The value of abdominal fat biopsy in the diagnostic search for amyloid substance

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Abstract

Considering their interest in amyloidosis, particularly of the primary type (AL), and the results described in the literature that indicate a high sensitivity yield in the localization of the amyloid substance in abdominal fat biopsy, the authors have developed and applied this technique, particularly after 1990.

They assess the results obtained from forty eight patients submitted to this study by abdominal subcutaneous fat biopsy, appreciating the technique sensitivity and specificity and dedicating special attention to the criteria that lead to its execution. They also discuss the value of the potassium permanganate test. With this work, the authors wish to communicate their experience and evaluate the criteria for the amyloid substance search, contributing to the increase in diagnosis of amyloidosis by use of a simple technique.

Key words: systemic amyloidosis, abdominal fat biopsy, diagnosis, potassium permanganate.

Introduction

The term "amyloid" was coined in 1838, by a German botanist, to describe the normal amylaceous constituent of plants (starch).[1] Although the disease has been described for more than 300 years, through the verification, in autopsies, of voluminous organs with a lardaceous and fatty appearance, it was in 1854 that Virchow used the term "amyloidosis" in humans, due to the sulfuric acid/iodine properties of the substance detected in the organs involved, and that was purportedly similar to cellulose.[1,2,3,4,5] Thus he established a name and a disease, the nosology of which continues in the 1990s, attracting the attention of clinicians and pathologists due to its multiplicity of clinical manifestations.

Initially designated lardaceous disease, it was found in patients with tuberculosis, syphilis, malaria, osteomyelitis or another bone diseases (secondary amyloidosis - AA). It was not until 1856 that Wilks described a case of a patient with lardaceous viscera not related to infection, marking the first suggestion of primary amyloidosis (AL).[1,2]

Amyloidoses are, therefore, a heterogeneous group of disorders, sharing the common characteristic of the presence of extracellular amyloid deposits in the various tissues and organs responsible for the alteration in their normal structure and function.[1,6,7]

Clinically, they are divided into localized or systemic, depending on whether there is involvement of just one organ, or simultaneous involvement of several tissues of the body. They may also be hereditary or acquired (secondary systemic, reactive or AA amyloidosis, and amyloidosis associated with immunocyte dyscrasia, primary or AL amyloidosis) (*Table 1*).

Their diagnosis is now performed by identifying the amyloid deposits, while until recently, it was only possible through a histological exam of the affected tissues.[7,8,9,10,11,12,13,14]

Structurally, the amyloid substance is made up of fibrillar aggregates that are linear, with a rigid, unbranched structure, typically arranged in a b--pleated sheet; it is insoluble and generally resistant to proteolytic digestion, constituting a vast group of protein complexes (*Table 1*) that are not related, yet share common chemical and physical characteristics.

It is traditionally recognized by its homogeneous eosinophilic appearance when observed under an optical microscope, while green birefringence, when seen under polarized light after Congo Red staining (*Fig. 1*), remains, until today, the standard process for

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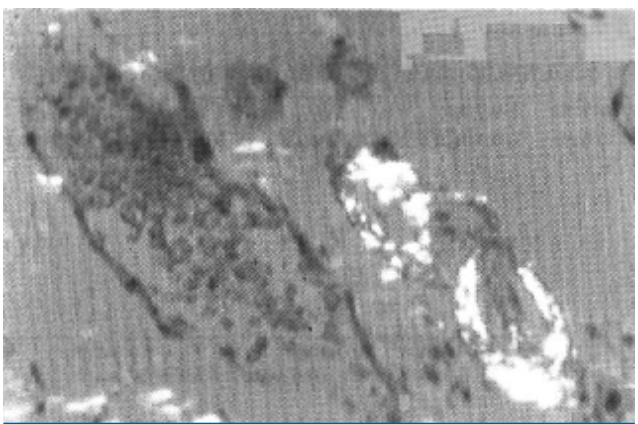
TABLE I

Systemic amyloidosis: immunohistochemistry identification

	Congo Red	Kappa/ Lambda	Protein A	β 2 M	Transthyretin (Prealbumin)
AL	+	+	–	–	–
AA	+	–	+	–	–
FMF	+	–	+	–	–
DRA	+	–	–	+	–
ATTR	+	–	–	–	+
SSA	+	–	–	–	+

Abbreviations: FMF – Familial Mediterranean fever; DRA – Dialysis-related amyloidosis; ATTR – Familial amyloidosis; SSA – Senile systemic amyloidosis
Adapted from Kyle and Gertz[1]



Birefringence under polarized light, corresponding to amyloid material surrounding the wall of the abdominal subcutaneous fat vessels (Congo Red with polarized light).

