Serum free light chains

1 Name and description of analyte

1.1 Name of analyte
Serum free light chains (SFLC)

1.2 Alternative name
Free light chains, kappa (κ) or lambda (λ) free light chains, paraprotein, M-protein (monoclonal protein).

1.3 This heading is not used

1.4 Description of analyte
SFLC are immunoglobulin light chains, produced in excess by the plasma cells of the immune system. The majority of light chains exist covalently bound to heavy chains as intact immunoglobulins. Free light chains are either kappa (22.5 kDa monomer) or lambda (45 kDa dimer), and are filtered at the glomeruli and reabsorbed for proteolysis in the proximal convoluted tubules. SFLC have a short half-life (2-4 h). Due to its smaller size, kappa is preferentially filtered at the glomeruli; therefore in health serum concetrations of lamba are approximately twice those of kappa. To overload tubular reabsorption, production must overcome the kidney's metabolism of FLC (10–30 g/24h); therefore increases in FLC are seen first in serum, rather than urine.

1.5. Function(s) of analyte
Serum free light chains have no known function; they are excess light chains arising from immunoglobulin synthesis.

2 Sample requirements and precautions

2.1 Medium in which measured
Serum is routinely used for quantification of SFLC. Electrophoresis is often performed on the same sample and requires serum to prevent the confounding peak of fibrinogen found when plasma is electrophoresed.

2.2 Precautions re sampling, handling etc.
After clotting, serum should be separated by centrifugation as soon as possible: there is no information on stability in un-centrifuged whole blood. Serum is stable for up to 21 days at 2–8°C, or frozen for long term storage.

3 Summary of clinical uses and limitations of measurements

3.1 Uses
SFLC measurement aids in the initial diagnosis and monitoring of multiple myeloma (MM), lymphocytic neoplasms, Waldenstrom's macroglobulinaemia, light chain deposition disease and AL amyloidosis. All of these disease states result from plasma cell dyscrasia, where a single (or select few) plasma cells proliferate clonally. Upon clonal plasma cell expansion, a particular FLC increases in concentration disproportionately to others, therefore giving rise to a raised 'involved' FLC (iFLC) concentration (either kappa or lambda) and an abnormal κ/λ ratio. Polyclonal rises in FLC may also give rise to raised light chain

concentrations; however, the κ/λ ratio should remain within reference intervals. This may occur during increased production of immunoglobulins such as during infection, or during decreased clearance of light chains as when glomerular filtration rate is reduced.

The majority of patients with a monoclonal gammopathy will have an intact paraprotein consisting of both heavy and light immunoglobulin chains. However, the majority of these will also secrete FLC and up to 20% of MM patients will secrete only light chains. As well as the malignant monoclonal gammopathies, non-malignant conditions such as monoclonal gammopathy of uncertain significance (MGUS) and smouldering multiple myeloma are also monitored with SFLC analysis due to the risk of progression to MM associated with these conditions.

In AL amyloidosis and light chain deposition disease, symptoms may be caused by precipitation of excess free light chains in various organs, particularly the kidneys and heart.

The measurement of SFLC in these conditions gives a more sensitive, and quantitative measure of paraprotein level than electrophoresis-based methods which are used in conjunction with SFLC analysis.

3.2 Limitations

- Detection of monoclonal gammopathy cannot be made using SFLC alone. NICE guideline NG35 recommends the use of serum protein electrophoresis in conjunction with SFLC measurement when screening for multiple myeloma, followed by immunofixation when a new paraprotein is detected.
- Absolute change in SFLC may be misleading as a marker of tumour burden owing to the non-linear results given by some samples at different dilutions, antigen excess, and polymerisation effects.
- In some patients, particular light chain epitopes may not be recognised by the SFLC assay given the diversity of light chains produced, but they may be visible on serum electrophoresis.
- The utility of SFLC has not been established in paediatric patients.

4 Analytical considerations

4.1 Analytical methods

Most laboratories use The Binding Site's Freelite® immunoturbidimetric assay or Siemens' immunonephelometric assay (in August 2017, of 171 labs registered on the NEQAS SFLC scheme, 128 labs used Freelite® and 35 labs used Siemens' nephelometric method; 8 labs were listed as using 'other' methods). These assays both use latex particle enhancement to improve sensitivity. Reagent antibodies bind to epitopes only accessible when light chains are free of heavy chains, thus reducing interference by intact immunoglobulins. Sandwich ELISA assays are also available. Assays may use either monoclonal or polyclonal antibodies to free light chains. Since the international guidelines for SFLC analysis in myeloma refer to the polyclonal Freelite® assay, this assay will be the focus of this monograph. Monoclonal antibody-based assays are suggested to have reduced cross-reactivity with intact immunoglobulins, but assays using polyclonal antibodies are able to detect diverse light chain variations and therefore are generally considered more sensitive.

