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Paraffin Immunofluorescence Increases Light-Chain Detection in Extra-Renal Light Chain Amyloidosis and Other Light-Chain–Associated Diseases

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Context.—Distinguishing the different types of amyloidosis is clinically important, because treatments and outcomes are different. Mass spectrometry is the new gold standard for amyloid typing, but it is costly and not widely available. Therefore, immunolabeling remains the first step in identifying the most common types of amyloidosis. In amyloid subtyping, direct immunofluorescence works well when applied to frozen sections, but immunohistochemistry on formalin-fixed, paraffin-embedded material often yields poor results, particularly for light chain amyloidosis. Recently, paraffin immunofluorescence has been described as a valuable salvage technique in renal pathology when frozen sections are not available but it has not been evaluated for extra-renal diseases.

Objectives.—To evaluate the use of paraffin immunofluorescence for light-chain detection in extra-renal light-chain amyloidosis and other light-chain–associated diseases.

Design.—First, we compared the staining intensity of both light chains between paraffin immunofluorescence and immunohistochemistry on a retrospective cohort of 28 cases of amyloidosis that have been previously typed. Then, we studied the role of paraffin immunofluorescence as an addition to our classical immunohistochemistry panel for amyloidosis typing.

Results.—In the retrospective cohort, we found that paraffin immunofluorescence outperformed immunohistochemistry for light-chain detection. Then, in the prospective part of the study, we showed that the proportion of correctly classified cases increased from 50% to 71.9% with the adjunction of second-intention paraffin immunofluorescence to the immunohistochemistry procedure.

Conclusions.—We therefore view paraffin immunofluorescence as a significant addition to the routine workflow for detection of light-chain–related diseases.

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Amyloidosis is a group of disorders associated with the presence of extracellular amyloid deposits constituted by fibrillar aggregates of misfolded protein. More than 35 protein types have been identified as putative causative agents.1 Because of common ultrastructural properties, all amyloid deposits stain red with Congo red and display a yellow-green birefringence under polarized light.2 Because treatment depends on the type of amyloidosis, identifying the chemical nature of the disease-causing amyloidotic protein is a key step in diagnosis. Over the last 10 years, amyloid typing methods based on mass spectrometry (MS) have become the new gold standard.3 Among its many advantages, MS does not rely on antibodies and can identify unknown causative proteins. Yet, because MS remains expensive and not readily accessible in most parts of the world, antibody-based techniques remain the first step in amyloid typing.4

When frozen material is available, immunofluorescence (IF) is the method of choice. It can successfully type approximately 85% of amyloidosis.4-5 Yet, except in renal pathology, frozen material is usually not available. In such cases, immunohistochemistry (IHC) on paraffin sections has traditionally been used to type amyloids, despite drawbacks that can make it unreliable. For light chain amyloidosis (AL amyloidosis) in particular, this method is known to be less sensitive and may yield results that are very hard to interpret, as contamination by serum creates a major background with commercial antibodies.6 In reference centers for amyloidosis typing, IHC can miss up to 25% to 30% of cases of AL amyloidosis.7,8 Given the major therapeutic implications, diagnosis of AL amyloidosis should not rely on the clinical context alone, as this may often lead to unnecessary treatment.9 As MS cannot be performed in all cases that IHC fails to determine, there is a
need to improve tissular light-chain (LC) detection on formalin-fixed, paraffin-embedded (FFPE) materials, to avoid further invasive procedures and delayed treatment for patients.

Paraffin immunofluorescence (IF-P) is now well recognized as a salvage technique in renal pathology, used when a fresh-frozen tissue section is not available. This technique is easy to implement, cost effective, and available in most countries. As formalin fixation induces protein cross-linking, which generally blocks antigenicity, IF-P requires an enzymatic digestion step that involves treating the tissue with a protease to expose antigenic extracellular immune complexes. This technique has proven valuable for most immune-complex–mediated glomerulonephritis- and dysproteinemia-associated kidney lesions. The most widely used enzymes are pronase and proteinase K. As the same enzymatic digestion step can also be applied to extrarenal tissue, we hypothesize that IF-P might be used to detect extrarenal AL amyloidosis and other LC-associated diseases, such as light chain deposition disease (LCDD).

