Routine Use of Amyloid Typing on Formalin-Fixed Paraffin Sections from 626 Patients by Immunohistochemistry

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Keywords
Amyloidosis • Amyloid prototypes • Formalin-fixed paraffin sections • Diagnosing amyloid • Congo red • Congo red fluorescence • Amyloid antibodies • Immunohistochemistry • Amyloid classification (=typing) • Expert evaluation • Pitfalls in amyloid typing • Mass spectrometry • Therapeutic implications

Introduction and Overview

All forms of amyloidosis are diagnosed using tissue sections and when amyloid has been diagnosed (see Chap. 13) it needs to be classified by identification of the chemical constituents of their amyloidogenic proteins. The currently known amyloid syndromes have been found to be associated with one of the approximately 30 chemically different proteins [1] by which the amyloid diseases differ. The amyloidotic protein therefore needs to be identified routinely in each patient to get information concerning the prognosis and to design a pathogenetically adequate therapy (see preceding chapters).

The classification of amyloid deposits can be performed directly on isolated amyloid fibril proteins by amino acid sequencing [2] or by proteomics on isolated amyloid fibrils [3]. Although these direct methods provide the highest level of precision, they are both laborious and time consuming and, hence, are less practical for routine clinical work. On the other hand, routinely fixed paraffin sections have been used for the typing of amyloid either by immunohistochemistry (IHC) [4, 5, 8, 10] or mass spectrometry (MS) [6, 7]. Both IHC and MS are much faster and are therefore applicable to routine practice. The IHC procedure identifies amyloidogenic proteins under the microscope by evaluating the antibody binding pattern obtained using amyloid antibodies in situ. In contrast, MS, the most recently devised method, analyses the likelihood of the presence of peptides generated from tissue extracts scraped or micro-sectioned from tissue slides [6, 7]. Since both IHC and MS examine unseparated amyloid fibrils, the results obtained are, therefore, indirect in these two latter methods. They were specifically developed for the classification of amyloid proteins in the formalin-fixed and paraffin-embedded tissue sections that are used in routine pathologic work,

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and have subsequently been introduced into clinicopathological practice [4–8, 10].

In this chapter, amyloid classification (or typing) through IHC is presented. The principal aim is to explain the special features of IHC typing of amyloid using amyloid antibodies, to show its validity, its ease of use, and its performance as a highly sensitive method applicable to routine practice, and also how to recognize pitfalls and avoid drawbacks [8, 9].

Finally, a note is added concerning the progress of two projects that compare (in double-blind studies) IHC and MS (Ringstudy I and II) to obtain well-substantiated data on a comparison of the two methods with respect to their performance and practicability for routine clinical work, at the international level [10].

Tissues, Amyloid Antibodies, Immunohistochemistry, and Execution

The basis for the development of a reliable classification of amyloid by IHC was dependent on two key features: firstly, the availability of prototype amyloid tissues and, secondly, the availability of amyloid antibodies. Prototype amyloid tissues are the tissues from patients whose amyloid type is known based on analysis of the extracted amyloid fibril protein by chemical or immunochemical means, such as partial or complete amino acid sequence analysis, or Western blotting [4]. We collected more than 153 prototype amyloids of various classes, mostly through the courtesy of colleagues and also as a result of chemical identification in our laboratory over many years [4].

The amyloid antibodies used were custom antibodies generated and produced by the author, which were selected based on their reactivity with prototype amyloids in formalin-fixed and paraffin-embedded tissue sections [5, 8, 10]. Briefly, precursor proteins or the ex vivo fragments thereof were used as immunogens while, in some cases, also synthetic peptides coupled to immunogenic carriers were used. Both, polyclonal and monoclonal antibodies were used as reviewed [4, 5, 8, 10].

An initial set of antibodies was tested with the common amyloid types [4]. Later, this antibody panel was extended to include also rare, and even very rare, amyloid types [5, 10]. It was important to test the antibodies against the amyloidotic proteins in situ since antibodies directed against the intact precursor proteins may show only limited reactivity with the corresponding amyloidotic proteins in tissue sections. Therefore, antibodies tested against the intact protein may not meet the full requirements for a safe IHC amyloid typing procedure [8, 9, reviewed in 4].