FIG. 1

its suspicion.[5,7,9,10,11,12,15,16] However, false positive and negative results have been found for this test, with greater frequency at less experienced centers.[7,17,18,19]

Other substances have been used as amyloid stains, but none of these methods has proven as reliable as the use of Congo Red.[7,9]

In an attempt to sub-classify the different types of amyloid substance, this method has undergone several modifications, through physical and chemical procedures that make it possible to modulate the congophilia according to the type of amyloid deposited in the tissue. This is the case of the potassium permanganate test, which characteristically produces a loss of tissue affinity for Congo Red in cases of AA or β2M amyloidosis, enabling a distinction to be made between these types of amyloidosis and AL, senile amyloidosis, familial amyloidosis and localized amyloidoses, which are resistant to digestion with potassium permanganate.[1,12,20]

They are frequently misleading methods and became obsolete with the appearance of the immunohistochemistry techniques (*Table 1*).[7,10,12,20] Through the use of specific antibodies for each type of amyloid, these techniques are reliable in the identification of the different types of amyloid substance, particularly of AA and β2M amyloidosis; for AL amyloidosis, they only produce positive results in 50% of cases, since there are no commercially available antiserums for all the types of light chain fragments, which are often unique.[7,12] These are techniques for amyloid characterization to be carried out subsequently, after identification of this substance by traditional methods.[7,10,12]

Electronic microscopy, although until recently considered the most sensitive and specific method in revealing amyloid tissue deposits,[3,11,15] even small ones, is today considered insufficient for the diagnostic confirmation of this disorder, especially when used in isolation.[10,21]

The identification of amyloid substance was initially performed in an autopsy, or in the case of clinical suspicion and where there was high sensitivity, in the product of invasive biopsy of symptomatic organs, (90-100%),[11,14,22] such as the kidney, liver, digestive tract, myocardium, carpal ligament, and sural nerve; they are, however, expensive methods, sometimes difficult to perform and not free from complications, particularly hemorrhagic complications.

Thus the need arose to resort to more innocuous and equally sensitive methods, particularly for use in large groups.

Based on the evidence that amyloid substance deposits can involve any organ, studies were undertaken using biopsies in non-symptomatic sites. Rectal submucosa biopsy was the traditional harvest site, with an incidence of positive results in 75-85% of cases.[9,11,22] It is, however, a technique that besides causing discomfort for the patient, may involve hemorrhagic complications and requires a high level of

Abdominal subcutaneous fat biopsy technique

FIG. 2

technical experience, due to the need to include the submucosa.[9,11,10,12]

On the other hand, less invasive biopsies, such as that of the bone marrow and of skin, have proven less sensitive (25-50% and 40-55% of cases, respectively).[9,11,12,17,15,18,22]

In 1973, Westermark and Stenkvist initiated the abdominal fat aspiration procedure (AFP) for the first time, to search for amyloid substance, based on the fact that amyloid deposits can be found in the connective tissue surrounding the adipocytes, and in particular, in the fat of the abdominal wall.[9,11,19] This simple method, which was easy to execute (technique described by Gerts et al.)[19] and posed no serious risks, proved to be equally sensitive to, or more sensitive than rectal biopsy (58-90%).[12,15,17,18,23,24] It is, moreover, a technique with excellent predictive value (specificity of 99%)[9,14,25] and, for this reason, very useful in the definitive diagnosis. Today it is the method of choice in the evaluation of systemic amyloidosis.

Other authors have obtained similar results with biopsy of accessory salivary glands, and stress the importance of its use, particularly in elderly patients, since like the case of abdominal fat aspirate, it has good sensitivity and specificity, without positive results for senile amyloidosis.[14]

We should not, however, forget that as deposits of amyloid substance are habitually focal, all these methods could produce negative results, which never exclude the presence of an amyloidosis.

Other techniques, especially that of radio-labeling of the amyloid P-component (SAP), have recently led to major advances in the knowledge of the natural history of amyloidoses, through the in vivo "labeling" of the amyloid deposits in the tissues, allowing their identification, evaluation of their distribution in the different organs/tissues, quantification, natural evolution and monitoring of the therapeutic approach. Unfortunately, this technique is not yet available here among us, and the histological examination remains the only method that can provide us with the definitive diagnosis of this condition.

In our Services, we initially perform the puncture, with aspiration of abdominal fat. Due to technical difficulties, the sensitivity of this method proved very low compared with the literature, therefore from the year 1990, we opted for the performance of a true abdominal subcutaneous fat biopsy (AFB) (*Fig. 2*), in an attempt to increase the number of amyloidosis diagnoses.