4.2 Reference method

No current reference method.

4.3 Reference materials

No current reference material.

4.4 Interfering substances

Due to the turbidimetric nature of the Freelite® SFLC assay, highly lipaemic or haemolysed samples may be subject to interference. Additionally, high concentrations of intact paraproteins (particularly those of the IgM type) may cause falsely elevated results. High concentration of circulating immune complexes may also cause non-specific light scattering. The extent and direction of interferences published by The Binding Site for the Freelite® assay are shown below:

- Haemoglobin (3 g/L): positive 2.1% κ, negative 1.6% λ
- Intralipid® (0.1 and 0.3%): negative 9.1% κ, negative3.0% λ respectively
- Bilirubin (342 μmol/L): negative5% κ and negative2.4% λ
- Immunoglobulins: 10 g/L IgG, 2 g/L IgA, 1 g/L IgM: <3% κ and <9% λ
- Rheumatoid factor (320 IU/mL): <16% κ and <9% λ

Methods using sandwich ELISAs use enzyme-coupled detection antibodies with a colourimetric end-point. Therefore, these assays are also subject to interference from icteric, lipaemic or haemolysed samples.

4.5 Sources of error

The quantitation of SFLC using immunoturbidimetry or immunonephelometry is subject to several limitations. Firstly, antigen excess can result in underestimation of SFLC concentration owing to the formation of smaller immune complexes and reduced light scatter. The Freelite® assay has an in-built algorithm that looks for antigen *excess* based on the reaction kinetics, and automatically dilutes the sample for re-testing. However, not all reactions with antigen excess will be identified this way, and consequently, some falsely low SFLC results may be reported.

Secondly, SFLC concentrations may appear non-linear when different dilutions are used (for example if a sample gives a 'greater than' result at the on-board dilution, an additional manual dilution pre-analysis may give a lower result). This is due to diluting-out interfering substances such as lipids, haemoglobin or other aggregating proteins. For non-linear samples, it is recommended that repeated measurements at increasing dilutions be performed until two similar results are obtained and any interferences are diluted out (Tate *et al*. 2009); however, a knowledge of which samples give non-linear results is required for this. Therefore it may be more realistic to have a strictly adhered to dilution protocol (either provided by manufacturer or developed in-house) in order to provide consistency in SFLC quantification.

Thirdly, some FLC will polymerise at high concentrations. Polymerisation can result in over-estimation of SFLC owing to the formation of larger immune complexes and greater light scatter.

These sources of error highlight the importance of interpreting SFLC results alongside both clinical details and other laboratory investigations such as serum electrophoresis. Additionally, a single measure of SFLC should not be used to monitor patient treatment or disease regression – the general trend in SFLC over time (bearing in mind dilutions used to obtain those results) should be used in conjunction with serum electrophoresis where possible.

5 Reference intervals and variance

5.1.1 Reference interval (adults)
(Method: Freelite®, The Binding Site. Serum from healthy males and females, age 21–90. Katzmann *et al*. 2002.)
κ/λ ratio: 0.26–1.65
κ light chains: 3.3–19.4 mg/L
λ light chains: 5.7–26.3 mg/L

5.1.2 Reference intervals (others)
The reference interval for κ/λ ratio in patients with renal dysfunction is higher than that for patients with adequate renal function as filtration of light chains by the glomeruli is impaired. Kappa light chain concentrations rise to a greater degree than lambda when excretion is impaired owing to reduced glomerular filtration because serum FLC concentrations are then more heavily influenced by the higher rate of kappa light chain synthesis.
Renal κ/λ ratio: 0.37–3.1 (Method: Freelite®, The Binding Site. 142 patients (male and female, age 19–88) with new dialysis-dependent renal failure. Hutchison *et al*. 2008.)

5.1.3 Extent of variation
Biological variation data for SFLC is limited. The values quoted below are from 21 healthy individuals (Braga *et al.* 2013). SFLC variation in patients with disease may vary to a greater extent. A biological CV of 27.8% (intraindividual) for the involved FLC in 52 patients with stable monoclonal gammopathy disease was established by Katzmann *et al*. 2011. This large biological variability is likely due to the short half-life of SFLC and the dependence of concentration on plasma cell numbers and renal clearance.

