28.2. Diagnosis of AL amyloidosis

Early diagnosis of AL amyloidosis is critical, to facilitate swift access to effective chemotherapy, and therefore suppress the production of amyloidogenic FLCs before irreversible organ damage occurs. Whilst the detection of a monoclonal protein does not provide a definitive diagnosis of AL amyloidosis, it does provide supportive evidence of an underlying plasma cell dyscrasia. Guidelines recommend that immunofixation of serum and urine in combination with sFLC analysis provides an efficient diagnostic screen for AL amyloidosis (Section 28.3).

By electrophoretic techniques, a serum monoclonal protein is detected in approximately 80% of patients, and a urine monoclonal protein is detected in approximately 70%. However, the underlying monoclonal gammopathy can be subtle and monoclonal proteins are undetectable in between 5 and 20% of patients, depending upon the sensitivity of the electrophoretic method used (Chapter 4). Figure 28.3 shows a typical serum protein electrophoresis (SPE) result from a patient with AL amyloidosis; it demonstrates a nephrotic pattern (low albumin, elevated α2 and low γ fraction) with no obvious monoclonal protein. Serum immunofixation electrophoresis (sIFE), however, reveals some polyclonal immunoglobulin in the γ region and a monoclonal λ FLC band in the β/γ region. This band is too small to be quantified by scanning densitometry of the SPE gel since it is undetectable against the background proteins. Figure 28.4.

shows the urine protein electrophoresis (UPE) from the same patient. It contains a considerable amount of protein, particularly albumin, and there is a small monoclonal spike. Urine immunofixation electrophoresis (uIFE) indicates a monoclonal λ protein against a background of polyclonal κ and λ FLCs. As with the serum protein, the urine monoclonal band is difficult to quantify (by UPE) and is of modest utility for the purpose of disease monitoring.
There are now numerous published studies comparing the diagnostic performance of sFLC and electrophoretic assays in screening for AL amyloidosis (Chapter 23). Katzmann et al. [2] compared diagnostic screening panels for identifying monoclonal gammopathy in patients suspected of having MM, AL amyloidosis and related monoclonal gammopathies. Focusing on the amyloid patients within this study, there were 581 with a confirmed diagnosis of AL amyloidosis and for these, the diagnostic sensitivity of the sFLC assays was 88.3%, which increased to 97.1% with the inclusion of sIFE (Figure 28.5). Importantly, addition of uIFE to the serum panel increased the sensitivity to 98.1% (representing an additional 6/581 patients), confirming that in only a minority of AL amyloidosis patients, monoclonal FLCs may be detected by urine studies alone (Section 7.7.1).

In a separate prospective study of 121 patients with biopsy-proven AL amyloidosis by Palladini et al. [3], the diagnostic sensitivity of the κ/λ sFLC ratio was 76%. By comparison, the diagnostic sensitivity of sIFE and uIFE was 96%. When AL amyloidosis patients were grouped according to monoclonal FLC type, the diagnostic sensitivity of the κ/λ sFLC ratio was significantly higher for κ clones than λ clones (97 vs. 69% respectively), whereas the diagnostic sensitivity of sIFE was lower for κ clones than λ clones (60 vs. 87%). The authors commented that this difference may be due to the formation of monoclonal κ FLC aggregates of variable size and electrophoretic mobility, resulting in the absence of a detectable monoclonal protein band by serum electrophoresis. They concluded that the diagnosis of AL amyloidosis should not rely on a single test, and that a screening algorithm comprising serum and urine IFE in combination with the κ/λ sFLC ratio had 100% diagnostic sensitivity for AL amyloidosis. A further study by the same group [4] compared the diagnostic performance of the renal reference interval for the κ/λ sFLC ratio (0.37-3.1) to that of the standard normal range (0.26-1.65) in patients with newly diagnosed AL amyloidosis. This is discussed in Section 6.3.

Previous studies on the diagnostic performance of the κ/λ sFLC ratio in AL amyloidosis reported a diagnostic sensitivity ranging from 75% to 98%. [5][6][7][8][9][10]. In the first published study of 262 AL amyloidosis patients at the National Amyloidosis Centre, London, the κ/λ sFLC ratio was associated with a greater diagnostic sensitivity than the combination of serum and urine IFE (98% [based on a 95% normal range] vs. 79%) [5] (Figure 28.6). In all published studies to date, sFLC analysis has proven to be an important complementary technique to IFE for screening for monoclonal gammopathy in patients with suspected AL amyloidosis.