In this study, we first focused on a retrospective cohort of known cases of the following conditions: extrarenal AL amyloidosis, extrarenal non-AL amyloidosis, and a few LCDD cases. The diagnostic value of IF-P with pronase or proteinase K pretreatment (respectively, IF-Pn and IF-Pk) was compared with that of conventional IHC, in order to evaluate the intrinsic validities of these 3 processes. Next, to evaluate the utility of IF-P in routine practice, we performed IF-Pk prospectively on all cases of amyloidosis and suspected LC-associated disease (without frozen material) seen at Lille University Hospital (Lille, France) between 2018 and 2019.

METHODS
Case Selection

Method validation study.—We retrospectively reviewed the case files of our institution and selected 20 cases of extrarenal AL amyloidosis (10 AL-λ and 10 AL-κ), 8 cases of extrarenal non-AL amyloidosis, and 3 cases of extrarenal LCDD. Because frozen material is available in routine practice after most kidney biopsies, we chose to exclude this location. Diagnoses had been established after a review of all the clinical and biological data and after standard technical tissue processing, namely Congo red staining and direct IF on frozen sections with the routine panel (κ, λ, Transthyretin [TTR], Serum Amyloid A [SAA]). To be included, cases were required to have been typed by direct IF or confirmed by MS, and there had to be enough material to perform IF-Pk, IF-Pn, and IHC. Staining intensity after IF or IHC was scored on a scale of 0 to 3+ (0, absent; 1+, mild; 2+, moderate; 3+, strong). A diagnosis of AL amyloidosis was made when the intensity difference between the 2 LCs on the staining scale was 2 or more.4

Prospective study.—From January 2018 through November 2019, IF-P and IHC were performed on all samples with amyloid deposits (confirmed by Congo red staining) or LC-associated deposit disease (amorphous eosinophilic Congo red negative material for LCDD and intracytoplasmic crystalline inclusions for crystal staining histiocytosis). By IHC, cases were classified as “non-AL amyloidosis” if high-intensity staining (3+) was observed with anti-SAA or anti-TTR, as “AL amyloidosis” if, between the 2 LCs, the difference in staining intensity on the staining scale was 2 or more and as “undetermined” for every other condition. By IF-P, only κ and λ were detected and a diagnosis of AL amyloidosis was made when on the staining scale, the difference in staining intensity between the 2 LCs was 2 or more.

The contribution of IF-P to the final diagnosis was then evaluated.

The methods we use for IF-Pn and IF-Pk are not exactly the same as the Mayo Clinic and Nephropath protocols published for renal pathology. Our modifications are based on internal validation and are mostly related to the fact that the Ventana BenchMark Ultra used for antigen retrieval does not allow complete customization of the protocol.

Paraffin Immunofluorescence.—IF staining of sections cut from FFPE tissue was performed as follows: FFPE tissue sections were deparaffinized in xylene and placed in alcohol. The staining procedure was carried out in a Ventana BenchMark Ultra (Roche, Tucson, Arizona). The following antibodies were used: anti-SAA (clone M0759; Dako), anti-TTR (clone A0002; Dako), anti-κ (clone A0193; Dako), and anti-λ (clone A0192; Dako).

The slides were examined under a light or epifluorescence microscope.

Paraffin Immunohistochemistry.—IF staining of sections cut from FFPE tissue was performed as follows: FFPE tissue sections were deparaffinized in xylene and placed in alcohol. Pronase from Streptomyces Griseus (PS147-1; Sigma-Aldrich) was used at 5 mg/mL and Proteinase K was bought ready-to-use (S302080-2; Sigma-Aldrich). Antigen retrieval steps were performed with Ventana BenchMark Ultra. For digestion, pronase was applied for 32 minutes at 36°C or Proteinase K for 8 minutes at 36°C. After retrieval of antigenic sites, the slides were kept in phosphate buffered saline. Direct IF was then performed manually for 1 hour with fluorescein isothyocyanate-conjugated anti-κ and anti-λ antibodies. The slides were then washed twice for 15 minutes in phosphate buffered saline and mounted with Fluoromount aqueous mounting medium (Sigma-Aldrich).