The requisite criteria for an antibody to be included in the IHC amyloid typing panel were as follows: (a) An antibody against a specific amyloid type should bind, by IHC, to all amyloids of this type in fixed tissue sections. (b) The IHC reaction should be strong, uniform, and consistent (see below). (c) The antibody should not bind to any other amyloid type strongly and consistently. (d) Two further considerations also influenced the selection of the antibodies for the standard panel: (d') when one antibody did not meet the above-mentioned criteria in full more than one antibody was used and (d'') a proper application and evaluation of a panel of antibodies for a definite classification of amyloidotic proteins in tissue sections requires a comparative evaluation (see below). (e) The antibody panel should be available to anyone, now and in the future, and the results obtained should be reproducible in other laboratories. Currently, these antibodies (and the protocols for their application) are available commercially [5, 10].

The antibody set used for routine typing in this chapter, and in prior reports [4, 7, 8, 10], comprises a panel of ten antibodies that are able to simultaneously classify eight different amyloid types. This panel covers 97.8% of all amyloid-containing tissues submitted consecutively to our center by physicians and patients for amyloid typing. The set of antibodies is directed against amyloid of the classes AA, ALα, ALκ, AHy, ATTR, Aβ, AFib, and ApoAI. Further antibodies against ALys, AGel, ACys, Aβ, APtP, AIAPP (see Table 17.1), and other types, are available when results from the standard panel indicate that they might be necessary [4, 7, 8, 10].
Table 17.1 Frequency of amyloid classes among 626 patients with amyloidosis typed, using comparative IHC amyloid typing

<table>
<thead>
<tr>
<th>Amyloid class</th>
<th>No. of patients</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL3</td>
<td>271</td>
<td>43.3</td>
</tr>
<tr>
<td>ALx</td>
<td>118</td>
<td>18.8</td>
</tr>
<tr>
<td>(AL, sum)</td>
<td>(389)</td>
<td>(62.1)</td>
</tr>
<tr>
<td>ATTR</td>
<td>93</td>
<td>14.9</td>
</tr>
<tr>
<td>AA</td>
<td>80</td>
<td>12.8</td>
</tr>
<tr>
<td>AFib</td>
<td>14</td>
<td>2.2</td>
</tr>
<tr>
<td>Aβ,M</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td>ALys</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>AApoAI</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>Aβ</td>
<td>4</td>
<td>0.6</td>
</tr>
<tr>
<td>APnP</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>ACys</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>AβP</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>AβP</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>AApolII</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>SAA</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Unknown</td>
<td>14</td>
<td>2.2</td>
</tr>
<tr>
<td>Sum total</td>
<td>626</td>
<td>100</td>
</tr>
</tbody>
</table>

The IHC technique used for amyloid typing by our laboratory (and also by others) is the unlabelled IHC technique of Sternberger (cited in [4]), in which a peroxidase–anti-peroxidase (PAP) complex is applied as the final amplification system, or its more sensitive variant—the ABC technique which uses an avidin–biotin peroxidase complex for amplification. Before beginning the IHC staining procedure, one section must be examined to verify the presence of amyloid in the submitted biopsy. When smaller amounts of amyloid are present, or if it is suspected that a sampling error might occur (see “sampling error” in Chap. 13, Fig. 13.2), approximately 15 sections are prefixed for 10–20 s with CR using a modified Puchler method [4]. This procedure incorporates the absolute minimum time necessary for staining, so that the CR staining will show up only by Congo red fluorescence (CRF), a more sensitive technique than the classical CR method employing bright light (see Chap. 13, Fig. 13.6c). This brief prewashing treatment therefore ensures that the CR staining will not interfere with the IHC procedure. Amyloid that fluoresces by CRF needs to be verified by polarization microscopy, when the pathognomonic green birefringence (GB) should be seen [4]. Sections that show the presence of amyloid are then chosen for IHC staining. Whenever the procedure needs to be changed, consequent to the inclusion of new solutions, other reagents, new personnel, etc., we routinely include seven positive controls (one for each of the five major amyloid classes) to make sure that the applied technique will be of a similar high standard and that the results will be comparable to former IHC staining. Positive controls, after evaluation, can be destained (using 80% acetic acid dropped onto the section for 2 min) and reused for IHC. In our laboratory, we routinely reuse positive control slides up to approximately six times [4]. The staining procedure utilizes routine methods with AEC (3-amino-ethyl carbazole) as the chromogen, followed by a weak counterstain with Mayer’s hemalum, and embedding in Kaiser’s glycerin jelly [4].