Material and methods

Analysis of the patients' processes submitted to AFB in the Medicine II Services of the HUCs (Coimbra University Hospitals), from 1990 until the end of 1993. Patients diagnosed with familial amyloidosis were excluded in advance. The goal was to retrospectively evaluate the selection criteria for biopsy performance, the sensitivity and specificity of the method, its morbidity and the agreement of the clinical criteria with the outcome of the potassium permanganate test.

The biopsies were performed in the lower quadrants of the abdomen, after anesthesia with 2% lidocaine, and immediately fixed in 10% formaldehyde. The Congo Red technique was used for the identification. The chemical test used for its characterization was potassium permanganate, defining AA amyloidosis when the congophilia disappeared and AL amyloidosis when no alteration was observed after digestion with the potassium permanganate.

Results

We obtained a population of 48 patients, made up of 22 males and 26 females. The mean age, at the time of the diagnosis, was 70 ± 10 years, ranging from 26 to 90 years.

The reasons for performing the biopsy were (*Fig. 3* and *Table 2*): in 14 patients, two of them with criteria of multiple myeloma; one monoclonal gammopathy (MG), the presence of a clinical history compatible with amyloidosis in 28 patients; nephrotic syndrome

TABLE II

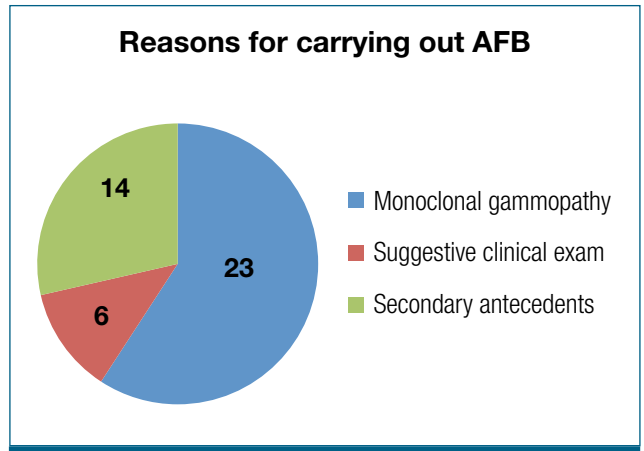
Reasons for performance

Monoclonal gammopathy		14
Myeloma	2	
Suggestive symptoms		28
Heart failure	10	
Kidney failure	12	
Hepatomegaly	4	
Nephrotic syndrome	4	
Changes in bowel movement	3	
Skin manifestations	3	
Peripheral polyneuropathy	3	
Non-nephrotic proteinuria	3	
Carpal tunnel syndrome	3	
Splenomegaly	1	
History of AA		6
Rheumatoid arthritis	3	
Hansen's disease	1	
Chronic osteomyelitis	1	
Chronic pyelonephritis	1	

in 4; non-nephrotic proteinuria in 3; kidney failure in 12; heart failure in 10; pericardial effusion or other suggestive echocardiographic alterations in 6, carpal tunnel in 3; peripheral polyneuropathy in 2, cutaneous manifestations in 3, hepatomegaly in 4, changes in the bowel movement in 3 and splenomegaly in just 1 patient; possible etiologies of secondary amyloidosis (AA) in 6 patients — rheumatoid arthritis in 3, chronic osteomyelitis in 1, Hansen's disease in 1 and chronic pyelonephritis in 1.

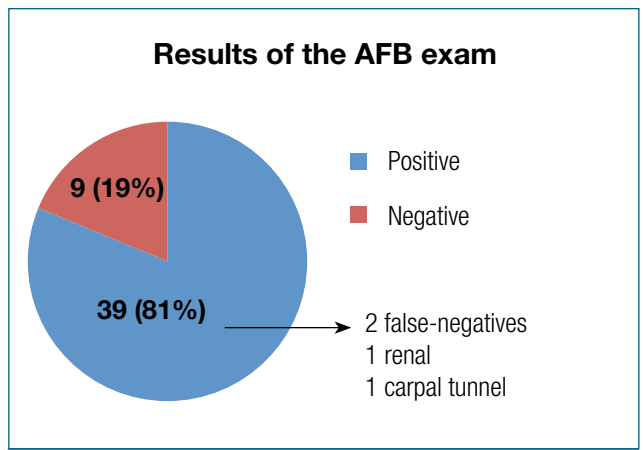
Fifteen of the patients had been submitted to two-dimensional echocardiography, some alterations compatible with amyloidosis having been found: pericardial effusion in 6 patients, valvular thickening in 3, thickening of the septum and of the ventricular walls in 1 and diastolic dysfunction in 3.