In a study by Kumar et al. [9] patients with AL amyloidosis had median concentrations of involved κ and λ FLCs of 314 mg/L and 194 mg/L, respectively which is considerably lower than those concentrations seen in MM [11]. The generally lower sFLC concentrations seen in AL amyloidosis contribute to a small proportion of patients with discordant sFLC and electrophoresis results. For example, occasionally, patients may have positive uIFE results (for monoclonal FLC) but normal sFLC ratios. This may be due to the timing of serum(urine sample collections or the loss of albumin through the renal glomeruli overloading the renal capacity for protein reabsorption. This and other apparently discordant results are discussed in Section 7.7. Rare patients with AL amyloidosis may be negative by all serum and urine tests, but this is more frequent for patients with localised AL amyloidosis (Section 28.2.1).

With the advent of alternative assays for sFLC measurement, discrepant sFLC quantitations can also occur. Comparisons of Freelite® with the Siemens N Latex FLC assays have highlighted significant variation in results for AL amyloidosis patients, confirming that data from the two assay formats is not interchangeable [12][13][14]. These results are presented in more detail in Section 8.5.8.

Initial reports on the use of mass spectrometry to type amyloid fibrils are promising [15]. For example, Muchtar and co-workers [16] from the Mayo clinic favourably report on the sensitivity of mass spectrometry to type amyloid deposits in a cohort of 557 patients. However Hill and Mollee [15] give more practical consideration to the implementation of this technique, and conclude that technical improvements and large clinical validation studies are required prior to its introduction into routine practice.

28.2.1 Localised amyloid disease

Rather than being a systemic disease, AL amyloidosis may also present as a localised disease, where amyloid deposition is limited to a single organ. The specific area of the body affected depends upon the biochemical nature of the amyloid fibril protein and, consistent with this, Kourelis et al. [17] demonstrated that IGVL gene usage is different between localised and
systemic forms of the disease. Localised AL amyloidosis may first be suspected on the basis of its location. Typical sites associated with localised AL amyloidosis include the brain, bladder, skin, urinary tract, conjunctiva, larynx and the tracheobronchial tree in the absence of systemic visceral dysfunction [16][19]. For patients with localised AL amyloidosis, localised therapy and life-long monitoring are necessary, although these patients have been shown to have a normal life expectancy [19]. The frequent association of multinuclear giant cells with localized amyloid deposits and the equal prevalence of κ and λ type deposits has led to the suggestion that the pathogenesis may differ from that of systemic amyloidosis [20].

Serum free light chains (sFLCs) have been evaluated in patients with localised amyloid disease attending the UK National Amyloidosis Centre, as presented in Table 28.1. An enlarged series of 235 cases, was reported by the same group in 2005 [21]. Of the 162/235 patients with tissue biopsy data available, the fibril type was classified as AL in 100 cases (27% κ, 73% λ). The study concluded that localised AL amyloidosis is associated with a generally excellent prognosis.

Overall, elevated levels of sFLCs are less commonly observed in localised amyloidosis than in systemic AL amyloidosis and, even when present, the concentrations are lower. sFLC concentrations may therefore assist in distinguishing the different types of amyloid disease and also systemic from localised light chain amyloid disease.

<table>
<thead>
<tr>
<th>Site of amyloid deposits</th>
<th>Number of patients</th>
<th>Monoclonal proteins*</th>
<th>Abnormal κ/λ sFLC ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>7</td>
<td>3 (43%)</td>
<td>6 (88%)</td>
</tr>
<tr>
<td>Bladder</td>
<td>25</td>
<td>1 (4%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Bowel</td>
<td>10</td>
<td>4 (40%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Bronchial</td>
<td>13</td>
<td>2 (15%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Nodular pulmonary</td>
<td>13</td>
<td>3 (23%)</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>Laryngeal</td>
<td>22</td>
<td>0</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>16</td>
<td>1 (6%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Skin</td>
<td>18</td>
<td>2 (11%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Ocular</td>
<td>10</td>
<td>2 (20%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>16</td>
<td>3 (19%)</td>
<td>5 (31%)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>6</td>
<td>0</td>
<td>1 (17%)</td>
</tr>
</tbody>
</table>

Table 28.1. Frequency of monoclonal proteins in patients with localised amyloid disease. *Serum monoclonal proteins or light chain proteinuria identified by electrophoretic tests [21]. (Courtesy of P. Hawkins)
SPE. (B) sIFE reveals a small (nonquantifiable) monoclonal λ protein in the β/γ region.

Figure 28.4. (A) UPE; and (B) uIFE from the same patient as in Figure 15.3, showing a monoclonal λ protein band.

Figure 28.5. Comparison of the diagnostic sensitivity of screening panels in 581 patients with confirmed AL amyloidosis.
28.2. Diagnosis of AL amyloidosis

Figure 28.6. sFLCs in 262 patients with AL amyloidosis at diagnosis.

References
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