**Evaluation of Immunohistochemical Reactivities**

The reading of IHC-stained slides is performed without any prior knowledge of either laboratory data or clinical picture. Positive standard slides are first evaluated to provide assurance that the “unknown” slides of submitted tissue sections have been stained properly with the set of antibodies employed. The second stage of the procedure is then to read the ten-stained slides of each patient and evaluate their IHC reactivities. This requires some experience since amyloid represents a complex mixture of many proteins and other constituents [4, 8, 10] that will show various reactivities. The latter need to be categorized by distinguishing strong, uniform, and consistent IHC reactions from the more inconsistent ones, to separate the specific and diagnostic from the nonspecific reactivities.

This is shown in Figs. 17.1–17.4. The amyloid typing is illustrated here using the four most prevalent amyloid types: AA, AL3, ALx, and ATTR (more types have been presented in Table 17.1 and in 10), CRF was used to identify the presence
of amyloid in each of the four illustrated cases, since it is the most sensitive method for confirming the presence of amyloid (see Chap. 13). Amyloid is shown by CRF in the “a-row” of all cases in Figs. 17.1–17.4. IHC typing of amyloid was performed on adjacent sections, to which the above-described panel of amyloid antibodies was applied. All three forms of illumination used for identifying amyloid (triple illumination) were applied to all amyloid types, as exemplified in the ATTR case in Fig. 17.4b, inset.

In Fig. 17.1a–e, characteristic reactivities are shown for renal autopsy tissue with glomerular AA amyloid (in a case of Muckle–Wells syndrome, see 4) as identified by CRF (Fig. 17.1a) and a very consistent and strongly congruent diagnostic reaction with anti-AA only (Fig. 17.1b, between arrows), while anti-ALκ and anti-ATTR (Fig. 17.1d, e) were nonreactive. The anti-ALκ antibody (Fig. 17.1c) showed an intense reactivity; however, this was weak in comparison to the reactivity shown in Fig. 17.1b which, being the strongest, was considered diagnostic of the amyloid type. Please note that light chain amyloid antibodies may also react strongly with amyloid-free tissue structures such as tubular cells (Fig. 17.1c, d), indicating that severe nephrosis contribute to the patient’s death.

In Fig. 17.2a–e, IHC identified ALκ as the amyloid type; here, amyloid is seen only in the cardiac vessels in a patient with cardiac decompensation and MGUS (free λ-L 343 mg/l serum). The cardiac functional decompensation resulted from severe narrowing of the vessels by amyloid (Fig. 17.2b–e, between arrows) leading to functional impairment, despite the cardiac muscle being virtually free of amyloid. The amyloid was barely stained with Congo red (not shown) but was visible with CRF illumination (Fig. 17.2a).

Diagnosis of the amyloid type was based on identification of a single, consistent, and very strong IHC reaction (corresponding to the location of amyloid deposits by CRF) with anti-ALκ only.

