR=23.7%, (≥VGPR and PR vs <VGPR, P=0.012) • *Day 21* o *iFLC* ▪ ≥VGPR=92.3% ▪ PR=59.2%, (≥VGPR vs PR, P=0.003) ▪ <VGPR=50.1%, (≥VGPR and PR vs <VGPR, P<0.001) o *M-protein* ▪ ≥VGPR=63.4% ▪ PR=45.5%, (≥VGPR vs PR, P=0.094) ▪ <VGPR=27.7%, (≥VGPR and PR vs <VGPR, P=0.007) • *Day 42* o *iFLC* ▪ ≥VGPR=96.4% ▪ PR=73.1%, (≥VGPR vs PR, P=0.002) ▪ <VGPR=63.1%, (≥VGPR and PR vs <VGPR, P<0.001) o *M-protein* ▪ ≥VGPR=80.5%	*iFLC > 75 mg/l."* page 412

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
▪ PR=62.2%, (≥VGPR vs PR, P=0.100) ▪ <VGPR=35.2%, (≥VGPR and PR vs <VGPR, P=0.006) ***Sensitivity and specificity (using ROC curves):*** • *Best detection limit* o iFLC reduction of 80% at day 21 if outcome was VGPR or better compared to <VGPR ▪ Sensitivity=94% ▪ Specificity=100%	
Jo, 2014[8]	
• Median follow-up time was 54.1 months ***Factors associated with OS (univariate analysis):*** • *With APB, OS in months:* o Not reached • *Without APB,OS in months:* o 38.3 (P<0.001) • Other statistically significant factors included: serum β_2-microglobulin, serum albumin, age, performance status, serum creatinine, serum calcium, blood hemoglobin, BMPC percentage, and treatment with ASCT ***Factors shown to be prognostic (multivariate analysis):*** • Occurrence of APB o HR=0.21 (95% CI, 0.08-0.52) • Other significant prognostic factors included β_2-microglobulin, serum albumin, serum creatinine, and treatment with ASCT ***Clinical features in patients with APB:*** • More frequently had FLC paraprotein at diagnosis (32.4%) compared to those without • Development of APB higher in those who received ASCT (18.2%) compared to those not receiving it (3.1%) • Median time to APB occurrence from diagnosis 7.9 months (range, 2.2-95.7 months)	• *"In this study, multivariate analysis demonstrated that the occurrence of APB was an independent prognostic indicator of prolonged OS in patients with MM. "* page 468 • *"In conclusion, the emergence ofAPB was associatedwith a favorable prognosis in MM patients."* page 468

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
Iwama, 2013[14]	
Prognostic impact of achieving normal FLCκ/λ ratio: *Patients with normal FLCκ/λ ratio:* • 3 year and median PFS = 64.8% and 40 months • 3 year and median OS = 96.3% *Patients without normal FLCκ/λ ratio:* • Median PFS = 18.4% and 13.5 months (P < 0.001) • Median OS = 38.7% and 29.3 months (P < 0.001) • Survival superior in patients achieving normal compared to those with abnormal FLCκ/λ ratio, regardless of high or low FLC at baseline (both P < 0.001) ***Prognostic impact of FLCκ/λ normalization in various IMWG response groups*** *Patients who obtained normal FLC κ/λ ratio (n=28) compared with those who did not (n=6):* • Normal FLC κ/λ ratio had better PFS survival curves in different groups but not statistically significant (CR, P=0.08; VGPR, P=0.13; PR, P=0.40) • OS significantly better with normal FLCκ/λ ratio (CR, P=0.023; VGPR, P=0.026; PR, P=0.021) *Pooled PR and VGPR results:* • PFS better with normal FLCκ/λ ratio (P=0.01) ***Prognostic factors by multivariate analyses:*** *Significant adverse factors for PFS:* • Age • Abnormal LDH • Not attaining CR • Abnormal FLCκ/λ ratio *Significant adverse factors for OS:* • Age • Abnormal LDH	• *"…normalization of FLCκ/λ ratio after treatment in patients with MM is an independent prognostic factor for longer overall survival regardless of baseline FLC concentration and treatment."* page 140

