ARTHRITIS & RHEUMATISM
Vol. 54, No. 6, June 2006, pp 2015–2021
DOI 10.1002/art.21902

Diagnostic Accuracy of Subcutaneous Abdominal Fat Tissue Aspiration for Detecting Systemic Amyloidosis and Its Utility in Clinical Practice

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Objective. **Aspiration of subcutaneous abdominal fat is a simple and fast method for detecting systemic amyloidosis; however, the sensitivity of this approach remains undetermined. The aim of this study was to assess the accuracy of fat tissue aspiration for detecting systemic amyloidosis and the utility of this method in clinical practice.**

Methods. **All consecutive patients with established and suspected systemic amyloidosis who attended our tertiary referral hospital between 1994 and 2004 underwent aspiration of subcutaneous abdominal fat. Congo red–stained tissue was assessed quickly in a single smear in a routine manner by a single observer, and was also assessed thoroughly in 3 smears by 2 independent observers.**

Results. **One hundred twenty patients with established systemic amyloidosis were studied (38 with AA amyloidosis, 70 with AL amyloidosis, and 12 with ATTR amyloidosis). Routine (quick) assessment was associated with a sensitivity of 80% (95% confidence interval [95% CI] 72–87%). Sensitivity increased to 93% (95% CI 87–97%) when 3 smears were thoroughly examined. The specificity of fat aspiration in 45 control subjects was 100% (95% CI 92–100%). One hundred sixty-two patients for whom there was a clinical suspicion of systemic amyloidosis were screened for amyloidosis by fat tissue aspiration and biopsy of at least 1 other tissue. In 69 (43%) of these 162 patients, a diagnosis of amyloidosis was established, and in 66 (96%) of these patients, the results of fat tissue aspiration were positive. The clinical utility of fat tissue aspiration was greater than that of biopsy of the rectum.**

Conclusion. **Subcutaneous abdominal fat aspiration is the preferred method for detecting systemic amyloidosis, with sensitivity of 80% associated with use of a routine approach. The use of a thorough assessment (3 fat smears, 2 observers) increased sensitivity to >90%. If the results of fat tissue aspiration are negative, the additional value of a subsequent biopsy of the rectum is negligible.**

The amyloidoses are a group of diseases characterized by deposition of proteinaceous fibrils with a cross–β-sheet molecular structure. This structure is responsible for the binding affinity of Congo red dye and the green birefringence observed with polarized light (1). Extracellular deposition of amyloid fibrils results in loss of organ function. Deposition of amyloid can be localized (restricted to 1 organ or site of the body) or systemic (in various organs and tissues throughout the body). Systemic amyloidosis is a disease with high mortality because of the progressive and widespread deposition of amyloid in vital organs (2).

Amyloid deposition in the kidneys, nerves, spleen, vitreous body, and abdominal fat is seen exclusively in patients with the systemic forms of amyloidosis. The detection of amyloid in the bone marrow, heart, liver, gastrointestinal tract, lung, or joint nearly always indicates systemic amyloidosis. The 3 major types of systemic amyloidosis are AA, AL, and ATTR amyloidosis. These types can be distinguished by the nature of the precursor protein of the fibrils. The underlying diseases require different treatment (2). In all of the

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Submitted for publication December 20, 2005; accepted in revised form March 3, 2006.

systemic amyloidoses, accurate and early diagnosis is extremely important for clinical management. Early effective treatment will retard or even stop further deposition of amyloid, but the potential effect of treatment will be dependent on the extent of disease progression at the time of diagnosis.

The diagnosis of amyloidosis is based on histologic analysis. A biopsy specimen should demonstrate positive staining with Congo red dye and characteristic apple-green birefringence observed with polarized light. Biopsy of an involved organ is the diagnostic gold standard. However, biopsy of a clinically suspected site (kidney, liver, or heart) is an invasive procedure, and significant complications, such as bleeding, are a potential risk. Both biopsy of the rectum and abdominal fat aspiration are frequently used to detect systemic amyloidosis in patients with signs or symptoms of the disease.