The methods we use for IF-Pn and IF-Pk are not exactly the same as the Mayo Clinic and Nephropath protocols published for renal pathology. Our modifications are based on internal validation tests and are mostly related to the fact that the Ventana BenchMark Ultra used for antigen retrieval does not allow complete customization of the protocol.

Statistics

Statistical analyses were performed with MedCalc for Windows software (version 17.4; MedCalc Software bvba, Ostend, Belgium). Categoric variables are expressed as number (percentage).

Method validation study.—A combination of routine IF and MS-based proteomic analysis was used as composite gold standard for subsequent analyses, the target diagnosis being AL amyloidosis. The index tests to be evaluated were routine immunohistochemistry and both Pn and Pk IF-P. Using the method validation cohort, we first estimated the sensitivity and specificity of the index tests. For each index test, performance and agreement levels were measured with McNemar’s test and Cohen’s k.

The study was performed in accordance with the Declaration of Helsinki. The Commission Nationale de l’Informatique et des Libertés consented to the study.
Secondarily, the value of each IF-P staining technique as part of a 2-step approach (for IHC-negative cases) was estimated.

**Prospective study.**—In the prospective study, the prospectively enrolled cases were classified by means of the 2-step approach evaluated in the validation study. The performance of the proposed methodology was evaluated as the proportion of correctly classified amyloidosis cases. All tests were 2-tailed, and the threshold for statistical significance was set at \( P < .05 \).

## RESULTS

### Validation Study: Comparison of IHC With IF-Pk and IF-Pn for the Diagnosis of Extrarenal Amyloidosis and LCDD

<table>
<thead>
<tr>
<th>Disease</th>
<th>Nature</th>
<th>Nb</th>
<th>IHC</th>
<th>IF-Pn</th>
<th>IF-Pk</th>
<th>IHC</th>
<th>IF-Pn</th>
<th>IF-Pk</th>
<th>Diag-IHC</th>
<th>Diag-Pn</th>
<th>Diag-Pk</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL amyloid (κ)</td>
<td>n = 10</td>
<td></td>
<td>IHC</td>
<td>IF-Pn</td>
<td>IF-Pk</td>
<td>IHC</td>
<td>IF-Pn</td>
<td>IF-Pk</td>
<td>Diag-IHC</td>
<td>Diag-Pn</td>
<td>Diag-Pk</td>
</tr>
<tr>
<td>Case 1</td>
<td>MSGB</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>UND</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
</tr>
<tr>
<td>Case 2</td>
<td>Digestive tract</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
</tr>
<tr>
<td>Case 3</td>
<td>MSGB</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
</tr>
<tr>
<td>Case 4</td>
<td>BSFT</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>UND</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
</tr>
<tr>
<td>Case 5</td>
<td>MSGB</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>UND</td>
<td>UND</td>
<td>κ</td>
<td>κ</td>
</tr>
<tr>
<td>Case 6</td>
<td>BM biopsy</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
</tr>
<tr>
<td>Case 7</td>
<td>Skin biopsy</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
</tr>
<tr>
<td>Case 9</td>
<td>BSFT</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
<td>κ</td>
</tr>
</tbody>
</table>