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
• Not attaining CR • Abnormal FLC κ/λ ratio • ISS (stage >2) • Hemoglobin (≤10 g/dl)	
Kastritis, 2013[5]	
Risk factors for progression from asymptomatic MM to symptomatic MM: *Extent of BM infiltration, (median time)* • 10-19% BM infiltration 90 months (95% CI of 62-117) • 20-49% BM infiltration 42 months (95% CI of 20-60) • ≥ 50% BM infiltration 31 months (95% CI, 13.5-49), P<0.001 • Increase in BM infiltration by 10% associated with 57% increase of risk to progression (95% CI 33-85%, P<0.001) *Characteristic of patients with "very high risk" AMM, progressing within 18 months (n=12):* • Kappa light chain = 67% • Lambda light chain = 33% • BMPC = 33% • FLC ratio ≥ 8 = 75% • FLC ratio ≥ 100 = 25% • M-protein ≥ 3 g/dl = 33% ***Univariate analysis:*** *Not associated with risk of progression:* • Type of heavy chain (IgG vs IgA) • Type of light chain (κ vs λ) *Associated with significant risk of progression:* • Pts with ≥3 g/dl of M-protein vs those with ≤3 g/l (HR: 2.36, 95% CI 1.001-5.4; P=0.046) *Time to progression, median time:* • 19 months in pts with BM plasma cells ≥19% and M-protein level ≥3 g/dl • 73 months in pts with BM plasma cells ≥19% and M-protein level <3 g/dl	• *"We believe that for patients at high risk for progression close monitoring, every 1–2 months for 1–2 years and MRI of the spine at diagnosis should be considered. Also, we believe that a FLC measurement should be performed at least at initial evaluation, and that at initial assessment and at 3–6 month intervals all patients should have a 24 h urine collection for protein electrophoresis."* page 952 • *"There is a subgroup of patients with extensive BM infiltration (≥60%) and/ or highly abnormal FLC ratio (≥100), who have a substantial risk of progression to symptomatic disease within the first two years from the diagnosis of AMM."* page 952

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
Pattern of progressive increase of M-protein levels leading to shorter time to progression: • Pts with increase ≥10% in at least 2 consecutive visits (within first year from diagnosis) vs those without M-protein increase (35 vs 66 months, P=0.1) *FLC ratio levels leading to shorter time to progression:* • FLC ratio ≥8 (or ≤1/8) o Median TTP was 55 months vs 73 months for those with FLC ratio <8 (or >1/8), P=0.005 *Abnormal MRI leading to increased significant risk of progression:* • Median time to progression 15 months; HR 5.8, 95% CI 1.84-18.35, P=0.001	
Larsen, 2013[6]	
Results for involved/uninvolved FLC ratio for the progression to symptomatic MM from SMM: *FLC ratio cut-point of ≥100:* • Sensitivity of 16% (95% CI, 11.3-21.9) • Specificity of 97% (94.6-98.4%) • Positive likelihood ratio of 5.1 (2.7-9.7) • Negative likelihood ratio of 0.87 (0.8-0.9) • Positive predictive value of 73 (57.4-85.4) • Negative predictive value of 68.1 (64.0-72.0) *TTP:* • Median TTP = 40 months (95% CI, 33-48), with 35% progressing within 2 years • FLC ratio ≥100 was 15 months (95% CI, 9-17) vs 55 months in the FLC ratio <100 group (95% CI, 46-65); P<0.0001 *Yearly progression to MM:* • In FLC ratio ≥100 o 43% at 1 year (RR=1.5, 1.3-1.8) o 72% at 2 years (RR=2.6, 1.8-3.6) o 87% at 3 years (RR=4.4, 2.7-7.4) • In FLC ratio <100	• *"Our data strongly support the conclusion that a serum involved/uninvolved FLC ratio ≥100 (or if κ/λ ratio is used, ≥100 or ≤0.01) is a highly specific independent biomarker with the ability to identify SMM patients at significantly increased risk of developing end-organ damage because of MM within 2 years."* pages 4 and 5 • *"…our findings show that patients with markedly high FLC ratio (≥100) are at high risk of progression to MM or related disorder within 2 years of diagnosis and hence may be considered candidates for intervention, especially as the mode of progression in this subset is likely to be renal failure in a substantial proportion of patients."* page 6