Fig. 17.1, a–e. Diagnosing and typing of AA amyloid: a. CRF; b. Anti-AA; c. anti-ALκ; d. anti-ALκ; e. anti-ATTR. For detailed description see text.
Fig. 17.2  ALκ amyloidosis. Diagnosing and typing, a–e, see above

Fig. 17.3  ALκ amyloidosis. Diagnosing and typing, a–e, see above
(Fig. 17.2c). In contrast, the anti-ALκ (Fig. 17.2d) stain was less strong and less uniform. There was almost no reactivity with anti-AA (Fig. 17.2b) and anti-ATTR (Fig. 17.2e), except for a few reactive and inconsistent spots in the latter. Note that considerable reactivity is present with anti-ATTR in cardiomyocytes, which does not, however, correspond in location to the areas positive for amyloid by CRF and, therefore, is not specific for amyloid.

Figure 17.3a–e displays severe interstitial cardiac ALκ-amyloid as identified in a patient with MGUS (free kappa-L 916 mg/l serum). ALκ amyloidosis was diagnosed based on CRF detection of an interstitial bright orange-red web of somewhat kinky amyloid bundles (Fig. 17.3a, between arrows). This corresponds with a similar kinky web-like reactivity with anti-ALκ in Fig. 17.3b (between arrows). Anti-AA (Fig. 17.3b) and anti-ATTR (Fig. 17.3e) were nonreactive, but some ALλ reactivity was seen (Fig. 17.3c, between arrows), which was, however, far weaker and more inconsistent when compared to the diagnostic kappa reaction in Fig. 17.3d.

Figure 17.4a–e shows IHC typing of pulmonary ATTR amyloid in a 91-year-old patient who died of pneumonia after a heart infarction. The amyloid seen in CRF illumination (Fig. 17.4a) gave a consistent, and therefore diagnostic, reaction with Anti-ATTR antibody only (Fig. 17.4b, between arrows), while anti-AA (Fig. 17.4b) was nonreactive, and anti-ALλ (Fig. 17.4c) and anti-ALκ (Fig. 17.4d) showed only minor reactivities. The inset in Fig. 17.4b shows the CRF and GB of adjacent amyloid deposits that were used for diagnosing this amyloid (indicated by two larger arrows in Fig. 17.4b–e). The smaller arrows in Fig. 17.4c, d point to intensive collateral reactivities, which do not represent amyloid. They can easily be distinguished from amyloid by CR prestaining followed by an IHC overlay and subsequent examination of the same frame with bright light as compared to fluorescent light illumination (by switching the light source) to verify which reactive areas represent amyloid. Using this technique, the amyloid specificity of the IHC stain is ensured [4].

In summary, in Fig. 17.1–17.4, the CRF (a-row) shows an intense bright orange-red
fluorescence of high sensitivity for all amyloid deposits, regardless of type. The b-row shows the specificity of the AA-antibodies across the different amyloid types. There is only a single, very strong reactivity against AA amyloid and no reactivity with the other amyloid types. AA-reactivity with monoclonal antibodies (mc series, 4) shows the highest level of selectivity. The c-row shows representative anti-AL\(\lambda\) reactivities. Although anti-AL\(\lambda\) reacts with most amyloids, the strongest reaction (which is therefore considered to be amyloid-specific) occurs only with AL\(\lambda\) amyloid deposits as shown in Fig. 17.2c. The \(\lambda\) reactivities of many amyloids have plagued amyloid IHC studies for many years [4, 9], leading to misdiagnoses, as discussed above, and have intensified a search for remedies. The resolution of this problem, some 15 years ago, led to the adoption of the method of “comparative IHC” (reviewed in 4; see below). As shown above, this method of comparative IHC is based on selection of the strongest amyloid-specific reactivity. The d-row presents the performance of the \(\kappa\) reactivities and the e-row presents the performance of theATTR antibodies across the four different amyloid types. In the latter, only the corresponding ATTR amyloid reacts strongly, as in the case of most ATTR amyloidoses seen in patients.