The bone marrow examination, carried out on 26 patients, revealed, in 13 cases, less than 5% of plasmocytes; in 7, between 5 and 10%; and, in 6, there were more than 10% of plasmocytes. Of the 48 biopsies performed, the search was positive in 9



Reasons for carrying out abdominal subcutaneous fat biopsy.

FIG. 3



Results of the abdominal subcutaneous fat biopsy.

FIG. 4

(19%) (*Fig. 4*).

MG was the criterion of performance that led to the highest number of positive results (3 in 14).

There was no subsequent histological confirmation in any other tissue or organ, or by autopsy, in any of these patients.

Using the criteria of Duston et al,[9] to subdivide the patients by the clinical/laboratorial evidence of systemic amyloidosis, we obtained 2 groups (*Table 3*). In the first, we included 6 patients with parameters "highly suggestive" of systemic amyloidosis, i.e., presenting one or more of the following characteristics:

TABLE III

Evidence of systemic amyloidosis in the 9 patients with positive biopsy

Patient	Basis of diagnosis
	High probability (clinical/laboratorial)
1	Polyneuropathy – M-component urine and serum
2	Carpal tunnel syndrome –M-component in serum
3	BM Changes + weight loss – echocardiography
4	Medullary plasmacytosis –M-component in serum
5	Arthralgias – echocardiography
6	Plasmacytosis – Delayed inflammatory syndrome
	Possible (clinical/laboratory)
7	Nephrotic syndrome, heart failure – pleural empyema
8	Kidney failure, history of chronic pyelonephritis – proteinuria
9	Hepatosplenomegaly, hypogammaglobulinaemia

Adapted from Duston et al.[9]

macroglossia, MG, light chains in the serum or urine, plasmacytosis in the bone marrow or characteristic "granulated" pattern in the echocardiography. We included the remaining 3 patients in the second group, with just a "possible" diagnosis of systemic amyloidosis.

The analysis of the 9 patients with positive biopsy enabled us to divide them clinically into: a) suggestive of AL (n=3); b) suggestive of AA (n=3); c) non-AA or AL oriented (n=3). In one of the cases submitted to the permanganate test, the result was not in agreement with the clinical suggestion (*Table 4*).

Two false negative results stand out; one patient suffering from secondary amyloidosis diagnosed by renal biopsy and the other with primary amyloidosis, with the amyloid having been identified in the carpal ligament following surgery for carpal tunnel syndrome (*Fig. 4*).

None of the cases developed any complications, such as infection or suture dehiscence.

Discussion and conclusions

AFB proved to be a method that is easy to carry out, not requiring the patient's hospitalization, with reduced costs, painless and without morbidity in the population of this study.

Unlike our previous experience with AFP, this technique seemed to us to be more effective, probably because it allows much larger samples to be harvested.

In 48 biopsies, we obtained 19% positive results (9 patients) that we consider significant, comparing them with other published series relating to AFP (7%).[9]

Although the definitive diagnosis requires the identification of amyloid substance in the tissues, there are certain clinical and laboratory manifestations which, although varied and nonspecific, should alert the clinician to the possibility of an amyloidosis. Examples, absence of an evident cause, and in particular, age over 40 years, are proteinuria, congestive heart failure, cardiomyopathy, peripheral neuropathy, carpal tunnel syndrome, macroglossia and hepatosplenomegaly.[3,18]

In our series, the most frequent reason for performing biopsy was the existence of a suggestive clinical context (in 28 patients). Renal involvement (occurring in 90% of the patients with systemic amyloidosis) resulted, in the majority of our cases, in kidney failure (12 patients); proteinuria with or without nephrotic syndrome, which is the form of manifestation most frequently found in amyloidoses (7-12% have amyloidosis),[11,18,19] was, respectively, the reason for the biopsy in 3 and 4 of our patients.

