16 - Nonsecretory multiple myeloma

In nonsecretory multiple myeloma, sFLC measurements:

- Are abnormal in the majority of patients and are important for diagnosis.
- Can be used for monitoring, without the need for frequent bone marrow biopsies or radiological scans.
- Are recommended by international guidelines for diagnosis and monitoring.
- Allow patients to be included in clinical trials from which they were previously excluded.

16.1. Introduction

Nonsecretory multiple myeloma (NSMM) accounts for 1 - 5% of all multiple myeloma (MM) cases [1][2]. The disease is characterised by the absence of detectable monoclonal proteins in serum and urine using immunofixation electrophoresis (IFE), although the degree of bone marrow plasma cell infiltration is similar to that found in secretory patients [1][3][4][5]. In nonsecretory patients monoclonal proteins can usually be detected immunohistochemically in bone marrow plasma cells (BMPCs) indicating that the tumour cells produce, but may not secrete, monoclonal immunoglobulins into the blood. Also, using highly sensitive tests such as isoelectric focusing, monoclonal proteins have been detected in the sera of some patients [6]. Ultimately, only 10 - 15% of NSMM patients are true “non-producers” in whom tumour plasma cells contain no detectable immunoglobulins [7].

As electrophoresis procedures for detecting monoclonal proteins have become more sensitive and reliable, fewer patients are now diagnosed with NSMM. Yet, even in expert hands, 2 - 3% of patients with MM have undetectable serum or urine monoclonal proteins by IFE and are classified as NSMM [3][8][9]. From a logical standpoint, such patients cannot be producing significant amounts of intact monoclonal immunoglobulins; IgG molecules accumulate in serum with a half-life of 3 - 4 weeks, so their production from even small clones of plasma cells can be visualised as monoclonal bands on serum protein electrophoresis (SPE) gels. By contrast, free light chains (FLCs) have a serum half-life of only 2 - 6 hours, 100- to 200-fold less (Section 3.5). Clonal production of monoclonal FLCs, therefore, needs to be correspondingly much greater to produce similar serum concentrations of monoclonal protein to those found in IgG-producing MM. NSMM patients are
therefore more likely to be producing low amounts of monoclonal FLC. Urinalysis may also be unhelpful because patients with NSMM usually have normal renal function. The modest monoclonal FLC production, typically seen, may not be sufficient to damage or overwhelm the reabsorption capacity of the kidneys and enter the urine (Section 3.5.2). Hence, more sensitive methodologies are required when the production of FLCs is too low for detection by electrophoresis techniques.

16.2. Diagnosis of nonsecretory multiple myeloma

The above arguments suggest that sensitive assays for sFLCs might detect monoclonal proteins in a proportion of patients with NSMM. The results from a large study are shown in Table 16.1. Archived sera were obtained from patients studied in MRC MM trials undertaken in the UK between 1983 and 1999. Of 2323 patients, 64 (2.8%) were diagnosed with NSMM and, of these, 28 were selected for study based on the availability of complete clinical records and the appropriate stored serum samples. In all patients, concentrations of κ and λ sFLCs were compared with results from SPE and IFE tests. The results showed that 19 of the 28 sera had abnormal κ/λ sFLC ratios and elevated κ or λ sFLC concentrations. A further four samples showed abnormally low levels of one or both sFLCs. sFLC concentrations in the remaining five samples were substantially normal (Figure 16.1 and Table 16.2).

<table>
<thead>
<tr>
<th>Classification based on sFLCs</th>
<th>κ sFLC (mg/L)</th>
<th>λ sFLC (mg/L)</th>
<th>κ/λ sFLC ratio</th>
<th>BMPC %</th>
<th>Other Results</th>
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<td>Serum reference interval</td>
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<td>8.1 - 33</td>
<td>0.36 - 1.0</td>
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<td>85</td>
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<td>12 patients: elevated free $\kappa$ and increased $\kappa/\lambda$ ratio</td>
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<td>4.5</td>
<td>6</td>
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5 patients: \(\kappa\) or \(\lambda\) normal or elevated and \(\kappa/\lambda\) ratios normal or borderline

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<th></th>
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<th>(\lambda)</th>
<th>Ratio</th>
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<td>25.2</td>
<td>0.341</td>
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</table>

BMPC: bone marrow plasma cell; Hist: immunohistochemical confirmation of MM; IFE +/-: weak diffuse bands; IFE +: weak narrow band; BJP +/-: low concentrations of urine FLCs; ND not detected; *trephine biopsy +ve for MM.