Comparative Immunohistochemistry as a Routine Method for Amyloid Typing

The following example is instructive of the use of this technique. Recently, in 2011, we received tissue sections of a reported AA amyloidosis with a request for a second opinion. The amyloid had been classified using a single monoclonal AA antibody, which was reported as reactive. Similar instances of amyloid typing, based on evaluation using a single antibody, have also been reported [8]. Subsequently, they were all found to be incorrect. In cases where an incomplete antibody panel is used, and the antibody that corresponds to the particular type of amyloid present in the specimen is missing from the panel, collateral nonspecific reactions can occur that have been misinterpreted as positive, diagnostic results. This type of false-positive result has been reported in cases that were subsequently diagnosed as AFib and AApolAI [8].

The deficiencies associated with the use of a single antibody in the typing of amyloid deposits can be further illustrated with reference to Fig. 17.1 and Fig. 17.2. It is apparent that the single reactivities seen in Figs. 17.1c and 17.2d would result in the misdiagnosis of AL\(\lambda\) and AL\(\kappa\) amyloidosis, if the homologous, diagnostic antibodies were not available, or if they had reacted inappropriately in Figs. 17.1b and 17.2c. This should be clear from the reasoning of the paragraph above: the only way to arrive at a firm identification of the correct chemical amyloid type is by comparing the different IHC reaction patterns and, thus, by separating the diagnostic from the nonspecific reaction. How this can be done is shown in Figs. 17.1–17.4 using comparative IHC with antibodies that meet the above-mentioned criteria. The results of many such comparative amyloid analyses are listed in Fig. 17.5. The visible reactions are graded based on their intensity as illustrated and described in Figs. 17.1–17.4. Thus, the homologous reactions in Figs. 17.1b, 17.2c, 17.3d, and 17.4e are graded as +++ since they are very strong and uniform. By contrast, the negative reactions in the b-row are graded negative 0, as are the reactions in Figs. 17.1e and 17.3e. However, Fig. 17.2d is graded as (++) in brackets to denote a reaction that is weaker and inconsistent as compared to the diagnostic reaction in Fig. 17.2c. Accordingly, Figs. 17.3c, and 17.4c, d are graded as inconsistent (++++) while Figs. 17.1d, 17.2e, and 17.3e are graded as (+), displaying the lowest level of reactivity with a signal that consists of only a few spots, as in Fig. 17.2e.

Figure 17.5 shows the combined results of the IHC typing of amyloid presented here using the described panel of antibodies and the above-mentioned way of evaluation not only on four of the most common amyloid types (Figs. 17.1–17.4) but also on additional amyloid classes as described in [5, 8, 10]. As can be seen from Fig. 17.5 (and additional publications), the comparative IHC evaluation scheme is able to discriminate the truly diagnostic from nonspecific
Reactions using amyloid antibodies, and this scheme has been applied to many different amyloid types, so far. However, particular attention must be paid to light chain amyloid antibodies, since the \( \lambda \)-light chains have a tendency to be present in many different amyloid types, however in variable amounts. Thus, it has been shown here, when an \( AL \lambda \) light chain reactivity is unspecific and it is specific in diagnosing the respective \( AL \lambda \) correctly [4, 8, 10]. Note that while the consistent and strong reactivities are readily apparent, the inconsistent reactivities are by far more variable.

The validity of the above IHC typing of amyloid is based on several key points: (a) All IHC reactions were tested with seven positive controls, representing prototype amyloids. (b) The ten different amyloid antibodies that were used intrinsically provided several built-in controls and typically yielded only one diagnostic reaction, while allowing the exclusion of all other amyloid types through the recognition of nonspecific reactions, as described above. (c) In all cases where clinical information was available, the IHC results were consistent with the type of amyloidosis suspected on clinical grounds. (d) In one patient (Figs. 11.1–11.6 in citation 4) more than one type of amyloid was detected by IHC, and this correlated with the microscopic morphology seen in the tissues, suggesting different sites for amyloidogenesis of each amyloid type. The recognition of such rare cases clearly shows the advantage of IHC as compared to MS, since the spatial separation of the two amyloids remains intact and can thus be evaluated separately. (e) This panel of antibodies has been used by numerous other laboratories, with similar results. Some of these cooperative studies are published and cited in [4]. (f) Additional data supporting the validity, high sensitivity, and precision of the comparative IHC typing of amyloid, using the above panel of amyloid antibodies, has been obtained from the first international blinded comparison of IHC versus MS ("Ring study I," in progress).
High-Sensitivity Classification of Single Minute Amyloid Deposits