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
o 16% at 1 year (RR=1.5, 1.3-1.8) o 28% at 2 years (RR=2.6, 1.8-3.6) o 40% at 3 years (RR not provided)	
Xu, 2013,[15]	
Baseline values: • FLC κ/λ ratio outside reference interval (range 0.27-1.35) in 99.2% • sFLC levels highest with IgA MM and light chain MM *Significant correlations of sFLC (κ; λ, respectively) with other clinical factors:* • *Negative correlations* o Hemoglobin (-0.331, P=0.012; -0.426, P<0.001) o Platelet count (κ only -0.342, P=0.009) • *Positive correlations* o BMPCs (0.302, P=0.022; 0.316, P=0.029) o LDH (0.411, P=0.002; 0.318, P=0.040) o CRP (0.617, P<0.001; 0.515, P=0.005) o β_2-microglobulin (0.336, P=0.013; 0.295, P=0.044) o Creatinine (λ only 0.329, P=0.029) o 24-hour urine (λ only 0.427, P=0.011) *sFLC predictability of OS (based on median light chain level (κ=180 mg/l, λ=592.5 mg/l):* • OS significantly inferior in higher levels (κ ≥180 mg/l or λ ≥592.5 mg/l) compared with lower levels (κ <180 mg/l or λ <592.5 mg/l), P=0.001) • 1 year OS = 70.1 ± 5.8% and 94.3 ± 3.2% in higher vs lower levels, respectively • 3 year OS = 30.5 ± 8.8% and 66.2 ±8.2% in higher vs lower levels, respectively • Median OS = 23 months vs not reached (P=0.001) in higher vs lower levels, respectively *FLC κ/λ ratio predicts OS (at FLC κ/λ ratio cut-off of <0.04 or >25):* • OS significantly inferior in pts with high a FLC κ/λ ratio (<0.04 or >25, n=59) at 48% compared with those with low FLC κ/λ ratio (between 0.04 and 25, n=62) at 51.2% (P<0.001) • Median survival was 21 months and not reached in high vs low FLC κ/λ ratio,	• *"Baseline sFlc levels and rFLC successfully predicted the overall survival for patients with NDSMM, even under the bortezomib therapy regimen. Furthermore, addition of the rFLC to the ISS improved the prognostic potential of the latter."* page 131

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
respectively (P<0.001) • 1 year OS = 71.7 ± 6.0% and 91.8 ± 3.5% in high vs low FLC κ/λ ratio, respectively • 3 year OS = 29.2 ±8.3% and 61.8 ± 9.4% in high vs low FLC κ/λ ratio, respectively *Conventional chemotherapy group:* • Median OS in low vs high FLC κ/λ ratio was 44.0 months (95% CI, 40.0-48.0) and 32.0 months (95% CI, 28.4-35.6), respectively (P=0.001) *Bortezomib group:* • Median OS in low vs high FLC κ/λ ratio was 56.0 months (95% CI, 33.3-78.7) and 39.0 months (95% CI, 24.4-53.6), respectively (P=0.005)	
Radovic, 2012[16]	
In MM group at diagnosis, n: • FLC concentrations deviating from reference interval = 89 (93.7%) • FLC ratios within reference interval (0.26-1.65) = 12 (12.6%) o Median survival = 51 months (range, 33-69 months) • Highly abnormal FLC ratios (<0.03 or >32) = 25 (26.4%) o Median survival = 22 months (range, 16-28 months) • Intermediately abnormal FLC ratios (<0.125 or >8) = 29 (30.5%) o Median survival = 30 months (range, 22-38 months) • Low abnormal FLC ratios (<0.26 or >1.65) = 29 (30.5%) o Median survival = 39 months (range, 27-51 months) *In NSMM group at diagnosis, n:* • FLC concentrations deviating from reference interval = 3 (100%) • Low abnormal FLC ratios (<0.26 or >1.65) = 3 (100%) o Lower RR of disease progression and rather good prognosis	• *"The existence of a significantly abnormal FLC ratio in the examined groups represents an independent risk factor for disease progression and poorer prognosis. The reduction of FLC ratio and monoclonal Ig to normal values, under the influence of the applied therapy indicates good response and adequate choice of therapy. Based on all the presented results, we can generally conclude that quantification of FLCs (FLC ratio) is necessary and represents a new diagnosis of paraproteinemias."* page 115
Kaplan, 2011[17]	
24-hour creatinine collections: • Coefficients of variation ranging from 12-30% *Maximum difference BJP excretion:*[c] • Differences ranged from -1588 to 2315 mg/dl	• *"Our data validate that 24-hour collections are inconsistent in practice and can lead to mistakes in quantifying BJP."* page 1050 • *"At a minimum, one should verify accuracy of 24-hour*