**Table 16.1. sFLC concentrations in 28 patients with NSMM** [1]. Serum reference intervals were the 95% ranges in use at the time of publication.

Careful repeat testing of the 28 sera by IFE, using optimal sensitivity (Table 16.1), showed monoclonal sFLCs in six, but the monoclonal bands were mostly weak and diffuse. Rather surprisingly, in nine of the 28 patients no monoclonal bands were seen using IFE even though the immunoassays indicated sFLC concentrations of >200 mg/L. In many of these samples, the elevated FLC concentrations should have been easily detectable by IFE. IFE gels from five of the serum samples containing high concentrations of \(\kappa\) sFLCs are shown in Figure 16.2. These are compared with three samples from patients with typical \(\kappa\) light chain multiple myeloma (LCMM). The sFLCs in the NSMM samples failed to focus into the same narrow monoclonal bands seen in the LCMM sera.
Two sera from NSMM patients with substantial concentrations of sFLCs measured by nephelometry (980 mg/L and 1700 mg/L), were subjected to size-separation gel chromatography and found to contain highly polymerised FLCs (40 - 200 kDa) (Figure 16.3). This suggested that variable polymerisation caused the monoclonal bands to smear on the SPE gels and this could account for their absence or diffuse appearance. Such large polymers would have minimal renal clearance compared with monomeric FLCs. Good renal function would be maintained (typical of these patients) and little FLC would enter the urine. These observations concur with other reports that describe polymerised or structurally abnormal FLCs in some patients with MM (Section 7.6).

Of additional interest, it was found that diffuse bands were more common in κ FLC-producing patients (Table 16.1). Hence, λ patients with low FLC production are more likely to produce discrete monoclonal bands and be classified as “secretory” LCMM. At one time, the lack of λ NSMM led to the suggestion that such patients may not exist. Moreover, the observed higher frequency of κ FLC polymerisation probably explains the 4:1 ratio of κ to λ NSMM patients reported in the literature.

There have only been two other large studies of sFLC measurements in NSMM. Chawla et al. found an abnormal κ/λ sFLC ratio in 19/29 (65%) NSMM patients at diagnosis, and concluded that overall survival was worse in patients with an abnormal sFLC ratio at baseline compared to those with a normal ratio, similar to findings for MM patients in general (Section 20.2). Migkou et al. reported an abnormal κ/λ ratio in 14/17 (82%) of patients at diagnosis, and nine of these had an involved FLC (iFLC) ≥100 mg/L, which exceeds the threshold required to assess haematological response in clinical trials (Sections 16.3 and 25.3.5).

Other reports have confirmed the diagnostic sensitivity of the sFLC ratio in smaller groups of NSMM patients. Cavallo et al. reported that four of five NSMM patients had abnormal FLC concentrations. Most recently, Jeong et al. found abnormal κ/λ sFLC ratios in two NSMM patients with normal serum and urine electrophoresis and immunofixation results. They commented that a combination of SPE and sFLC analysis was effective in screening for monoclonal gammopathies, including NSMM (Chapter 23).

Whilst elevated sFLC concentrations are found in a high proportion of patients conventionally thought of as non-secretory, normal sFLC results can be indicative of true non-secretory patients or non-producing patients. Papanikolaou et al. reviewed flow cytometry data for 210 NSMM patients and identified 19 that had no cytoplasmic immunoglobulin detected. sFLC analysis of these patients revealed normal sFLC ratios for 18/19, demonstrating concordance with the flow cytometry results. In the one patient with an abnormal sFLC ratio, elevated sFLCs were attributable to grade 3 renal failure. Application of the renal reference interval for the κ/λ sFLC ratio may aid interpretation of results in such cases (Section 6.3). Ma et al. also reported a true ‘non-producing’ NSMM patient in whom normal sFLC results were in agreement with electrophoretic and immunohistochemical analysis.

