18.3. Other uses of sFLC analysis in IIMM response assessment

18.3.1. Rapid assessment of response

The short serum half-life of sFLCs (2 – 6 hours) compared with that of intact immunoglobulins (5 – 7 days for IgA/IgM, or several weeks for IgG; Chapter 3) means that they often provide an earlier indication of response to treatment. In addition, at low serum IgG concentrations, the half-life of IgG is prolonged by FcRn receptor recycling (Chapter 3). In such cases, serum IgG levels are particularly slow to indicate responses to treatment, and sIFE may remain positive long after a tumour has been eradicated (as demonstrated by bone marrow immunophenotyping) [2][3][4].

Early sFLC responses to chemotherapy have been reported by many researchers [3][4][5][6][7][8][9][10]. Such responses are most apparent in IIMM patients treated with drugs that produce a rapid tumour response, such as bortezomib (Velcade). As an example, IgGλ and λ FLC levels for a patient with IgGλ MM are shown in Figure 18.5 [1]. During initial therapy with bortezomib, doxorubicin and dexamethasone (VDD), the serum λ FLC concentration fell rapidly, and had normalised by 21 days following treatment. In contrast, IgGλ only gradually decreased (with a serum half-life of around 30 days), and treatment was continued.

Measurement of sFLCs at short time intervals can provide additional information on the kinetics of response to treatment. For example, Das et al. [4] reported rapid responses to bortezomib in 6 of 8 patients, 3 of whom exhibited repeated falls and rises of...
sFLCs co-incident with treatment cycles (Figure 18.6). The sFLC relapse was very rapid, with doubling times of less than 10 days. By comparison, the intact monoclonal immunoglobulin did not show the same peaks and troughs. Similar observations have been made in other studies using bortezomib. These rapid patterns of response can only be observed with the use of tumour markers - such as sFLCs - that are quickly cleared from the serum, together with short sampling intervals. Early indication of tumour responses by sFLCs could facilitate changes of treatment strategy, which may have a major bearing on cost of treatment and utilisation of resources.

The validity of sFLC measurements as a marker of tumour response has been illustrated by comparison with bone marrow plasma cell counts. In a study comprising 45 IgG MM patients, Mead et al. showed that, during treatment, monoclonal plasma cell counts correlated better with changes in sFLCs (and serum β2-microglobulin) than they did with intact monoclonal IgG (Figure 18.7). κ/λ sFLC ratios had the highest concordance with bone marrow plasma cell counts, and were more accurate than both urine FLCs and SPE/sIFE for assessing disease status (Table 18.1).

<table>
<thead>
<tr>
<th></th>
<th>Bone Marrow</th>
<th>Concurrency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Serum FLCs</td>
<td>Normal</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>5</td>
</tr>
<tr>
<td>Urine FLCs</td>
<td>Normal</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>3</td>
</tr>
<tr>
<td>Serum IFE</td>
<td>Normal</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>14</td>
</tr>
</tbody>
</table>

sFLCs were classified as abnormal if the κ/λ sFLC ratio was outside the normal range; urine FLCs >40 mg/L were classified as abnormal; and bone marrow assessment was classified as abnormal if there were ≥5% plasma cells.

Table 18.1. Comparison of bone marrow plasma cell counts in MM using different tests for monoclonal proteins.

18.3.2. Prediction of overall response

Many studies have indicated that the reduction in sFLCs after one or two cycles of treatment is highly predictive of overall response. For example, Dispenzieri et al. reported that a 90% reduction in sFLCs (90% reduction in the difference between the involved and uninvolved FLC [dFLC]) within 7 days post-ASCT predicted for haematologic complete response (p<0.001). Other studies have similarly highlighted the value of early sFLC reductions in predicting overall response. Looking at a different measure of outcome, Orlowski et al. reported that rapid normalisation of the FLC κ/λ ratio - after the first or second cycle of therapy - was highly predictive of prolonged time to progression. The prognostic value of the sFLC response is discussed further in Chapter 20.

18.3.3. Early detection of ineffective therapy
Monitoring with sFLCs allows early detection of ineffective treatment, and the identification of patients who may benefit from alternative chemotherapy regimens. One such example is shown in Figure 18.8. In this patient with IgGλ MM, the sFLC concentration initially decreased in response to treatment, with a negligible corresponding change in IgG concentration. Subsequently, λ sFLC levels stabilised, indicating that the patient was no longer responding to treatment. This was in contrast to the IgG levels, which were continuing to fall, suggesting a continuing response. Only after several months did IgG levels start to increase, signifying a tumour regrowth.

18.3.4. Early detection of disease relapse

Relapse with paraproteins in the absence of other symptoms of end-organ damage has been termed a ‘biochemical relapse’. Current IMWG guidelines suggest initiating treatment in patients with a rapidly rising paraprotein level with a “doubling time of 3 months or shorter”[19], or by an increase in the serum M-protein ≥ 10g/L, or the urine M-protein by ≥ 500 mg/24hr, or the iFLC ≥ 200 mg/L (with an abnormal sFLC ratio), in 2 consecutive measurements separated by ≤ 2 months[20].

Early detection of disease relapse or progression is important to identify ineffective treatment and to limit severe end-organ damage. Katodritou et al.[21], have demonstrated that median PFS in patients treated at biochemical relapse was significantly increased in comparison to those treated at clinical relapse (24 vs. 13.2 months, p=0.006). This was supported by Sidana et al.[22], who also demonstrated, in a retrospective study, a significant increase in median OS for patients treated at biochemical relapse over those treated at clinical relapse (125 vs. 81 months, p=0.001). Interestingly, this study also identified 13 patients who were treated at clinical relapse but were previously documented to have biochemical relapse, indicating that an observable paraprotein relapse may be detected prior to a clinical relapse.