Table 6: Summary of Findings of Included Studies	
Main Study Findings	**Author's Conclusions**
o Strong correlation between these differences and differences in 24-hour creatinine values (correlation coefficients - range 0.43-0.99)[d] *Using random urine samples (no less than 10 days from 24-hour urine samples) to estimate BJP:* • 23 samples from 11 pts o 18 samples (78%) had excellent agreement with clinical scenario and normalized BJP on corresponding 24-hour sample[e]	*urine collection by checking 24-hour creatinine excretion before using it to estimate 24-hour BJP excretion in the traditional way."* page 1050 • *"It appears likely that one can use protein to creatinine ratios from random urine samples to estimate 24-hour BJP excretion."* page 1051
Uljon, 2011,[18]	
• *N=17, 1005 measurements taken, n:* o sFLC=704 o SPEP=897 o IgGA=851 *Monitoring of disease status:* • No significant differences in ability between sFLC and electrophoretic M-spike measurement o Both measurements trended together, regardless of disease advance or regression on therapy (14/17) o In n=1, sFLC lagged behind M-spike o In n=1, sFLC indicated earlier change in disease course o In n=1, sFLC did not detect progression	• *"…in all but one of 17 cases reviewed, SFLC measurements did not show changes earlier than M-spike measurements. Additionally, we observed short-term minor fluctuations that reversed on subsequent measurement, some correlated with bortezomib administration, but without obvious correlation to tumor burden or quantitative M-spike."* page 567 • *"Therefore, for practical patient management in this population of patients with refractory/relapsed intact Ig myeloma, SFLC measurements did not appear to add value as an adjunct to conventional SPEP with M-spike quantitation."* page 567

AMM = asymptomatic multiple myeloma; ASCT = autologous stem cell transplant; BJP = Bence-Jones proteinuria; BM = bone marrow; BM = bone marrow; BMPC = bone marrow plasma cells; CI = confidence interval; CR = complete response; CRP = C-reactive protein; FLC = free light chain; FLCκ/λ = free light chain kappa/lambda; Hb = hemoglobin; HR = hazard ratio; IgGA = individual Ig heavy chain assessment by nephelometry; IMWG = International Myeloma Working group; ISS = international staging system; LDH = lactate dehydrogenase; MM = multiple myeloma; M-protein = monoclonal protein; NSMM = non-secretory multiple myeloma; OS = overall survival; PCD = plasma cell dyscrasias; PFS = progression free survival; PR = partial response; rFLC = FLC κ/λ; RR = relative risk; ROC = receiver operating characteristic; SD = stable disease; sFLC = serum free light chain; SMM = smoldering multiple myeloma; SPEP = serum protein electrophoresis; TTP = time to progression; VGPR = very good partial response; vs = versus.

[a] "Achievement of CR was defined as the absence of M-component by immunofixation and less than 5%plasma cells in the bone marrow."

[b] "Achievement of CR was defined as the absence of M-component by immunofixation and less than 5%plasma cells in the bone marrow. For stringent CR, patients also required normalization of the sFLC ratio."

[c] "For each patient, the maximum difference between BJP excretion based on the submitted collection and normalized to the patient's mean creatinine excretion."

[d] "The correlation coefficient here represents the relationship between (submitted BJP 2 normalized BJP) and (submitted 24-hour creatinine 2 mean 24-hour creatinine). A value of 1.0 indicates that the change in BJP was completely related to changes in sample collection; a value of 0 indicates that the change in BJP was related entirely to other factors (eg, a genuine change in tumor burden)."

[e] "…defined excellent agreement in 2 ways: values whose protein to creatinine ratios were within 0.2 (10 cases) or values different by more than 0.2 but consistent with improvement or deterioration as described in clinical notes or reflected in independent laboratory data (8 cases)."

Table 7: Summary of Guideline Recommendations[a]
Recommendations
BSH, 2010[2]
• *"Monitoring of patients with asymptomatic myeloma should include regular (typically 3 monthly) clinical assessment for the emergence of ROTI and measurement of serum and urinary M-protein (and SFLC when indicated). Repeat bone marrow examination and skeletal imaging should be considered prior to the start of treatment. (Grade C; IV)"* page 9 ***Measuring Response to Therapy:*** • *"The SFLC assay should be used to assess response in all patients with light chain only, non-secretory and oligosecretory disease. (Grade C, level IV)"* page 15

ASCT = autologous stem cell transplant; BSH = British Society for Haematology; MSAG = Medical Scientific Advisory Group; ROTI = myeloma-related organ and tissue impairment; SFLC = serum free light chain.
[a] Recommendations are provided verbatim.