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## **Diagnosis of Amyloidosis**

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### **1. Introduction**

Despite a strong clinical suspicion of amyloidosis, the diagnosis must be confirmed by tissue biopsy. Histological examination of biopsy specimens demonstrates an amorphous, eosinophilic substance that stains pink with the Congo red, and displays characteristic apple-green birefringence by polarized microscopy [1]. The histological analysis is the only method for establishing the diagnosis of amyloidosis [2,3]. The deposition of amyloid occurs in extracellular matrix, and often in a perivascular distribution with some degree of heterogeneity [1-3].

Although in the systemic amyloidosis the biopsies can be obtained from any organ affected, the blood vessel fragility associated with amyloid deposition carries a risk of bleeding [2,3]. Thus, in the clinical routine, biopsies from non-symptomatic sites are more commonly used [2,3]. In the past, rectal and gingival biopsies were considered the gold standard for the diagnosis of amyloidosis, but actually, abdominal fat pad aspiration has been the preferred due its simplicity, low cost, minimal complications, and good accuracy [1,4].

### **2. Abdominal fat pad aspiration or biopsy**

Westermarck and Stenkvist in 1973 described a method to remove pieces of subcutaneous abdominal fat for diagnosis of amyloidosis[3]. Although some variants has been described, normally the aspiration is done using an 18-23 gauge needle, with 2-5 aspirations [3,5]. The sensitivity reported range from 55-75% and specificity is over than 90% [2,6]. Guy and Jones, analyzing the performance of the abdominal fat pad aspiration in 45 patients with systemic amyloidosis found sensitivity of 58%, specificity of 100%, positive predictive value of 100% and negative predictive value of 85%, confirming the accuracy of the methodology [7].

The clinician and pathologist must be familiarized with the methodology, histological pitfalls and the possibility of false negative, as possible in preferential deposition in terms of organ involvement of amyloid depending of its subtype, as the transthyretin type, with its predilection to deposit in the heart [1,3]. Another situation that can result in false negative, for example, is when the disease is an early stage with amyloid deposits in plaques [8].

### 3. Rectal biopsy and others gastrointestinal tract sites

The rectal biopsy was the most used diagnostic method in the past. Actually it has been replaced by abdominal fat pad aspiration, because this is more feasible in the clinical practice with low cost and lack of complications. Analysis of deep fragments including the submucosa, obtained during a rectoscopic examination, the sensitivity ranges from 75-85% [3,9].

Other sites of gastrointestinal tract can be biopsied. Tada studied 42 patients with gastrointestinal amyloidosis and found amyloid deposition especially in the duodenum and jejunum [10]. Okuda Y et al had similar results assessing rheumatoid arthritis patients, where the proportion of amyloid deposition was 76.5% for duodenal cap and 88.6% for second portion of the duodenum, suggesting a good efficacy of duodenal biopsy in this population [11].

Labial and gingival biopsy has been shown useful in the amyloidosis diagnosis, but the latter is less sensitivity [3]. Several studies have confirmed the usefulness of labial biopsy, such as Fatihi et al that evaluated labial biopsy in patients with renal amyloidosis and found amyloid deposits in 80% of accessory gland biopsy and 75% of rectal biopsy [12]. Lechapt-Zalcman et al performed labial salivary biopsy in 32 patients with polyneuropathy of unknown origin and detected amyloid deposits in 7 (transthyretin in five and AL in two), proposing this technique as routine in investigation of axonal polyneuropathies [13]. Hachula et al detected amyloid deposits in 26 of the 30 patients with systemic amyloidosis using labial salivary gland biopsy, emphasizing the importance of this procedure, even in the absence of oral symptoms [14].

### 4. Others biopsy sites

Because there is risk of life-threatening bleeding, biopsy from others sites is used only whether abdominal fat pad aspiration, rectal or labial salivary gland biopsy fail to establish the diagnosis [3].

The kidney is the most frequently involved organ in systemic amyloidosis and although kidney biopsy is fundamental for diagnosis, this procedure has been contraindicated in some situations, for example, bleeding diathesis, and can be complicated by perirenal hematoma or arteriovenous fistula [15]. Before performing a kidney biopsy, less invasive biopsy procedures from easily accessible tissues should be considered. Yilmaz M et al studying 78 patients with chronic kidney disease found the frequency of amyloid deposition was 100% in

the duodenum, 83% in the rectum, and 29% in the gingiva, without complications related to endoscopy or biopsies [15].

Since the cardiac involvement is the major prognostic determinant in systemic amyloidosis [16], the evidence of cardiac lesion is crucial to therapeutic decisions. The gold standard test for diagnosing cardiac amyloidosis is the endomyocardial biopsy, however, it is not performed routinely due risk of complications, although infrequent, such as ventricular wall perforation, cardiac tamponade, pneumothorax, and arrhythmias [17]. Therefore, the cardiac amyloidosis is normally established by echocardiographic evidence of amyloidosis and histologic confirmation of amyloid on noncardiac tissue [17]. The changes observed in the echocardiography are those of restrictive cardiomyopathy with concentric ventricular hypertrophy, especially in the interventricular septum and posterior wall of the left ventricle [3]. Low voltage on electrocardiography and interventricular septal thickness of  $> 19.8\text{mm}$  on echocardiography together have a sensitivity of 72% and specificity of 91% for cardiac amyloidosis [18].