At one time, it was requested that we perform IHC typing on a single tissue section that contained one small area of amyloid of approximately the size of two macrophages. We attempted, for the first time, the sequential application of one amyloid antibody after another, with destaining (see above) between each application. The resultant diagnosis was ATTR amyloidosis (published in 1984, Feuerle et al. cited in 4). This amyloid was later genotyped as a rare ATTR variant. Similarly, the detection of very small, single amyloid deposits in the initiating phase of AA amyloidosis in juvenile rheumatoid arthritis, missed by former evaluators, has been summarized and illustrated in Chap. 13, Fig. 13.4. Over the years, numerous patients have been diagnosed, based on the evaluation of small deposits of amyloid present in biopsies with very early amyloidosis that were too small to be evaluated by other typing methods.

Performance and Prevalence

This section presents the overall results of amyloid typing, using comparative IHC with a diagnostic panel of amyloid antibodies. Only patients with a single amyloid type have been included. The results of amyloid typing in patients with more than one amyloid type, and the typing of amyloids in animals (which both follow the same principles as discussed above) is presented elsewhere (manuscript in preparation). In addition, also the non-amyloidotic protein thesauroses, such as α- and κ-LCDD, have not been included in this list.

In these studies, 153 prototype amyloids were employed to ensure the specificity of the amyloid antibodies [4, 10]. All 153 (100%) of the prototype amyloids could be typed correctly using comparative IHC. The results of amyloid typing in 626 patients evaluated at the Reference Center of Amyloid Diseases are presented in Table 17.1. Of the 626 samples received, in 612 patients (97.8%) the amyloid type could be diagnosed and verified by the use of appropriate controls. Light chain-derived amyloid was the most prevalent form, being diagnosed in 389 patients (62.1% of all samples submitted), with 271 patients having ALκ (43.3%) and 118 having ALλ (18.8%). The second and the third most numerous classes were ATTR in 14.9% and AA in 12.8%, respectively. All other amyloid classes were rare, or very rare, with AFFib (2.2%) representing the most numerous among the rare amyloid classes encountered.

Among the 14 unknown samples, in 2 patients amyloid derived from semenogelin I was subsequently diagnosed using our specific antibody to define which of the two proteins identified by MS was the amyloidogenic one, in 2003 as published in [11]. Another novel amyloid was identified immunohistochemically as SAA4 [12] and verified by MS by courtesy of Alan Solomon. Two other amyloids were analyzed by amino acid sequence analysis and identified as ALκ amyloid; one was published [13] and the other indentified by courtesy of J.J. Liepniaks and M.D. Benson. Against both novel ALκ amyloidogenic proteins polyclonal antibodies have been developed which are now part of our panel and part of an extended panel in case of unreactivities. In addition, five other unknown amyloids could neither be typed by IHC nor by MS. Finally, these approximately 2.2% of unreactive samples show that novel amyloid diseases can be selected using the presented panel of antibodies directed against known amyloids. Chemical analysis has resulted in the production of novel amyloid antibodies and this will continue with the discovery of even more novel amyloid diseases.

In approximately 60% of patients, the clinical records were available for evaluation and in almost all cases the clinicopathologic correlation was excellent. In many cases, the IHC typing of amyloid was instrumental in guiding the subsequent clinical management of patients with seemingly uncharacteristic, and even obscure, minor symptoms [2]. Results similar to those presented in this chapter were also obtained independently, in several other laboratories, using the same sets of antibodies [4].
In summary, amyloid typing on tissue sections using comparative IHC does not require any prior clinical knowledge or laboratory data. It provides a definitive diagnosis of the amyloid type by detection of the chemical identity of the amyloidotic protein in the fibril in situ without antigenic retrieval in approximately 97.8% of submitted tissue samples. This amyloid typing technique is reliable, very fast, easy, and affordable for all institutes competent in performing IHC. In addition, it is of the highest sensitivity since one amyloid spot in a single slide is virtually sufficient for a full classification (see Chap. 13, Fig. 13.4).

