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VIEWPOINT

EDUCATIONAL CORNER

Abdominal Fat Biopsy for the Diagnosis of Cardiac Amyloidosis

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Amyloidoses comprise a group of disorders caused by extracellular deposition of misfolded proteins, which aggregate into insoluble fibrils constituting the amyloid substance (1). Immunohistochemistry or proteomic analyses allow the classification of the different types of amyloidosis on the basis of the specific amyloidogenic precursor. The International Society of Amyloidosis recognizes 36 different amyloidogenic proteins in humans. According to the nomenclature established by International Society of Amyloidosis, the amyloid protein is defined by the letter "A," followed by a suffix indicating the specific protein. From a clinical perspective, amyloidoses can be classified into acquired conditions or conditions related to mutations and into systemic or localized forms (1). **Table 1** summarizes the characteristics of some of the most common forms of systemic amyloidosis. Cardiac involvement in amyloidosis is more common in immunoglobulin light chain (AL) amyloidosis and in transthyretin amyloidosis (ATTR) because of either wild-type or mutated (variant) transthyretin (1–4).

The most frequently used method for amyloid identification in tissues remains the demonstration of the characteristic apple-green birefringence pattern on Congo red–stained sections under a polarized light microscope. Immunohistochemistry and mass spectrometry techniques are useful for amyloid subtyping, which is crucial for a timely initiation of targeted treatments, including chemotherapy regimens in AL amyloidosis (5,6).

Biopsy of the affected organs has a high diagnostic yield, but it is invasive and may cause severe and potentially life-threatening complications (7). Gingival and rectal biopsies were proposed in the 1960s as less invasive approaches. A possible alternative is abdominal fat pad fine-needle aspiration biopsy, which has excellent specificity and positive predictive value (both 100%), but low sensitivity, ranging from 84% in AL amyloidosis to 15% in wild-type ATTR, and a high rate of inadequate specimens (11%) (8). Abdominal fat pad excisional biopsy has been reported to have a higher accuracy; it is only slightly more invasive than fine-needle aspiration biopsy and can ensure adequate material for amyloid typing when electron microscopy is not available, given that proper sample processing is warranted (7,8). Nonetheless, because of its limited sensitivity, particularly in ATTR, a negative fat biopsy result does not exclude the diagnosis of amyloidosis.

This paper and Video 1 describe how to perform an abdominal fat biopsy to diagnose amyloidosis.

EQUIPMENT

The material needed to perform a small incision with sterile technique and to fix tissue samples should be prepared, including sterile gloves; sterile towels or drapes; 1% lidocaine (10 ml); 2 25- to 27-gauge needles; a non-Luer-lock syringe; a surgical kit including needle driver, scalpel, and toothed forceps; sterile gauze pads; nonabsorbable monofilament suture (e.g., polyester); a plaster; a box with 10% formalin;

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TABLE 1 Characteristics of the Main Forms of Systemic Amyloidosis

Type	Acquired or Hereditary	Abnormal Protein	Heart	Kidney	Liver	Neuropathy	Other Organs
AL	Acquired	Immunoglobulin light chains	+++	+++	++	+	GI tract, soft tissues
ATTR	Acquired	TTR wild type	+++	-	-	-	Carpal tunnel syndrome
	Hereditary	Mutated TTR	++	-	-	+++	
AA	Acquired	SAA	±	+++	+ (late)	-	-
ALECT2	Acquired	LECT2	-	+++	++	-	-
Aβ2M	Acquired or Hereditary	β_2-microglobulin	-	-	-	±	Carpal tunnel syndrome, joints
AFib	Hereditary	Fibrinogen	-	+++	±	-	-
AApoAI	Hereditary	Altered ApoA-I	+	++	++	+/−	Testes
ALys	Hereditary	Mutated lysozime	-	+	++	-	GI tract, skin

AA = inflammatory/reactive amyloidosis; AApoAI = apolipoprotein A-I amyloidosis; Aβ2M = β_2-microglobulin amyloidosis; AFib = amyloidosis with deposition of the α chain of fibrinogen A; AL = amyloid light chain amyloidosis; ALECT2 = LECT2 amyloidosis; ALys = lysozyme amyloidosis; ApoA-I = apolipoprotein A-I; ATTR = amyloid transthyretin amyloidosis; GI = gastrointestinal; LECT2 = leukocyte cell-derived chemotaxin-2; SAA = serum amyloid A; TTR = transthyretin; +++ = very common; ++ = common; + = uncommon; ± = rare; − = not applicable or not occurring in this condition.

and a box with 2.5% glutaraldehyde (in case electron microscopy is planned).