Cardiac involvement appears more frequently in primary amyloidosis (10% in secondary amyloidosis and 90% in primary amyloidosis),[26] and is the main cause of death; this situation alerted us to the possibility of amyloidosis in 10 patients presenting congestive heart failure. Even in patients without cardiac complaints, echocardiography can lead to the diagnosis of an amyloidosis, having proven, in some series, the most sensitive noninvasive method in the detection of cardiac involvement.[10,23] These are, however, nonspecific alterations, including the finding of the classical granular pattern of the ventricular myocardium, but which can also be found in other situations, specifically in left ventricular hypertrophy due to other causes.[26]

Hepatomegaly (in 4 of our patients) and/ or splenomegaly (in just 1), as already mentioned above, might be related to systemic amyloidosis, particularly if associated with proteinuria (frequent association in primary amyloidosis), MG, Howell-Jolly bodies in

TABLE IV

Comparison between the clinical classification and the results of the permanganate test

Patient	Clinical/laboratory	Permanganate test
1 M-component urine and serum, polyneuropathy	A L	A L
2 M-component in serum, carpal tunnel syndrome	A L	
3 Echocardiography, transit alterations + weight loss	AL/ AA ?	A A
4 M-component in serum, medullary plasmacytosis	A L	A A
5 Echocardiography, arthralgias	AL/ AA ?	A L
6 Delayed inflammatory syndrome, plasmacytosis	AL/ AA ?	
7 Nephrotic syndrome, heart failure, pleural empyema	A A	A A
8 Proteinuria, kidney failure, history of chronic pyelonephritis	A A	A A
9 Hepatosplenomegaly, hypogammaglobulinaemia	A A	

peripheral blood smear (by spleen infiltration) or with normal or slightly increased values of the liver function tests (in disproportion to the hepatomegaly).[3]

Peripheral polyneuropathy (in 3 patients) and carpal tunnel syndrome (in 3 patients) are more frequently related to primary amyloidosis. It is emphasized that in one of our cases with carpal tunnel syndrome, the AFB gave a false negative result, with the diagnosis having been performed by histology of the carpal ligament. This situation has been observed in other series[19] in which the test for amyloid in the abdominal fat, rectal submucosa and bone marrow frequently proved negative, making it necessary to resort to the carpal ligament. Unfortunately, many surgeons do not propose this diagnostic hypothesis when sending the surgical specimen for histological study, often delaying the diagnosis.[13] The sensitivity of this method is 95%, even in very early stages of the disease, and can precede the diagnosis by two years.[3,13]

In three of our patients, cutaneous manifestations led to the amyloid test. These may be varied, and are more frequent in AL amyloidosis.[18]

Finally, three of the patients had altered intestinal transit possibly caused by involvement of the autonomic nervous system or by direct deposition on the walls of the digestive tract, producing obstipation, occlusive/sub-occlusive pictures and later on, diarrhea. None of the patients exhibited malabsorption syndrome or digestive hemorrhage, which may also be form of presentation of an amyloidosis.[21]

We found possible etiologies for an AA amyloidosis in 6 patients, infectious pathology in 3, and the presence of rheumatoid arthritis in the others.

But among the reasons that led to the biopsy, MG was the one that produced the largest number of positive results in this series of ours (3 in 14).

The presence of monoclonal protein in the serum or urine appears in between 80 to 90% of the cases of AL amyloidosis, requiring its screening, particularly in a given clinical context (as was the case in 9 of the 14 patients – 64%).[12,23,27] The performance of a bone marrow examination or of a bone biopsy is necessary in these cases, and it is possible to find bone marrow plasmacytosis (above 5% in most cases)[1,12,17] and, in 30 to 50% of the patients, the presence of amyloid substance, doing away with the need for biopsy at other sites for diagnosis.

Two of the 14 patients with MG had criteria for multiple myeloma. As is known, these two disorders constitute different spectrums of the same disease[3] (immunocyte dyscrasia), and are sometimes hard to distinguish (habitually based on bone marrow plasmacytosis, quantification of monoclonal protein in the serum and urine and the presence of bone lesions).[1,5]

As mentioned, the clinical manifestations can suggest the type of amyloidosis.[4,22,28] In secondary amyloidosis (AA), there is renal involvement in more than 90% of the patients, with hepatosplenomegaly also appearing frequently. The heart and the peripheral nerves are rarely involved. In AL, on the contrary, there is a predominance of cardiac involvement (90% of the cases), macroglossia, peripheral neuropathy and carpal tunnel syndrome, besides the very frequent presence of monoclonal protein in the serum/urine.

Based on these criteria, we attempted to classify the 9 patients with positive biopsy, later comparing the results with those of the permanganate test (*Fig. 3*). We experienced certain difficulties in the clinical clas-

sification of some patients, due to the overlapping of syndromes and because the permanganate test did not always prove in agreement. However, we emphasize that none of these criteria has an absolute value, and there references to frequent exceptions[4,12] in which only the immunocytochemical study of the amyloid fibril can enable a distinction to be made between the different types of amyloidosis. ■

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