Finally, the first two blinded international comparisons between IHC and MS (Ringstudies I and II) have been concluded with Ringstudy I using microdissection and MS. Reports in progress will confirm the high sensitivity and precision of IHC, but also determine the relative strengths and the weaknesses of each method [10].

### Pitfalls and Remedies

(a) An inherent problem in the classification of amyloid is that amyloid is not a pure substance but a very heterogeneous complex, which is comprised of various structural and soluble, aggregated and polymerized proteins, and their fragmented or point-mutated variants. In addition, this complex is saturated with various, variable, extracellular constituents, including, in particular, serum proteins that can be adsorbed to this amyloid complex to varying extents [4, 5, 8–10, see Figs. 17.1–17.4]. Both methods, IHC and MS, have to cope with this situation. Since the pathogenetically most important and unique constituents are the amyloid fibril proteins, we have produced antibodies that preferentially recognize these. In this report, it is described how these antibodies can distinguish amyloid from its contaminants.

(b) The histomorphological evaluation of amyloid that is possible with IHC is very helpful in coping with the plethora of constituents found associated with amyloid deposits. Since the amyloid fibrils remain intact and in place during the typing process, even in tissues with the smallest deposits, a spatial correlation between the site of antibody reactivity and the amyloid deposit can be made (see Figs. 17.1–17.4).

(c) The rare instances when more than one amyloid type can be diagnosed in a single patient [9] can also be addressed by using this panel of antibodies for comparative amyloid typing as illustrated in [4].

(d) In instances where the antibodies produce a stronger stain with structures other than amyloid deposits (a common occurrence, in particular, in the immunoglobulin-derived amyloids, AL[1], since the extracellular space is washed with IgGs, see Figs. 17.1–17.4) prewashing of the section with CR, followed by an IHC overlay, allows correlation of the spatial distribution of the CRF and IHC staining patterns (i.e., whether they occur in the same area of the section) and determination of which of the strongest reaction is congruent with the amyloid deposit.

(e) Lack of experience in the interpretation of immunohistochemical staining is a very important pitfall of this method [9]. Since the diagnosis of amyloidosis is not a trivial procedure, in cases where problems arise, consultation with an expert center for diagnosis of the amyloid type should be considered [9].

### Take Home Message

1. The immunohistochemical diagnosis of amyloid type is easy, fast, and very precise when performed by an expert laboratory. It can be performed in every institute that is competent in the techniques of IHC, after some degree of training.

2. For immunohistochemical typing, an appropriate panel of antibodies is needed for comparative evaluation without antigenic retrieval, since “one antibody—one diagnosis” does not lead to a safe assessment of the amyloid type.

3. Evaluation of the IHC patterns generated by these antibodies needs a certain amount of histopathologic training to recognize a true

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1 AL[1] is proposed here as a practical acronym for the combination of AL and AH.
diagnostic reaction, which is characterized by its strong and consistent appearance, and to distinguish the latter from the inconsistent and weak reaction typical of a false-positive result (see Figs. 17.1–17.4).

4. To distinguish non-amyloidotic structures from amyloidotic ones, prestaining with CR and the use of CRF in combination with IHC is very helpful and even some small amyloid deposits can be correctly typed, see chap. 13.

5. When problems arise, an expert reference center needs to be consulted [9].

Report for Clinicians

The report to the clinician should be candid and present both the strengths and the limitations of the procedures under which the classification was performed. This ensures that the clinician has confidence in the findings on which he or she must base the appropriate therapy. The first issue to be addressed should be the quality of the biopsy, its size, and the amount of amyloid. In cases where the presence or absence of amyloid is not clear, an independent expert should be consulted to clarify the issue. Amyloid typing should always be performed as a double-blind study, preferably without any prior knowledge of the clinical data, since the latter can, at times, also mislead.

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