STEP 1: PREPARATION OF THE BIOPSY SITE

Shave the lower abdominal area. Using the preferred agent, clean the skin on the lower quadrants of the abdomen, below the umbilicus. Disinfect the skin through an antiseptic agent such as povidone-iodine. Aspirate approximately 10 ml of 1% lidocaine. Attach a 25- to 27-gauge needle to the syringe, and remove any trapped air. Make multiple injections in the subcutaneous tissue around the site of biopsy, and inject a total of 3 to 4 ml of lidocaine. Remember to aspirate before injecting, to avoid intravascular injection. If needed, stop bleeding by firm application of sterile gauze pads. Wait 3 to 4 min for the effect of lidocaine to develop. Use povidone-iodine for further disinfection, and isolate the area with sterile towels. For most patients undergoing minor skin surgery, no antibiotic prophylaxis is recommended (9).

STEP 2: BIOPSY

Perform a linear incision approximately 1.5 cm (0.59 inch) long, approximately 5 cm (1.97 inch) below the umbilicus. The incision should reach at least the middle layers of subcutaneous fat. Apply firm pressure on the area of procedure with a pad of gauze to prevent extravasation of blood. Grasp the fat and pull it gently upward. Use the scalpel to excise the fibrofatty tissue (at least 2 to 3 fragments with a volume of 0.1 to 0.2 cm^3 each). Suture the skin incision with a simple interrupted technique; 3 to 4 stitches are usually needed.

STEP 3: SPECIMEN PROCESSING

Transfer the fibroadipose tissue into the 10% formalin container while taking care that the tissue is completely immersed in formalin. If electron microscopy is available, place 5 to 6 other fragments of fibroadipose tissue in glutaraldehyde solution. Label the containers appropriately. Submit the formalin for histology; a short fixation time is recommended to avoid overfixation artifacts.

For electron microscopy, tissue samples must be fixed with 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer, pH 7.4, for 2 h at 4°C (39°F). These samples will then be rinsed in phosphate buffer, post-fixed in 1% phosphate-buffered osmium tetroxide for 30 min at 4°C (39°F), and then dehydrated in a graded series of ethanol, transferred to propylene oxide, and embedded in an epoxy resin.

WHAT TO REMEMBER

The biopsy can be performed in an inpatient or outpatient setting. Patients should be asked about allergies to antiseptics, local anesthetic agents, and adhesives. Dual antiplatelet therapy, combined antiplatelet and anticoagulant therapy, or thrombocytopenia is not a contraindication to periumbilical fat biopsy, but hemostasis may be more difficult to achieve in these patients. The biopsy should not be performed in the site of scars, bruises, rashes, and demonstrated or possible infection. The patient can stand up after 10 min from the end of the procedure.

POSSIBLE COMPLICATIONS

Complications include bleeding and hematoma, infection, allergic reactions, and aesthetically suboptimal scar formation (10). Bleeding or the formation of a hematoma after the procedure can often be treated by first applying manual pressure and then a pressure dressing. Persistent bleeding may require removal of the dressing and sutures, evacuation of any hematoma, and meticulous hemostasis with