## 5. Determining the type of amyloid protein

Effective medical treatment needs an accurate diagnosis with demonstration of amyloid deposition in the tissues and accurate molecular classification of amyloidosis [1,19,20]. For example, in AL amyloidosis, derived from immunoglobulin light chain, the cornerstone of treatment is the aggressive treatment of the underlying neoplastic process, and in AA amyloidosis, the target of treatment is the underlying inflammatory disease [20-23].

Immunohistochemistry is currently the standard methodology for amyloid typing in routine clinical practice; it has been able to identify amyloid deposits through binding antibodies directed against most of the amyloid molecules identified to date. In patients with systemic amyloidosis, studies with antibodies to AA and to the immunoglobulin light chains are usually sufficient [2,20]. Some pitfalls are present in the clinical practice, and in some cases, misdiagnoses may occur, especially when immunohistochemical staining is performed in the absence of standardized antibodies and appropriate positive controls [24].

The majority of cases of AA can be reliably typed in frozen and/or paraffin sections, but immunohistochemical typing of AL is still challenging, due commercial antibodies are raised against the constant regions of the respective immunoglobulin light chains, and whether a subset of AL, in which amyloid fibrils are derived from a truncated light chain (ie, containing only variable regions), will be expected to be nonreactive with commercial antibodies [25-27].

Another important pitfall is the presence of background stain in the tissue, which in paraffin sections in particular can be significant due the “locking-in” of serum proteins during fixation [20]. The use of frozen specimens and immunofluorescence stains considerably increases the reliability and reproducibility of labeling with antibodies to immunoglobulin light chain, due provide a cleaner background [20,28]. Picken emphasizes that the interpretation of immunohistochemistry performed in paraffin sections and immunofluorescence in frozen sections is not a simple matter and also depends on the experience and expertise of the operator [20].

Since early diagnosis is a very important step to appropriate treatment of transthyretin (TTR) amyloidosis, and this amyloidogenic protein causes two different forms of the disease (hereditary amyloidogenic TTR [ATTR] amyloidosis and senile systemic amyloidosis [SSA]), we should accurately distinguish them. For instance, to detect Val30Met mutation in TTR gene, which is the most frequent pathogenic mutation in hereditary ATTR amyloidosis, some researchers use real-time PCR genotyping assay, considering reliable, rapid, cost-effective, and suitable analysis, however, to achieve accurate results the application of both genetic and proteomic methods is preferable to compensate the disadvantages and possible pitfalls in each of the techniques used [19]. Using proteomics techniques, amyloid typing can be successful in small samples, including biopsies [29,30]. Several TTR variants can be detected in serum specimens using mass spectrometry or sophisticated electrophoresis techniques [31,32], however, this methodologies, and others new technologies, such as laser microdissection, are frequently available only at specialized centers.

6. Assessing the extension of involvement in systemic amyloidosis

The amyloid typing must be followed by distinction between localized and systemic amyloidosis [20]. While the treatment of localized forms is mainly conservative, the treatment of systemic forms has been more aggressive, and the prognosis is directly related with the disease extension, and the organs affected [2,20]. To determine the extension of the disease, some investigations are necessary, and it is presented in Table 1.

Organ	Performed routinely	Performed as clinically indicated
Kidneys	Proteinuria, serum creatinine, ultrasonography	Renal vein Doppler ultrasound
Heart	Chest radiography, ECG, echocardiography, MRI, NT- <sup>99m</sup> Tc-pyrophosphate scan, 24-h Holter proBNP/troponin	
Gastrointestinal tract	Serum protein electrophoresis	Gastrointestinal endoscopy, oesophageal manometry
Liver	Liver enzymes	Ultrasonography
Spleen	Ultrasonography, blood cell counts	Howell-Jolly bodies in blood smears
Nerves	-	EMG
Respiratory system	Chest radiography	Blood gas analysis, bronchoscopy, CT scan of the chest
Endocrine glands	ACTH test, TSH	-
Eyes	Fundoscopy	Slit-lamp examination
Haemostasis	PT, X factor	-

ECG – electrocardiography; MRI – magnetic resonance imaging; NT-proBNP – N-terminal pro-brain natriuretic peptide; EMG – electromyography; ACTH – adrenocorticotrophic hormone; TSH – thyroid stimulating hormone; PT – prothrombin.

Table 1. Determining site and extent of amyloidosis [3,17]

## 7. Conclusion

The clinical suspicion must be confirmed with histological examination, and the amyloid typing is crucial to determine the correct treatment. Although the apparently simplicity of the abdominal fat pad aspiration has facilitated the diagnosis of amyloidosis, the physicians should be aware to pitfalls, especially in the amyloid typing, requiring an expert pathologist to correct analysis.

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