| AL amyloid (λ) | n = 10 |     | IHC | IF-Pn | IF-Pk | IHC | IF-Pn | IF-Pk | Diag-IHC | Diag-Pn | Diag-Pk |
| Case 11 | BSFT        | 3  | 3   | 3     | 1    | 1   | 1     | λ    | λ        | λ       | λ       |
| Case 12 | Skin biopsy | 2  | 2   | 2     | 1    | 0   | 0     | UND  | λ        | λ       | λ       |
| Case 13 | Cardiac biopsy | 3  | 2   | 2     | 1    | 0   | 0     | λ    | λ        | λ       | λ       |
| Case 14 | MSGB         | 3  | 2   | 3     | 1    | 0   | 1     | λ    | λ        | λ       | λ       |
| Case 15 | Cardiac biopsy | 2  | 3   | 2     | 0    | 0   | 0     | λ    | λ        | λ       | λ       |
| Case 16 | Digestive tract | 1  | 2   | 2     | 1    | 1   | 1     | UND  | UND      | UND     | UND     |
| Case 17 | MSGB         | 3  | 2   | 2     | 1    | 0   | 0     | λ    | λ        | λ       | λ       |
| Case 18 | BM biopsy    | 3  | 3   | 3     | 1    | 1   | 0     | λ    | λ        | λ       | λ       |
| Case 19 | MSGB         | 2  | 2   | 3     | 0    | 0   | 0     | λ    | λ        | λ       | λ       |
| Case 20 | Synovial     | 2  | 2   | 2     | 0    | 0   | 0     | UND  | λ        | λ       | λ       |

| Non-AL amyloid | n = 8 |     | IHC | IF-Pn | IF-Pk | IHC | IF-Pn | IF-Pk | Diag-IHC | Diag-Pn | Diag-Pk |
| Case 21 | Digestive tract | 1  | 0   | 0     | 1    | 0   | 0     | UND  | UND      | UND     | UND     |
| Case 22 | BM biopsy     | 2  | 1   | 1     | 1    | 1   | 1     | UND  | UND      | UND     | UND     |
| Case 23 | Digestive tract | 1  | 0   | 0     | 0    | 0   | 0     | UND  | UND      | UND     | UND     |
| Case 24 | MSGB          | 1  | 1   | 0     | 1    | 1   | 1     | UND  | UND      | UND     | UND     |
| Case 25 | Lung          | 1  | 0   | 1     | 1    | 0   | 1     | UND  | UND      | UND     | UND     |
| Case 26 | Skin          | 0  | 0   | 0     | 1    | 1   | 0     | UND  | UND      | UND     | UND     |
| Case 27 | BSFT          | 1  | 0   | 1     | 1    | 0   | 1     | UND  | UND      | UND     | UND     |
| Case 28 | MSGB          | 1  | 0   | 0     | 0    | 0   | 0     | UND  | UND      | UND     | UND     |

| LCDD (κ) | n = 3 |     | IHC | IF-Pn | IF-Pk | IHC | IF-Pn | IF-Pk | Diag-IHC | Diag-Pn | Diag-Pk |
| Case 1  | Lung         | 1  | 0   | 0     | 3    | 2   | 3     | κ    | κ        | κ       | κ       |
| Case 2  | Lung         | 1  | 1   | 1     | 2    | 3   | 3     | UND  | κ        | κ       | κ       |
| Case 3  | Lymph node   | 2  | 2   | 2     | 2    | 3   | 2     | UND  | UND      | UND     | UND     |

Abbreviations: AL, light chain amyloidosis; BM, bone marrow; BSFT, biopsy of subcutaneous fatty tissue; Diag, diagnostic; IHC, immunohistochemistry; IF, immunofluorescence; MSGB, minor salivary gland biopsy; LCDD, light chain deposition disease; Pk, Proteinase K; Pn, Pronase; UND, undetermined.

* Cases tested by mass spectrometry.

A comparison of the performance of each of the 3 index tests with the gold standard diagnoses revealed that none of the 3 techniques gave false-positive results. They all thus displayed 100% specificity. There were differences in sensitivity, however, for the diagnosis of AL amyloidosis. Overall, IHC correctly classified only 12 of 20 cases of AL, corresponding to 60% sensitivity (95% CI, 36.1–80.9). By comparison, IF-Pn and IF-Pk correctly identified, respectively, 16 (sensitivity 80%, 56.3–94.3) and 18 cases (sensi-
tivity 90%, 68.3–98.8) of 20 (Table 1). As compared with the gold standard, McNemar’s test evidenced a significant difference for IHC diagnosis ($P = .008$), in line with its limited sensitivity. No difference was observed between the gold standard and either IF-P procedure ($P = .12$ and $P = .50$ for IF-Pn and IF-Pk, respectively). Consistently with the results of McNemar’s test, Cohen’s $\kappa$ was low for IHC (.46, .20–.73), moderate for IF-Pn (.70, .43–.96), and high for IF-Pk (.84, .62–1.0).