Since its introduction in the 1960s, biopsy of the rectum has been considered by many investigators to be the gold standard for screening, with a sensitivity of ~80% (3–6), but this method is rather inconvenient and time-consuming. Aspiration of abdominal fat tissue is a simple, safe, and less inconvenient method, which was originally described in 1973 (7). It is a fast bedside or outpatient procedure (8). As previously mentioned, amyloid deposition in abdominal fat tissue is seen exclusively in the setting of systemic amyloidosis. The specificity of this test, provided that the staining procedure is performed correctly, approaches 100% (9–12). Sensitivity values vary greatly, from 52% to 88% (5,9–18). This wide range might be attributable to the amount of fat tissue, differences in experience with the staining and scoring of biopsy specimens, characteristics of the patients, the size of the study group, and the type of systemic amyloidosis.

Because of the wide range of sensitivity reported in the literature, the clinical utility of abdominal fat aspiration for detecting systemic amyloidosis remains to be determined. In clinical practice, other more invasive biopsies often are performed in order to establish the diagnosis. In the present study, we analyzed our experience with abdominal fat aspiration over a 10-year period. The aim of the study was to assess the diagnostic accuracy of abdominal fat aspiration and the clinical utility of this method.

PATIENTS AND METHODS

Study design. All consecutive patients seen at our tertiary referral university hospital between 1994 and 2004 were prospectively studied. In patients and control subjects, subcutaneous abdominal fat tissue was aspirated and stained with Congo red dye in order to detect amyloid. To assess the diagnostic accuracy and clinical utility of the stained fat tissue, 3 study groups were defined. The sensitivity of fat tissue aspiration was studied in patients with well-established systemic amyloidosis (standard group). The specificity of this procedure was studied in control subjects without systemic amyloidosis; this group comprised healthy individuals and patients with typical localized amyloidosis (control group). Localized amyloidosis was defined by the clinical presentation of only 1 typical tissue (e.g., the eyelid or larynx) affected with amyloid in a patient in whom no sign or symptom of systemic amyloidosis developed for at least 5 years despite a rigorous search for amyloid in other sites, such as the rectum or bone marrow. Specificity and sensitivity were determined for both routinely and thoroughly assessed fat smears.

The clinical utility of fat tissue aspiration was studied in patients for whom there was a clinical suspicion of systemic amyloidosis (screening group). In this screening group, biopsy of at least 1 tissue other than abdominal fat had to be performed in order to compare the performance of fat tissue with that of other tissues. In all groups of subjects, fat tissue was compared concurrently with other biopsy samples, especially those obtained during biopsy of the rectum. The local ethics committee approved the study, and all patients and controls who gave informed consent were included.

Detection and classification of amyloidosis. In the standard group, a diagnosis of systemic amyloidosis was established if the patient had either positive results of a biopsy at a site typically involved in systemic amyloidosis, such as the kidney, liver, heart, rectum, or nerve, or a positive result of a biopsy at a different site combined with signs or symptoms indicating systemic amyloidosis (19). Obviously, fat tissue from this group was excluded from diagnosis. Patients with systemic amyloidosis were classified as having AA, AL, or ATTR amyloidosis, as described elsewhere (20).

In the screening group, the criteria for diagnosis and classification were similar to those used in the other groups, but in the screening group, fat tissue positivity alone was sufficient for establishing the diagnosis of amyloidosis. If the findings in fat tissue and other tissues were negative, amyloidosis was considered to be absent. For all patients, the minimum clinical followup period was 2 years.

Fat aspiration, staining with Congo red dye, and assessment of fat smears. Skin and subcutaneous tissue on both sides of the umbilicus of patients and controls were anesthetized with lidocaine. On both sides of the umbilicus, fat tissue was aspirated with a 16-gauge needle connected to a 10-ml syringe. The aim was to aspirate at least 30 mg of fat tissue. At least 4 visible fragments of fat tissue were put on each of 3 glass slides and crushed into a single-cell layer by pressing a second slide perpendicularly to the first, as previously described (21). The 3 smears were dried in air at room temperature, fixed with acetone, and stained with alkaline Congo red dye according to the method described by Puchtler et al (22). The affinity of tissue for Congo red staining was analyzed by apple-green birefringence as observed with polarized light, using a 100-watt Olympus BX50 microscope (Olympus, Hamburg, Germany).