Since sFLC assays are intrinsically more sensitive than sIFE for detection of monoclonal FLC production (Chapter 4), earlier detection of tumour relapse should be possible in IIMM patients who relapse with FLCs alone or potentially, a proportion of those who relapse with intact immunoglobulins and FLCs. In a study of 187 MM patients, Willenbacher et al.[23] found that relapse was detected a median of 3 months earlier by sFLC levels than by conventional monoclonal protein measurements. Similar findings were reported by Dejoie et al.[24], who reported that of the 228 IIMM patients who progressed by SPE, 6.25% showed an increase in sFLCs that preceded the increase in intact immunoglobulin, by a median of 63 days. In a smaller study by Mösbauer et al.[25], relapse was identified earlier in eight of nine IIMM patients when sFLC ratios were used rather than IFE (median, 98 days; range 35 - 238 days) (Figure 18.9).

In situations where relapse occurs relatively rapidly after successful treatment (i.e. within a few months), due to their relatively long half-life, monoclonal IgG levels may not normalise before relapse. Therefore, falling concentrations of IgG hide the early increases caused by tumour relapse. In contrast, because of their short half-life, an increase in sFLCs at relapse occurs from lower baseline levels and can be more readily detected. This is illustrated in a case study by Fuchida et al.[26] (Figure 18.10).

18.3.5. Monitoring patients treated with monoclonal
Monoclonal antibody (mAb)-based therapies represent a major advance in the treatment of multiple myeloma (MM). However, they can present a challenge to diagnostic laboratories when the therapeutic antibody migrates as a discrete band on serum protein electrophoresis (SPE) and serum immunofixation electrophoresis (sIFE)\(^{[25][26]}\). For example, daratumumab (an IgG\(_κ\) anti-CD38 mAb) migrates as a discrete band in the \(γ\)-region\(^{[26][27][28]}\). Tang et al.\(^{[29]}\) observed that therapeutic mAbs produce a false positive electrophoretic result in the majority of treated patients, which may persist for several months after therapy has ended. The authors noted that in approximately 20% of cases, the mAb was indistinguishable from the tumour-derived monoclonal protein, and therefore may prevent assessment of complete response. The limitations of SPE are further discussed in Section 17.4.

Murata et al.\(^{[26]}\) demonstrated that daratumumab also produces an increase in IgG\(_κ\) concentrations measured by the Hevylite assay. In such cases, Freelite sFLC assays may offer an alternative means of monitoring patients, as they do not recognise intact monoclonal antibodies (Section 5.1). Rosenberg et al.\(^{[28]}\) investigated if daratumumab produced significant interference on Freelite sFLC assays by spiking serum samples from MM patients containing monoclonal intact immunoglobulins (IgG\(_κ\) \(n=20\); non-IgG\(_κ\) \(n=10\)) with the drug. There was no significant effect of daratumumab on the measured \(κ\), \(λ\) or \(κ/λ\) sFLC ratio when the maximal clinically relevant concentration of the drug (1 g/L) was added. In addition, super-therapeutic concentrations of the drug (2 g/L) gave very low \(κ\) sFLC values (0.4 – 1.4 mg/L). The authors concluded that it is unlikely that daratumumab interacts with the FLC assay, and represents a practical alternative to SPE and sIFE to monitor response to therapy. Similar results were reported by Jenner et al.\(^{[30]}\). The use of sFLC assays to monitor intact immunoglobulin patients may become increasingly important as the use of mAb therapy become more widespread and there is increasing awareness of the ability of mAbs to potentially interfere with serum electrophoretic assays.

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**Figures**

**Figure 18.10.** \(λ\) sFLC levels decrease more rapidly than IgG levels in a patient with IgG\(_λ\) IIMM treated with dexamethasone (Dex).

Relapse from initial treatment was masked by slowly decreasing IgG levels but detected by an increase in \(λ\) sFLC. The patient was subsequently treated with bortezomib/dexamethasone (Bor/Dex) and short term falls and rises in sFLC levels correlated with treatment cycles. Such changes were not evident from IgG levels. (This research was originally published in International Journal of Hematology [27] © 2012 by The Japanese Society of Hematology).
18.3.4. Early detection of disease relapse

Figure 18.5. A rapid decrease in $\lambda$ sFLCs gives an earlier indication of response than intact immunoglobulin measurements in a patient with IgG$\lambda$ IIMM.

VDD: Velcade, doxorubicin and dexamethasone.

18.3. Other uses of sFLC analysis in IIMM response assessment

Figure 18.6. Changes in the $\kappa/\lambda$ sFLC ratio during 6 cycles of bortezomib (V) showing rapid responses to treatment and subsequent relapses.

Figure 18.7. Accuracy of different blood tests for assessing bone marrow plasma cell volume in MM.
Because of slow catabolism, IgG concentrations lag behind reductions in bone marrow plasma cell content during treatment.

View source:

- 18.3. Other uses of sFLC analysis in IIMM response assessment

Figure 18.8. A rapid drop in $\lambda$ sFLCs provides an early indication of response to treatment.

By contrast, IgG levels remained high for almost 2 months. Subsequently, a lack of treatment effect was indicated by stable $\lambda$ sFLC levels despite decreasing IgG levels. Several months later, relapse was confirmed by an increase in both $\lambda$ sFLC and IgG concentrations.

View source:

- 18.3.3. Early detection of ineffective therapy

Figure 18.9. sFLCs and IFE during relapse of a patient with MM

[18] (Obtained from Haematologica Journal website: haematologica.org)
References