For the IHC-misclassified samples ($n = 8$), IF-Pn and IF-Pk exhibited re-calculated sensitivities of 50% (15.7–84.3) and 75% (34.9–96.8), respectively. It is worth noting that most cases missed by IHC, and to a lesser extent by IF-Pn, were AL-κ (Table 1).

With regard to other LC-related diseases, 3 cases of LCDD were included. In all cases, the deposits were monotypic for LC, as determined by IF on frozen sections. There were 2 cases of lung involvement and one case of lymph node resection. IHC correctly classified only 1 of 3 cases, as compared with 2 of 3 for both IF-Pk and IF-Pn (Table 1). Given the low number of cases, statistical analyses were not performed.

### Prospective Study

On the basis of the results of the first part of the study, proteinase K was considered the best IF-P modality. We thus performed IF-Pk on all cases of suspected LC-associated diseases (without frozen material available) seen at Lille University Hospital between January, 2018 and November, 2019. Overall, there were 39 cases of amyloidosis and 3 additional cases of suspected LC-associated disease (2 LCDD, 1 crystal storing histiocytosis [CSH]). Seven cases were excluded from the study because at least 1 of the techniques could not be performed for lack of sufficient material (Figure 1).

It is worth mentioning IF-Pk (and also IF-Pn) exhibited lesser background intensity than IHC (Figures 2 and 3), but in all cases some background staining was observed in the vessel lumen, as expected for formalin-fixed material.

Eventually, the study included 32 cases of amyloidosis from various locations as follows: 7 lung biopsies, 12 minor salivary gland biopsies, 2 subcutaneous fat biopsies, 1 surgical spleen removal, 4 digestive tract biopsies, 1 total hepatectomy, 2 bone marrow biopsies, 1 lymph node biopsy, 1 periorbital mass biopsy, and 1 transurethral bladder biopsy.

For each included case of amyloidosis, a routine IHC panel was performed with anti-SAA, anti-TTR, anti-κ, and anti-λ antibodies. Cases were classified as AL, non-AL (AA or TTR), and undetermined. Among the 32 included amyloidosis cases, first-intention IHC allowed identification of 10 AL and 6 non-AL amyloidosis (2 ATTR and 4 AA), whereas 16 cases were deemed “undetermined.” The IF-Pk and IHC results were in agreement for all the IHC-classified amyloidosis cases. Among the 16 cases classified as undetermined by IHC, IF-Pk allowed reclassification of 7 cases as AL amyloidosis (43.8%, including 4 AL-κ and 3 AL-λ cases Figures 1–3).

Concerning the 3 additional LC-associated disease cases, IF-Pk successfully revealed monoclonal deposits not seen by IHC in 1 case of pulmonary localized CSH$^{13}$ and 1 case of pulmonary LCDD.

Regarding the 7 cases of amyloidosis reclassified as AL amyloidosis by IF-Pk, 2 specimens were sent for MS at the request of the clinicians and confirmed as AL amyloidosis. Three patients had a second biopsy for IF on frozen sections, which also confirmed the diagnosis of AL amyloidosis (Figure 1). For the remaining 2 patients, compelling clinical and biological evidence led to the diagnosis of localized AL amyloidosis of the lung. In 1 of these 2 cases, furthermore,
the monotypia of the lymphoplasmocytic infiltrate surrounding the amyloid deposits corresponded with the LC incriminated by IF-Pk.