If a particular patient had more than 1 fat tissue sample, the first sample obtained was used. Fat smears from

Table 1. Sensitivity of Congo red–stained fat smears from 120 patients with established systemic amyloidosis, assessed in 3 different ways*

Amyloidosis type (n)	1 smear fast, 1 observer		3 smears fast, 1 observer		Thorough, 2 observers	
	Sensitivity	95% CI	Sensitivity	95% CI	Sensitivity	95% CI
AA (38)	30 (79)	63–90	31 (82)	66–92	35 (92)	79–98
AL (70)	56 (80)	69–89	61 (87)	77–94	66 (94)	86–98
ATTR (12)	10 (83)	52–98	10 (83)	52–98	10 (83)	52–98
All (120)	96 (80)	72–87	102 (85)	77–91	111 (93)	87–97

* Except where indicated otherwise, values are the number (%). See Patients and Methods for a description of the 3 different methods. 95% CI = 95% confidence interval.

patients were randomly combined with those from controls, and all smears were assessed in a manner blinded for clinical data. Smears from all subjects were assessed in 3 different ways: a single smear in a routine manner by a single observer, 3 smears in a routine manner by a single observer, and 3 smears in a thorough manner by 2 observers. All aspirates from individuals in each of the 3 groups were reviewed by 2 observers in a blinded manner.

The first method was designated "single smear fast." A single experienced observer (BPCH) scored amyloid as being absent or present in a single smear, at 40× magnification, for a maximum observation period of 30 seconds, looking for red-stained particles or areas and, only if such particles or areas were present, subsequently looking for typical green birefringence. The second approach was designated "3 smears fast." The difference between this and the former method was that all 3 smears were scored per patient for a maximum of 90 seconds for all 3 smears together. The third technique was designated "thorough examination." Two experienced observers (JB and BPCH) independently scored all smears at 40× magnification, looking at all 3 smears per person for a maximum of 5 minutes for all 3 smears together. During a thorough examination procedure, the polarization filter was turned almost continuously in order to detect even minimal tissue fragments that showed birefringence from red to green. If the 2 observers disagreed, the 3 smears were reviewed and the findings discussed to obtain consensus.

Assessment of other biopsy specimens. The results for all other biopsy specimens stained with Congo red dye that had been obtained within 3 months before or after the first fat aspiration and routinely examined by pathologists for detection of amyloid were compared with the results of fat tissue analysis.

Statistical analysis. Statistical analysis was performed using the statistical software package GraphPad Prism, version 4.02 (GraphPad Software, San Diego, CA). The Mann-Whitney test was used to detect differences between variables for the patient groups. Fisher's exact test was used to calculate the differences present in 2×2 tables, where appropriate. For all tests, 2-tailed P values less than 0.05 were considered significant.

RESULTS

Specificity and sensitivity of fat tissue aspiration. One hundred twenty patients had established systemic amyloidosis at the time the first fat aspiration was performed: 38 patients (15 men and 23 women) had AA type, 70 patients (36 men and 34 women) had AL type, and 12 patients (5 men and 7 women) had ATTR type. The median ages of these patients were 58.5 years (range 13–77 years), 60 years (range 33–84 years), and 53 years (range 33–77 years), respectively. Underlying diseases in the patients with AA amyloidosis were inflammatory arthritis (n = 28 patients), chronic infection (n = 4), familial Mediterranean fever (FMF) (n = 3), and other (n = 3). The control group of 45 subjects (21 men and 24 women) comprised 27 healthy volunteers and 18 patients with typical localized amyloidosis. The subjects in this group were younger than those in the groups with AA, AL, and ATTR amyloidosis ($P < 0.05$), with a median age of 41 years (range 21–67 years).

Amyloid was not detected in fat tissue aspirated from any of the 45 control subjects. Therefore, the specificity of fat aspiration in the control group was 100% (95% confidence interval [95% CI] 92–100%).