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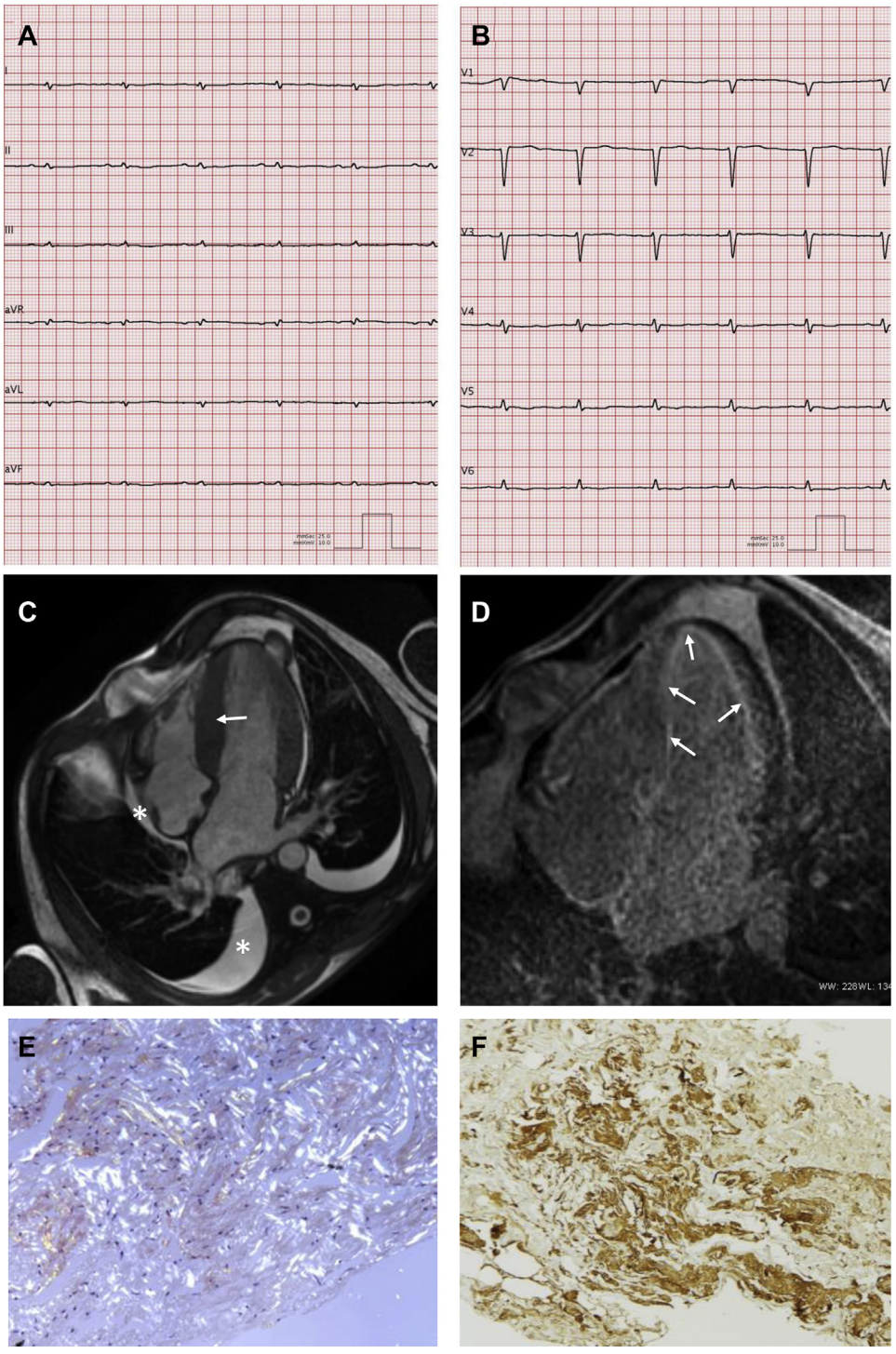
FIGURE 1 ECG, CMR, and Histological Features of the Patient



(A and B) Peripheral and precordial leads of a resting electrocardiogram (ECG) showing diffuse low QRS voltages. **(C)** Pre-contrast cardiac magnetic resonance (CMR) 4-chamber view showing increased wall thickness **(arrow)** and pericardial and pleural effusion **(asterisks). (D)** Late post-contrast cardiac magnetic resonance view showing diffuse subendocardial enhancement **(arrows). (E)** Congo red staining from a periumbilical fat specimen showing apple-green birefringence (original magnification 10×). **(F)** Lambda light chain immunostaining from a periumbilical fat specimen (original magnification 10×).

chemical agents, such as aluminum chloride, or with electrosurgery. Wound infection is usually noted several days after the procedure. The patient should be instructed to monitor the procedural area for signs and symptoms of infection, such as erythema, pain, swelling, purulent discharge, and complete or partial wound dehiscence. The patient should be advised to seek immediate medical attention if these problems develop. In an infected wound, sutures should be removed, cultures of the wound should be obtained, and pus drained. Appropriate antibiotic treatment should be started, and the wound must be left open to heal by secondary intention.