Among the 9 cases of amyloidosis that remained “undetermined” after both IHC and IF-Pk, 4 were sent for MS, which determined ATTR amyloidosis in 1 case, β2M amyloidosis in 1 case, ALECT2 amyloidosis in 1 case, and fibrinogen amyloidosis in 1 case Figure 1. The 5 remaining cases were definitely declared “undetermined.” All these patients had a second biopsy, sent fresh for IF during follow-up. The second sample was diagnosed as AL amyloidosis in 4 cases and as ATTR amyloidosis in 1 case (Figure 1).

Overall, among the 21 patients of our cohort with a final diagnosis of AL amyloidosis, IF-Pk correctly identified 17 (80.9%) versus only 10 (47.6%) for conventional IHC. When the latter was used as a first-intention technique, the use of IF-Pk on undetermined samples allowed reclassification of 7 of 11 cases of AL amyloidosis (63.6 %), with no false-positive result.

The flow chart for the prospective validation study is available in Figure 1.

**DISCUSSION**

Amyloidosis typing has always been a challenge in pathology, particularly when amyloidosis is not clinically suspected, because in this case it is frequent to have only FFPE material. Although IHC is commonly used as a first-line option, it is known to be less sensitive than IF. MS-based methods have emerged as the new gold standard for amyloid typing, performing well on FFPE material. Yet, they are expensive and not accessible in most places. MS is thus restricted to selected cases with undetermined results on multiple biopsies, and there remains a need for alternative methods of typing on FFPE.

Over the last few years, IF-P has proved to be of great utility as a salvage technique in kidney pathology. In the studies concerned, IF-P on sections digested with pronase or proteinase K showed an excellent performance in amyloid typing and more generally typing of paraprotein-associated lesions within the kidney. One recent study on a limited number of cases suggested that IF-P might also be used for extrarenal locations of amyloidosis. Its results were seen with an intriguing, as in the past, pronase pretreatment has been associated with loss of amyloid antigenicity and

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**Figure 2.** Amyloidosis of the digestive tract. (A) Congo red staining magnification ×200. (B) Congo red staining magnification ×400, amyloid deposits are visible along the basement membranes and within the submucosal space. (C) Congo red staining, magnification ×400, deposits show a yellow-green birefringence when the Congo red stain is examined under polarized light. (D) Congo red staining, magnification ×400, Congo red staining viewed with a Texas Red filter on an epifluorescence microscope highlights the distribution of amyloid deposits.
retrieval methods for amyloid subtyping have never proven their utility in routine practice. We have thus sought to evaluate the use of IF-P for amyloidosis and other LC-associated diseases.

Our first step was to validate IF-P as a robust and semiautomated immunolabeling technique for amyloidosis typing on FFPE material. To do so, we studied the diagnostic performances of both IF-Pn and IF-Pk, as compared with conventional IHC, for the detection of LCs within deposits of well-documented cases of AL amyloidosis and 3 cases of LCDD. Conventional IHC exhibited a sensitivity of only 60% (36.1–80.9), consistent with the literature data. Some expert centers have a different experience, reporting almost equivalent results for IHC and MS. Yet, achieving such performances required the use of specific homologous antibodies and multiple steps of standardization with defined amyloid prototypes. Such measures could only be implemented in a few reference centers. In the present work, IF-P yielded better results than IHC, especially when performed on proteinase K-digested sections. In this case, the sensitivity was 90% (68.3–98.8), with 100% specificity. Proteinase K was slightly better than pronase: 2 cases of AL amyloidosis were missed with proteinase K and 4 with pronase (sensitivity 90%, 68.3–98.8 versus 80%, 56.3–94.3). Because of fixation, residual serum stains induce a background signal in both IF-P and IHC, as opposed to IF. Background noise with anti-λ antibodies is almost absent, as compared with immunohistochemistry.