Table 1 shows the sensitivity of fat tissue aspiration in the standard group of 120 patients with established systemic amyloidosis, as assessed by 3 different approaches. Fast assessment of a single smear by a single observer yielded a sensitivity of ~80% for the different types of systemic amyloidosis. Increasing the number of smears from 1 to 3 increased sensitivity by 3–7%, and thorough examination of the 3 smears by 2 observers further increased the sensitivity to >90% for both AL and AA amyloidosis.

A significant difference in sensitivity was detected between men and women ($P < 0.05$). The findings of fat tissue aspiration were negative in only 1 of 64 women (sensitivity 98%), compared with 8 of 56 men (sensitivity 86%).

Clinical utility of fat tissue aspiration. The screening group comprised 162 patients. Fat smears obtained from this group were thoroughly assessed to detect amyloid. The findings in fat tissue aspirates were

Table 2. Characteristics of 162 patients with clinical suspicion of systemic amyloidosis (screening group)*

Characteristic	AA type		AL type		ATTR type		None, amyloid−
	Amyloid+	Amyloid−	Amyloid+	Amyloid−	Amyloid+	Amyloid−	
No. of patients	25	42	27	24	17	7	20
Age, median (range) years	61 (27–81)	64 (24–84)	65 (43–84)	61 (34–77)	52 (24–65)	37 (28–59)	54 (24–73)
No. men/no. women	6/19	17/25	17/10	12/12	9/8	5/2	15/5
Indication for fat aspiration							
Neuropathy	3 (12)	2 (5)	6 (22)	8 (33)	10 (59)	0	15 (75)
Proteinuria	11 (44)	22 (52)	11 (41)	3 (13)	0	0	1 (5)
Renal failure	7 (28)	13 (31)	2 (7)	6 (25)	1 (6)	0	0
Cardiomyopathy	0	1 (2)	5 (19)	2 (8)	0	0	3 (15)
Macroglossia	0	0	3 (11)	1 (4)	0	0	0
Carpal tunnel syndrome	0	0	0	0	3 (18)	2 (29)	1 (5)
Gastrointestinal	1 (4)	1 (2)	0	0	1 (6)	0	0
Other	3 (12)	3 (7)	0	4 (17)	2 (12)	5 (71)	0

* Except where indicated otherwise, values are the number (%). None = no suspicion of a specific type of systemic amyloidosis.

positive in 66 patients (41%), and in 53 (80%) of these 66 patients the diagnosis was confirmed by the results of at least 1 other biopsy. In 13 patients (20%), only the findings for fat tissue were positive (4 AA, 7 AL, and 2 ATTR type). In 3 patients (2 with AL and 1 with ATTR type), the findings in fat tissue were negative whereas the findings in other tissue (i.e., kidney [twice] and duodenum) were positive. Eventually, systemic amyloidosis was diagnosed in 69 (43%) of 162 patients, of whom 66 (96%) had positive findings in fat tissue aspirates. In the screening group, the negative predictive value of fat tissue aspiration was 97% (93 of 96 patients had negative findings in fat tissue aspirates).

Table 2 shows the characteristics of patients in the screening group. The age and sex of these patients did not differ from those of patients with the corresponding type of amyloidosis in the standard group. Underlying diseases in the patients with suspected AA amyloidosis included inflammatory arthritis (n = 21), chronic infection (n = 1), and other (n = 3); none of the patients had FMF. Among patients with suspected AA amyloidosis, the diagnosis was confirmed in 37%; among those with suspected AL amyloidosis, the diagnosis was confirmed in 53%; and among those with suspected ATTR amyloidosis, the diagnosis was confirmed in 71%. During followup, clinical systemic amyloidosis developed in none of the 93 patients in whom no amyloid was detected in any biopsy specimen.

Amyloid was not detected in any of the 20 patients in whom no obvious form of the 3 main types of amyloidosis was suspected (no chronic disease, no plasma cell dyscrasia, no family history) before the fat aspiration was performed. In the majority (75%) of this group of 20 patients, neuropathy was the indication for fat aspiration.

In the screening group of patients with suspected AA amyloidosis, proteinuria as an indication for biopsy was not seen more frequently in those with amyloid than in those without amyloid. However, patients suspected of having AL amyloidosis differed: proteinuria was more often ($P < 0.05$) the indication for biopsy in patients with amyloid than in those without amyloid.