5.1.3.1 Interindividual CV
In healthy individuals:
Kappa: 14.1%, lambda: 27.5%, κ/λ Ratio: 15.3%

5.1.3.2 Intraindividual CV
In healthy individuals:
Kappa: 8.1%, lambda: 7.0%, κ/λ Ratio: 4.5%

5.1.3.3 Index of individuality
In healthy individuals (recalculated from CVA, CVI and CVG):
Kappa: 0.58, lambda: 0.26, κ/λ Ratio: 0.31

5.1.3.4 CV of method (for Freelite® immunoturbidimetric assay (The Binding Site)
across three different reagent lots on three different analysers:
Total precision: kappa 7.3–12.5%, mean 9.7%; lambda 5.0–8.2%, mean 6.9%

Within-run precision: kappa 1.6–3.3% average 2.2%; lambda 2.0–3.4%, average 2.6%
Between-run precision:
Kappa 1.9–4.2% mean 2.8%; lambda 0.0–2.4%, mean 1.5%
Between-day precision:
Kappa 6.6–11.2% mean 8.9%; lambda 4.4–7.2%, mean 6.1%

5.1.3.5 Critical difference

The reference change value has been reported as 55% in patients with a plasma cell disorder (Katzmann et al. 2011). Variability is likely due to the large biological variation in SFLC. Together with the technical limitations of SFLC measurement, critical difference is likely not a good measure of relapse or treatment response. Monitoring SFLC trend over time rather than on the basis of the difference between two samples is preferable.

5.1.4. Sources of variation

- Biological variation
  Impaired clearance owing to renal glomerular dysfunction and increased gamma globulin synthesis in infection/inflammation can give rise to biological variation in SFLC given their short half-life.

- Analytical variation
  The Freelite® assay aims to reduce batch-to-batch variation by using a 'rolling pool' of antisera that contain 90% identity between consecutive batches. However, there is significant variation between different batches of polyclonal antisera. The median between-reagent lot variation for FLC measurement was 19–20% where the 'master antisera lot' was not changed (Tate *et al*. 2007). This source of variation may be reduced in assays that utilise monoclonal antisera.

  Analytical specificity: cross-reactivity with binding of intact immunoglobulins is reported to cause potential overestimation of 10% at normal FLC and immunoglobulin concentrations (Tate *et al*. 2009).

6 Clinical uses of measurement and interpretation of results

6.1 Indications and interpretation

SFLC analysis is indicated for suspected multiple myeloma, known multiple myeloma or monoclonal gammopathy, as well as suspected/known amyloidosis and light chain deposition disease. Patients with non-malignant gammopathies also require repeated SFLC analysis in order to monitor for disease progression (rate of progression is 1% per year for MGUS patients).

Due to the assay limitations discussed above (section 4.5), the concentration of SFLC cannot be used as a definitive marker of malignant disease burden; trends over time may be used as an indicator for effectiveness of therapy or disease relapse.

Up to 20% of multiple myelomas may be light-chain only and undetectable on serum protein electrophoresis, highlighting the importance of SFLC analysis in the diagnosis of these patients. However, not all patients with monoclonal gammopathy have abnormal SFLC results – some may be only detectable with serum protein

electrophoresis, and some may require urine protein electrophoresis if substantial excretion of the light chains is occurring. Therefore, SFLC results must be considered in conjunction with the results from electrophoretic analyses and clinical details.

6.2 Confounding factors

If the patient sample contains a polymerising light chain, the sample does not dilute linearly or if antigen excess is present, spurious results may be obtained (see section 4.5). See section 4.4 for interferences to be aware of when interpreting SFLC results.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

Raised kappa FLC, lambda FLC or a raised κ/λ ratio: malignant plasma cell proliferation such as multiple myeloma, plasma cell leukaemia, Waldenstrom's macroglobulinaemia. AL amyloidosis, smouldering multiple myeloma and monoclonal gammopathy of uncertain significance (MGUS) are also causes of a raised SFLC.

Raised kappa or lambda FLC in the presence of a normal or slightly abnormal κ/λ ratio may indicate renal disease or polyclonal hypergammaglobulinaemia. Therefore, consideration of clinical details and other laboratory findings is essential when interpreting SFLC results.