In most places, the first step in amyloidosis typing on FFPE material is IHC with a minimal comparative panel, including anti-SAA, anti-TTR, anti-κ, and anti-λ antibodies. Here, to evaluate the performance of IF-Pk on unselected cases in routine practice, we chose to perform it in parallel with the IHC panel, on all amyloidosis cases (without frozen material) seen at our institution. On the one hand, all cases of AL amyloidosis diagnosed by IHC were also correctly recognized by IF-Pk. Furthermore, IF-Pk allowed reclassification of 7 of 11 (63.6%) AL cases that first-intention IHC failed to identify. Because IF-Pk is a relatively new technique, most cases classified as AL amyloidosis by IF-Pk were confirmed during follow-up, either by IF on a second sample or by MS. Even though the sensitivity of IF-Pk clearly enhances the detection of LCs in

Figure 3. Comparison of immunofluorescence on proteinase K-digested sections and immunohistochemistry. (A, B) Results of immunohistochemistry, amyloid deposits are slightly more stained with anti-κ (A) than with anti-λ antibodies (B), but the difference in staining intensity between the 2 light chains is insufficient to make a diagnosis of light chain amyloidosis. A lymphoplasmocytic infiltrate served as an internal control. (C, D) Results of paraffin immunofluorescence on proteinase K-digested sections, staining of the deposits is more intense with anti-κ (C) than with anti-λ (D). Background noise with anti-λ antibodies is almost absent, as compared with immunohistochemistry.
FFPE material, some cases can still be missed; among the patients whose status remained undetermined after both IHC and IF-Pk, 4 were secondarily diagnosed as AL amyloidosis by IF on frozen sections. Overall, for amyloidosis, the proportion of correctly classified cases increased from 50% to 71.9% with the addition of second-intention IF-Pk to the classical IHC procedure.

Regarding other LC-associated diseases, we studied only a limited number of cases. Our results suggest, however, that IF-Pk may outperform IHC in these cases also, as IF-Pk revealed monoclonal deposits undetected by IHC in 1 case of LCDD and 1 case of CSH. In CSH, the crystalline nature of the inclusion suggests that IF-Pk could even be more sensitive than conventional IF, as described for LC proximal tubulopathy. More studies are necessary, however, to test this possibility.

Why is IF-Pk more sensitive than IHC for the detection of LCs within deposits? Several hypotheses might be considered. First, proteolytic digestion may be more effective than traditional heat-based antigen retrieval steps (performed in citrate or ethylenediaminetetraacetic acid buffer) at denaturing protein tertiary or quaternary structures that prevent antibodies from reaching their target. In the case of amyloidosis or LC-associated crystalline structures, the proteins present in deposits have lost their native structure through major conformational transformations, and peptides may be required to expose a sequestered epitope. Specific enzymatic digestion steps are also required for anti-TR IFHC. Another possibility is that the antibodies used in IF recognize free LCs more readily than complete ones in intact immunoglobulins. In support of this view, commercial anti-κ and anti-λ antibodies designed for IHC are mostly used to assess the monoclonality of lymphoplasmacytotic proliferation, unlike the fluorescein isothiocyanate-conjugated antibodies used in renal pathology.

Our study has some limitations. First, we report a monocentric experience, and our findings need to be reproduced in other centers. Moreover, the small number of cases included in the prospective part of the study should also prompt future works. Next, not all the cases that remained undetermined after both IHC and IF-Pk benefited from subsequent MS. Instead, most of the patients were classified by IF on a second sample sent fresh. Although IF performs well in amyloid typing, recent studies have shown it can lead to rare cases of misclassified AL amyloidosis, as compared with MS. On the other hand, these limitations highlight the difficulty of gaining access to MS in some countries and the need for alternative typing methods.

Our results support the idea of performing IF-Pk instead of IHC for LC immunolabeling in amyloidosis. It is true, however, that mixing different detection techniques could slow down the typing process, as compared with an IHC-only panel. Moreover, not all pathologists use an epifluorescence microscope in their daily practice. For these reasons, and because external validation has not yet been done, we recommend IF-Pk as an additional technique in cases of undetermined results with the first IHC panel. Only in cases of very limited material should it replace κ and λ detection by IHC.

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