Comparison of fat tissue with tissue obtained from concurrent biopsies. In the standard group of 120 patients with established systemic amyloidosis, the sensitivity of biopsies of other tissue was also calculated, as shown in Figure 1. Table 3 shows in detail the number of

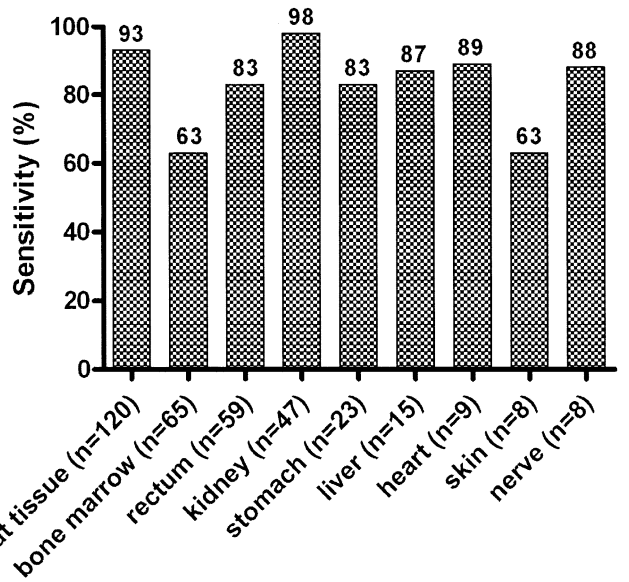

Figure 1. Sensitivity of the various tissues (n = number of samples) stained with Congo red in the standard group of 120 patients with established systemic amyloidosis.

Table 3. Results of thoroughly examined fat tissue and routine rectal biopsy in 120 patients with established systemic amyloidosis and 69 patients with amyloidosis in the screening group*

	Fat		Rectum		Rectum and fat			
Amyloidosis type	No. of patients	F+	No. of patients	R+	F+, R+	F−, R+	F+, R−	F−, R−
Established group								
AA	38	35 (92)	26	24 (92)	23	1	1	1
AL	70	66 (94)	22	17 (77)	15	2	4	1
ATTR	12	10 (83)	11	8 (72)	8	0	1	2
All	120	111 (93)	59	49 (83)	46	3	6	4
Screening group								
AA	25	25 (100)	22	18 (82)	18	0	4	0
AL	27	25 (93)	18	9 (50)	9	0	7	2
ATTR	17	16 (94)	9	6 (67)	6	0	2	1
All	69	66 (96)	49	33 (67)	33	0	13	3

* Values are the number (%). F = fat tissue; R = rectal biopsy.

patients in this group for whom biopsy specimens from the rectum were assessed concurrently with fat tissue. In only 3 patients were the results of the rectal biopsy positive while findings in fat tissue were negative, whereas in 6 patients the findings in fat tissue were positive and the results of rectal biopsy were negative. In 4 patients, the findings in both rectal tissue and fat tissue were negative.

Table 3 also shows details regarding the 69 patients with suspected amyloidosis (screening group) for whom biopsy specimens from the rectum were assessed concurrently with fat tissue. None of the 3 patients with negative findings in fat tissue had positive results of rectal biopsy, whereas the 13 patients with negative results of rectal biopsy had positive findings in fat tissue. In 6 of these 13 patients, the diagnosis was confirmed by the results of biopsy of tissue other than fat.

Invasive biopsies that were performed in the standard group of 120 patients versus the screening group of 69 patients, all of whom had systemic amyloidosis or suspected systemic amyloidosis, involved the following major organs: kidney, 39% versus 10%; liver, 13% versus 9%; and heart, 8% versus 6%.

DISCUSSION

Accurate and early diagnosis of systemic amyloidosis is essential in the clinical management of the disease. Fat tissue analysis is the best detection method and is associated with high clinical utility.

