

Fat aspiration procedure for the detection of amyloid

Aspiration of abdominal subcutaneous fat tissue

Aspiration of abdominal subcutaneous fat tissue is a simple outpatient procedure (1). It should be noticed, however, that it takes at least 10 - 15 minutes to avoid unnecessary pain and bruising and to get ample material. The patient should know that bruising might occur.

Equipment used for fat aspiration

See figure 1A for the equipment: Chlorhexidine solution for skin cleaning, a 5 ml syringe connected to a 22 Gauge needle for lidocaine anaesthesia (figure 1B), two 10 ml syringes connected by a valve system to 16 Gauge needles for fat aspiration (figure 1C), small band-aids, gauzes, and protective gloves.



Fig 1A. Equipment for procedure



Fig 1B. Equipment for anaesthesia



Fig 1C. Equipment for fat aspiration

Fat aspiration technique

A syringe of 10 ml is connected by a valve system to a needle of 16 Gauge (figure 2A). After closing the valve the plunger is pulled out, fixed with squeezed fingers, and the cap of the lidocaine needle can be reused elegantly by positioning it upside-down inside the plunger ("Tarek's trick") to fix firmly the position of the plunger and thus maintain negative pressure in the syringe during aspiration (see figure 2B and 2C).



Fig 2A. Closed valve, reuse cap



Fig 2B. Pull plunger, (Note cap) ...

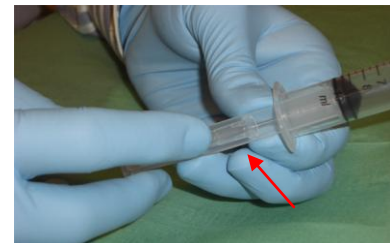


Fig 2C. ... place cap, fix plunger.

The skin of the patient is marked (figure 3A) and cleansed (e.g. with chlorhexidine) at both sides of the umbilicus at about 7-10 cm distance. Skin and subcutaneous tissue (three directions, see below) are then anaesthetized with lidocaine (each side 2 ml=20 mg)(figure 3B). Check first that the patient is not allergic to lidocaine. After inserting the needle beneath the skin the valve can be opened to start aspiration of fat tissue (figure 3C).



Fig 3A. Marking the skin

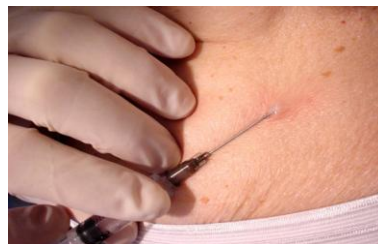


Fig 3B. Anaesthesia with lidocaine



Fig 3C. Opened valve, aspiration

The needle can be moved into three directions (Northeast, East, and Southeast) at the left side of the abdomen and mirror-wise at the right side. The aspiration procedure should be performed slowly and gently into each of the three directions, going to and fro with some rotation, and one should realise that it takes some time before the needle will be filled with fat tissue and the first fat can be seen passing the valve and entering the top of the syringe. This can be continued until enough fat tissue has been collected (figure 4A). After finishing the procedure press the puncture site for a while and cover the puncture site with a band-aid (figure 4B). Aim of the procedure is to obtain an adequate quantity for microscopic analysis (30 mg) and further at least 30 mg of fat tissue for immunochemical quantification of SAP and the specific amyloid proteins. Aspiration can be done at both sides of the umbilicus in order to obtain at least 60 mg of fat tissue (figure 4C).



Fig 4A. Result in syringe



Fig 4B. Gross weight and size



Fig 4C. Cover puncture site

When you are finished and have collected enough fat tissue, the easiest solution for you is: **Seal the syringes and send them to Groningen for analysis at room temperature; see below ➤**

Two technical problems can be encountered during aspiration: no tissue at all or much blood entering the syringe.

1. If no fat appears in the syringe or the aspiration has stopped completely, the needle may have become obstructed. The simplest way to check this is to remove the needle out of the patient. Normally, fat tissue present in the needle is then directly and audibly forced into the syringe because of negative pressure. If this is not the case and fat tissue obstructs the needle completely, tissue in the needle can be removed by using positive pressure in the syringe. This may lead to a rather explosive evacuation ("firing fat tissue") and should therefore be carried out carefully. Tissue is evacuated into a clean container (e.g. sputum or urine) or empty syringe, while fixing the needle firmly to the syringe to prevent the needle leaving the syringe ("firing needles").

2. If much arterial or venous blood enters the syringe by accident, the needle should be removed out of the body. The puncture site should be pressed for at least one minute, and the procedure can be repeated into a different direction or at a different site. Pain is infrequent, localised, and seldom a real problem necessitating the use of more lidocaine. If bruising is suspected to be present at the end of the procedure, the patient him/herself may press the puncture site for a couple of minutes before rising from the supine position.

- Fat tissue analysis in short -

After extracting the plunger, fat tissue can be collected from the syringe on an empty glass slide to separate fat tissue from accidentally obtained blood.

Preparing slides for microscopy

At least four visible fragments of fat tissue (not fat droplets!) should be put on each of three glass slides (preferably with a frosted edge which can be used to write on it with a pencil). These fragments are crushed into a single layer by squeezing a second slide placed perpendicularly to the first ones (figure 5). It is important to press in the middle of the glass slides to prevent breaking of glass. The resulting six smears are marked for identification, dried in the air at room temperature for one hour, and subsequently fixed with acetone for 10 minutes. After drying and fixation, all slides can be stored at room temperature until (shipped to a reference centre for) staining with Congo red and further study if positive for amyloid. Fat tissue should not be frozen before slides have been made: freezing of fresh and unfixed tissue may affect the quality of the tissue.