A PROCEDURE IN CONTEXT

The patient shown in Video 1 was a 69-year-old man referred to cardiological evaluation because of worsening dyspnea. He had hypertension, diabetes, chronic obstructive pulmonary disease, and a history of multiple myeloma treated with autologous stem cell transplantation in 2017. His electrocardiogram showed sinus rhythm and low QRS voltages (**Figures 1A and 1B**). On echocardiographic examination, left ventricular ejection fraction and volumes were normal, but wall thickness was increased (interventricular septum 15 mm, posterior wall 12 mm), and there was grade II diastolic dysfunction. Renal function was preserved (estimated glomerular filtration rate 98 ml/min/1.73 m^2), and serum protein electrophoresis showed a monoclonal peak. Both N-terminal fraction of pro-B-type natriuretic peptide (766 ng/l) and high-sensitivity troponin T (50 ng/l, reference value <14 ng/l) were increased. Bence Jones proteinuria and increased serum lambda free light chain (52.80 mg/dl; reference value 0.57 to 2.63) were also found. Cardiac magnetic resonance demonstrated increased left ventricular mass, increased precontrast T_1 signal, early darkening of the blood pool, and subendocardial circumferential late gadolinium enhancement (**Figures 1C and 1D**). The patient was eventually diagnosed with cardiac AL amyloidosis after a periumbilical fat biopsy sample showed Congo red-positive deposits composed of lambda light chains (**Figures 1E and 1F**). The patient started hematologic treatment with lenalidomide and dexamethasone, together with furosemide (to relieve pulmonary congestion) and an angiotensin-converting enzyme inhibitor (as cardioprotective therapy) (1).

CONCLUSIONS

Abdominal fat biopsy is a minimally invasive and safe procedure that can lead to the histological confirmation and typing of amyloid deposits. Despite its limited sensitivity, periumbilical biopsy can obviate the need for endomyocardial biopsy in the diagnostic flowchart of cardiac amyloidosis.

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REFERENCES

1. Aimo A, Buda G, Fontana M, et al. Therapies for cardiac light chain amyloidosis: an update. Int J Cardiol 2018;271:152–60.

2. Aimo A, Castiglione V, Borrelli C, et al. Oxidative stress and inflammation in the evolution of heart failure: from pathophysiology to therapeutic strategies. Eur J Prev Cardiol 2020;27:494–510.

3. Vergaro G, Aimo A, Barison A, et al. Keys to early diagnosis of cardiac amyloidosis: red flags from clinical, laboratory and imaging findings. Eur J Prev Cardiol 2019 Oct 4 [E-pub ahead of print].

4. Wechalekar AD, Gillmore JD, Hawkins PN. Systemic amyloidosis. Lancet 2016;387:2641–54.

5. Kourelis TV, Gertz MA. Improving strategies for the diagnosis of cardiac amyloidosis. Exp Rev Cardiovasc Ther 2015;13:945–61.

6. Mollee P, Renaut P, Gottlieb D, Goodman H. How to diagnose amyloidosis. Int Med J 2014;44:7–17.

7. Shidham VB, Hunt B, Jardeh SS, et al. Performing and processing FNA of anterior fat pad for amyloid. J Vis Exp 2010;44:1747.

8. Guy CD, Jones CK. Abdominal fat pad aspiration biopsy for tissue confirmation of systemic amyloidosis: specificity, positive predictive value, and diagnostic pitfalls. Diagn Cytopathol 2001;24:181–5.

9. Wright TI, Baddour LM, Berbari EF, et al. Antibiotic prophylaxis in dermatologic surgery: advisory statement 2008. J Am Acad Dermatol 2008;59:464–73.

10. Saun TJ, Truong J, Dev SP, Wanzel K. How to perform an elliptical excision of the skin. N Engl J Med 2019;380:e34.

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APPENDIX For a supplemental video, please see the online version of this paper.