7.1.2 Investigation

Further laboratory investigation should include serum protein electrophoresis (concurrent with SFLC), urine electrophoresis and immunofixation where a paraprotein is seen on these analyses, to identify the type of paraprotein. Full blood count, calcium and a renal profile may also contribute to differential diagnosis of multiple myeloma given the classic symptoms of raised calcium, renal dysfunction, anaemia and bone lesions (CRAB).

Bone marrow trephine and aspirate biopsy with quantification of plasma cells is typically performed where malignant plasma cell disease is suspected.

Where amyloidosis or light chain deposition disease is suspected, deposits may be seen in specific organ biopsies, particularly from the kidneys. Diagnosis of these conditions is most commonly confirmed with histological staining for deposits.

7.2 Low values

7.2.1 Causes

Low κ/λ ratio, with concurrent raised lambda FLC: monoclonal gammopathies as above for raised ratio.

Low kappa or lambda FLC may be seen in conditions where plasma cell production is suppressed such as in bone marrow failure.

7.2.2 Investigation

As above.

7.3 Notes

The κ/λ ratio is indicative of which FLC is involved and which is uninvolved. Where the ratio is high, kappa is the involved FLC and lambda is the uninvolved FLC, and vice versa.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

κ/λ ratio in patients with MM, AL amyloidosis or light chain deposition disease: specificity 100%, sensitivity 97% (when using the reference range 0.26–1.65). However, if the patient has an infection or a flare of rheumatoid disease, the assay should be repeated at a later date as significantly raised polyclonal FLC may give an abnormal ratio.

The combined sensitivity of FLC ratio when used alongside serum immunofixation was assessed to be 99.5% in 428 patients with urinary monoclonal protein (Katzmann et al, 2006).

Sensitivities for specific conditions:

- intact immunoglobulin multiple myeloma: 95% sensitivity
- light chain only multiple myeloma: 100% sensitivity
- AL amyloidosis: 91% sensitivity, improved to 99% when used in conjunction with immunofixation
- light chain deposition disease: 100% sensitivity
- 'non-secretory' multiple myeloma (diagnosed as such by serum electrophoresis): 68–100% sensitivity (variable between studies).
- smouldering multiple myeloma: 88% sensitivity
- MGUS: 44% sensitivity.

9 Systematic reviews and guidelines

9.1 Systematic reviews

9.2 Guidelines

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; 23:215-224.

Myeloma: diagnosis and management. NICE guideline NG35 February 2016.

International Myeloma Working Group (IMWG) Criteria for the Diagnosis of Multiple Myeloma. October 2015.

9.3 Recommendations

1. Braga F, Infusino I, Dolci A, Panteghini M. Biological variation of free light chains in serum (letter). Clin Chim Acta 2013;415:10-11.

2. Jenner E. Serum free light chains in clinical laboratory diagnostics. Clin Chim Acta 2014; 427:15-20.

Tate J, Bazeley S, Sykes S, Mollee P. Quantitative Serum Free Light Chain Assay – Analytical Issues. Clin Biochem Rev 2009; 30:131-140.

Katzmann J, Snyder MR, Rajkumar S *et al*. Long-term biological variation of Serum Protein Electrophoresis M-Spike, urine M-spike, and monoclonal serum free light chain quantification: implications for monitoring monoclonal gammopathies. Clin Chem 2011; 57(12):1687-1692.

Katzmann J, Clark R, Abraham R 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: 1437-1444.

Katzmann J, Dispenzieri A, Kyle R *et al*. Elimination of the need for urine studies in the screening algorithm for monoclonal gammapathies by using immunofixation and free light chain assays. Mayo Clinic Proc 2006; 81(12):1575-1578.

Hutchison C, 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: 11.

Tate J, Mollee P, Dimeski G *et al*. Analytical performance of serum free light-chain assay during monitoring of patients with monoclonal light-chain diseases. Clin Chim Acta 2007; 376(1-2):30-36.

10 Links

10.1 Related analytes

Serum immunoglobulins, total protein (for quantification of paraprotein bands on electrophoresis), β_2-microglobulin (used in the International Staging System for myeloma).

10.2 Related tests

Serum electrophoresis, urinary protein electrophoresis (Bence-Jones protein electrophoresis), immunosubtraction, immunofixation.
These electrophoresis-based assays are used in conjunction with SFLC to detect and type paraproteins during the diagnosis and monitoring of monoclonal gammopathies. No single assay should be used in isolation for this purpose.

Author: Dr Helen Valentine

9.ix.2017