Fat aspiration procedure



Fig 5A. Four lumps of fat tissue



Fig 5B. Perpendicular glasses

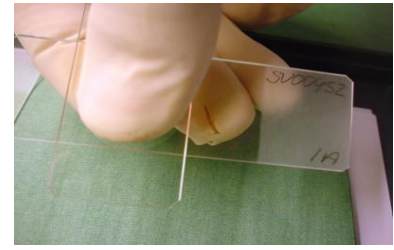


Fig 5C. Squeezing

Congo red stain and microscopy

Staining with alkaline Congo red should be performed according to the classic method described by Puchtler (2). In short:

Prepare stock solution I: saturated solution of NaCl in 80% ethanol

Prepare stock solution II: saturated solution of NaCl and Congo red in 80% ethanol

Prepare working solutions I and II by adding NaOH (final concentration 0.01%) just before use and then filter.

- Stain for 30 sec with Mayer's Hematoxylin
- Rinse in running tap water for 10 min
- Stain for 30 min in freshly filtered working Solution I
- Stain for 30 min in freshly filtered working Solution II
- Rinse briefly in ethanol (100%) 2x
- Rinse briefly in demineralised water 2x
- Cover the slides with Kaiser's glycerol gelatin and a cover glass.

The affinity of tissue for Congo red can be analysed by the apple-green birefringence in polarised light using a good microscope. In our institution we use the Olympus BX 50 microscope, a strong (100 Watt) light source, and two investigators score the slides blinded to the clinical data and in a semi-quantitative grading system: 0 (negative, no apple-green birefringence detectable), 1+ (minute, <1% of surface area), 2+ (little, between 1% and 10%), 3+ (moderate, between 10% and 60%), 4+ (abundant, >60%) (figure 6).

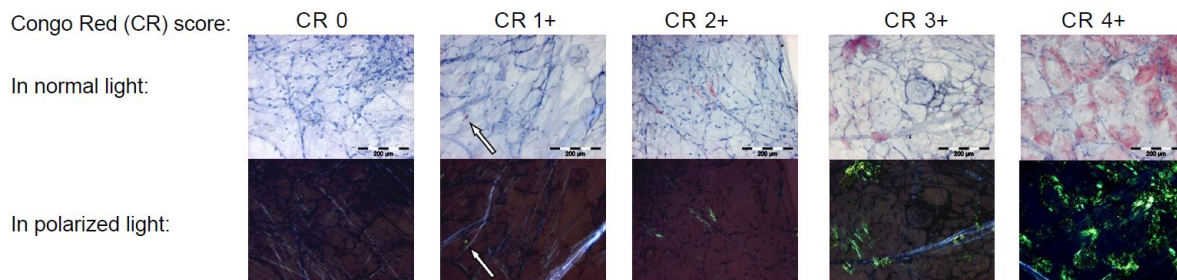


Fig 6. Congo Red grading system

Preparation and extraction of fat tissue aspirates

The remaining fat tissue can be stored in an 2 ml Eppendorf cup (at -20 C or -80C) until shipment (at room temperature preferably within three days, but ultimately within one week) to our hospital in Groningen, The Netherlands (see the address below ➡).

Immunochemical quantification of the concentration of SAP and the amyloid precursors AA, TTR, kappa, and lambda in fat tissue extracts

Before quantification, the amount of fat is weighed to get the so-called wet weight. It is then washed three times in a Tris buffer with calcium to remove possible remnants of blood still present. Then SAP is extracted by incubation with a Tris buffer with EDTA and the SAP concentration can be measured by ELISA. Subsequently the washed fat tissue is extracted in

a solution of Tris and guanidine, centrifuged, and the supernatant of the fat tissue extract is collected. The total protein concentration and amyloid A protein concentration is measured by ELISA as has been described (3). Dutch reference values of patients without AA amyloidosis: < 12 ng/mg fat tissue or < 1.3 µg/mg protein. TTR and immunoglobulin kappa and lambda light chain concentrations can also be measured by ELISA, Western blot or Nephelometric methods.

Information and addresses

Clinical questions and information:

Dr. Bouke P.C. Hazenberg (b.p.c.hazenberg@reuma.umcg.nl)

Tel: +31 50 3613432 (secretary) or +31 50 3616161 (operator call)

Fax: +31 50 3619308

Laboratory questions and information:

Mr. Johan Bijzet (j.bijzet@lc.umcg.nl)

Tel: +31 50 3613421

➤ **Address for sending glass slides or syringes for amyloid detection and precursor quantification:** (Please notify us by phone or e-mail before sending the material)

University Medical Center Groningen

Mr. Johan Bijzet, BSc

Lab. Rheumatology, T2.238

HPC EA41

Hanzeplein 1,

9713 GZ Groningen

The Netherlands

Tel: +31 50 361 3421

Fax: +31 50 361 3591

E-mail: j.bijzet@lc.umcg.nl

References

1. Westermark P. Diagnosis and characterization of systemic amyloidosis by biopsy of subcutaneous abdominal fat tissue. *Internal Medicine Specialist* 1984;5:154-60.
2. Puchtler H, Sweat F, Levine M. On the binding of Congo red by amyloid. *J Histochem Cytochem* 1962;10:355-63.
3. Hazenberg BP, Limburg PC, Bijzet J, van Rijswijk MH. A quantitative method for detecting deposits of amyloid A protein in aspirated fat tissue of patients with arthritis. *Ann Rheum Dis* 1999; 58:96-102.

© Bouke P.C. Hazenberg, MD, PhD, consultant rheumatology
GUARD (Groningen Unit for Amyloidosis Research & Development)
University Medical Center Groningen, The Netherlands

Revised version, 18 April 2011