The widespread routine use of sFLC immunoassays has made the identification of true NSMM even rarer than previously observed, with a frequency in the order of only one in 200 myeloma cases.
16.3. Monitoring nonsecretory multiple myeloma

The assessment of disease burden using routine marrow histology and flow cytometry is notoriously inaccurate, due in large part to the patchy nature of marrow involvement and issues with sampling [2]. The sFLC assays facilitate disease response assessment in most NSMM patients, and can be done with routine blood testing rather than requiring imaging or bone marrow assessment [2]. As sFLC concentrations assess FLC production from all of the bone marrow and extramedullary sites, they are likely to provide a better indication of overall tumour activity than bone marrow aspirations or skeletal surveys. The use of sFLC analysis for monitoring oligosecretory patients including those previously classified as NSMM, is now recommended in international guidelines (Chapter 25).

In the first study of sFLCs in NSMM patients, limited monitoring samples were available from six patients, and revealed elevated sFLC levels at clinical presentation, reduced concentrations during plateau phase, and increased levels at relapse (Figure 16.4) [1]. One patient (no. 2) showed some discordance between the clinical assessment and sFLC concentrations; whilst still being classified as being in remission, rising concentrations of sFLCs indicated imminent disease relapse.

Historically, randomised trials have generally tended to exclude patients with NSMM because they do not have easily measurable disease [19]. However, since the incorporation of sFLC analysis into the International Uniform Response Criteria (IURC) (Section 25.3.5), the majority of NSMM patients can now be enrolled into clinical trials. For example, in the study of 28 NSMM patients described above, 17/28 (61%) patients had measurable monoclonal sFLC concentrations (defined as iFLCs ≥100 mg/L, provided that the sFLC ratio is abnormal) [1]. Similar findings were reported by Migkou et al. [5]. Many patients with NSMM have been studied prospectively since sFLC analysis has been routinely available [20]. Two examples are described in the clinical case histories below.

Clinical case history 1

NSMM with “difficult to assess” symptoms during clinical relapse.
A 38-year-old woman presented with a fractured rib following mild trauma. Over the
following months, the pain subsided but non-specific symptoms including breathlessness,
vague chest pains and tiredness persisted. During this time, full blood counts, erythrocyte
sedimentation rate (ESR) and biochemistry were all normal, as were chest X-rays and lung
function tests. In the absence of a diagnosis, the general practitioner considered a
psychiatric assessment.

Seven months after the initial presentation, she remained symptomatic and was re-investigated, whereupon bone scans and X-rays showed extensive osseous lesions.
Immunoglobulin measurements showed immunoparesis, but no serum monoclonal protein was
detected. She was noted to have hypercalcaemia (2.85 mmol/L - NR: 2.08 - 2.67) but had
normal renal function. In view of the absence of monoclonal immunoglobulins, MM was still
considered unlikely. However, a skull X-ray and CT scan showed osteolytic lesions (Figure 16.5)
so a skull biopsy was performed which was reported as ‘plasmacytoma/NSMM’. She was
given chemotherapy for the following 8 months that resulted in clinical remission.

Seven months later, and over 2 years after the initial presentation, she re-attended hospital
because of chest pains and breathlessness. Again, clinical examination was normal, as were
routine biochemistry and haematology tests. Immunology tests showed reduced
immunoglobulins but no detectable monoclonal protein by serum electrophoresis. A bone
marrow biopsy showed 5% plasma cells that were morphologically normal. Chest X-ray, a
ventilation perfusion scan and lung function tests revealed no evidence of pulmonary disease.
Blood tests were requested for sFLCs, the results of which were: κ 330 mg/L, λ 6.5 mg/L and
an abnormal κ/λ ratio of 51 (Figures 16.1 and 16.6 [week 67]). Doubt was expressed regarding
the validity of the results so sFLC measurements were repeated 2 and 3 weeks later and
showed κ increases to 470 mg/L and then 525 mg/L with a rising κ/λ ratio, confirming
disease recurrence.
sFLC concentrations were assessed retrospectively from archived samples and then the patient was monitored prospectively. Figure 16.6 shows that the κ sFLC concentrations had increased rapidly during the tumour recurrence, with an apparent doubling time of 30 days as indicated by the κ/λ ratio. κ sFLC concentrations subsequently reduced during treatment. During the period of relapse, the λ sFLC concentration increased suggesting reduced renal clearance of FLCs from impaired glomerular filtration (Section 6.3). After a peripheral blood stem cell transplant (PBSCT) κ and λ sFLC concentrations and the κ/λ sFLC ratio returned towards normal as the patient went into clinical remission.

Clinical case history 2

A patient with NSMM/plasmacytoma who was excluded from clinical trials.

A 37-year-old man with pelvic pain was found to have a solitary plasmacytoma located in the right iliac crest. Bone marrow biopsy of the opposite iliac crest was normal and no monoclonal protein was identified in serum or urine. Treatment comprised surgical resection followed by irradiation (5,000 Gy). Subsequently, he remained asymptomatic, but 5 years later a routine skeletal survey showed a thoracic spine lesion at T2, which was irradiated. Over the following 7 years further painful lesions developed. These were identified using different scanning techniques (particularly positron emission tomography) and were treated with irradiation or melphalan and prednisolone.

Throughout this period, and in spite of repeated testing, no monoclonal protein was identified by SPE and UPE. Finally, 12 years after the initial presentation, sFLC immunoassays became available and showed κ 7.5 mg/L, λ 632 mg/L and a κ/λ ratio of 0.01. These results identified a λ-producing tumour with no associated suppression of the κ sFLCs (Figure 16.7). One month later, λ sFLC concentrations had increased to 700 mg/L, prompting treatment with thalidomide (50 mg/day) and dexamethasone (40 mg/week). Over the subsequent 7 months, the λ sFLC concentration gradually fell to 33 mg/L and the κ/λ sFLC ratio began to normalise. Based on the sFLC results, dexamethasone was reduced to 12 mg/week and he remained well and in complete remission.
Figure 16.7 shows the changes in sFLC concentrations over a 12-month period. The effectiveness of the drugs and the doses required can all be monitored during this period of therapy. This has produced clear benefits for the patient and avoided costly scans and painful bone marrow biopsies. Furthermore, since the patient has measureable monoclonal sFLCs (>100 mg/L \[^{[19]}[21]\]) he can be entered into clinical trials of new treatments. The patient has been monitored successfully using sFLC assays for many years since the original tests were performed.

**Figures**

**Figure 16.1.** sFLC concentrations in patients with NSMM compared with normal individuals and patients with LCMM.

- Clinical case history 1.

**View source:**
- 16.2. Diagnosis of nonsecretory multiple myeloma
- 16.3. Monitoring nonsecretory multiple myeloma
Figure 16.2. Serum IFE from five patients with NSMM and three patients with LCMM.

Samples were applied at similar FLC concentrations.

View source:
- 16.2. Diagnosis of nonsecretory multiple myeloma

Figure 16.3. Size-separation gel chromatography showing the FLC size variation in a serum sample from a patient with NSMM.

The sample contained 1754 mg/L of $\kappa$ FLCs by Freelite® immunoassay but was negative by SPE and IFE (Figure 16.2).

View source:
- 16.2. Diagnosis of nonsecretory multiple myeloma

Figure 16.4. Changes in sFLCs and clinical status in 6 patients with
Figure 16.4. Changes in sFLCs and clinical status in 6 patients with NSMM. Numbers refer to patients in Table 16.1. NR: upper limit of normal range. (This research was originally published in Blood © the American Society of Hematology).

View source:
- 16.3. Monitoring nonsecretory multiple myeloma

Figure 16.5. X-ray and computerised tomography (CT) scans of the skull in NSMM.

View source:
- 16.3. Monitoring nonsecretory multiple myeloma

Figure 16.6. sFLC concentrations during the course of the disease in a patient with NSMM.
The changing $\kappa/\lambda$ sFLC ratio is related to the tumour growth. ABCM: adriamycin (doxorubicin), busulphan, cyclophosphamide, melphalan; VAD: vincristine, adriamycin (doxorubicin), dexamethasone; HDM: high dose melphalan; PBSCT: peripheral blood stem cell transplant.

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