Amyloidosis is a histologic diagnosis, and it would be advantageous to have a specific test that does not require biopsy of a major organ (such as the kidney, liver, or heart), because of the risk of complications associated with such procedures. Even biopsy of the rectum, which for a long time has been considered the gold standard for screening, is an inconvenient, time-consuming, expensive, and risky procedure compared with fat tissue aspiration. Although in our study the groups were small and subjects in the control group were relatively young, we confirmed the specificity of thoroughly examined fat tissue to be 100% (95% CI 92–100%). The utility of this test is based on its excellent positive predictive value, i.e., that fat tissue positivity actually proves the presence of systemic amyloidosis. Despite these excellent values, one should keep in mind that high specificity depends on correct application of standard protocols using the Congo red staining method described by Puchtler et al (22–24). In clinical practice, it is good to recognize that false-positive results might occur. First, pale-yellow birefringent fibrin, collagen, or elastin fibers should be distinguished from the typical apple-green birefringence of amyloid. Second, exogenous polysaccharide materials, such as plant cell walls, starch, cotton fibers, and various fungi, stain with Congo red dye and show green birefringence (23,24).

According to the literature, the sensitivity of fat tissue for diagnosing systemic amyloidosis is still undetermined, because of the wide range in the values for sensitivity (52–88%) (14,15). Therefore, in routine clinical practice, invasive biopsies of visceral organs are still performed in many cases. Our study shows that in routine clinical practice, the sensitivity of subcutaneous fat tissue analysis is 80–85%, provided that an adequate amount of fat tissue (at least 30 mg) is properly processed and examined, and provided that 3 glass slides

per patient are examined instead of only 1. Systematic use of rectal, stomach, bone marrow, and especially abdominal fat tissue, as practiced in the screening group in this study, results in a diminished need to perform biopsies of major organs compared with the need in the standard group, as illustrated for the kidneys, for which the necessity to perform biopsy decreased ~30% (from 39% to 10%).

Thorough assessment of fat smears stained with Congo red dye increases sensitivity to >90% in AA and AL amyloidosis, without decreasing specificity. This is probably attributable to the extent of experience in assessing fat tissue, as discussed by Linke et al (25). These values are sufficiently high to propagate a more prominent role in clinical practice for fat tissue analysis in the detection of systemic amyloidosis. In patients with FMF, the sensitivity of fat tissue analysis seems to be lower than that in patients with other inflammatory diseases (26). Therefore, the high sensitivity of fat aspiration for AA amyloidosis in this study may be partly explained by the low number of patients with FMF.

Pitfalls in the analysis of fat tissue are false-negative results due to an insufficient amount of material (too little fat tissue aspirated), inadequate staining technique, improper use of polarizing instruments, and insufficient light intensity. Therefore, in case of negative findings in the fat aspirate from a patient with a persistently high clinical suspicion of amyloidosis or progressive disease for which there is no other explanation, fat aspiration should be repeated, and the aspirate should be sent for further analysis to a center with experience in this procedure. Recently developed techniques such as (immuno)chemical analysis of fat tissue may facilitate automated detection of amyloid in fat tissue (21,27,28). The development of generally available techniques for automated detection of amyloid in fat tissue with high accuracy would be a major step forward, because amyloid detection would then become independent of the experience and dedication of the observer.

The sensitivity of fat tissue aspiration turns out to be at least as good as (and, in the case of thorough examination, even better than) the sensitivity of biopsy of the rectum. Notably, if the findings in fat tissue are negative, the profitability of subsequent biopsy of the rectum is negligible. Therefore, if findings in a thoroughly examined fat smear are negative and clinical suspicion remains high, the next step is a directed biopsy of an organ suspected to be involved. As expected, the sensitivity of bone marrow biopsy turned out to be rather low (63%), whereas the sensitivity of biopsy of the kidney, liver, and heart was high (87–98%), as shown in Figure 1 (5). The 63% sensitivity associated with bone marrow biopsy does signify that when detection of an M protein suggests the need for a bone marrow biopsy in order to look for plasma cell dyscrasia, additional staining with Congo red dye may help to detect approximately two-thirds of the patients with amyloidosis, with minimal additional effort.

The utility of fat aspiration in clinical practice is high, as shown in the screening group, because the method easily identifies a group of currently treatable disorders. As reported above, the results of fat tissue aspiration were positive in 66 (41%) of the 162 patients. In 13 (20%) of these patients, fat tissue was the only tissue positive for amyloid. Only 3 (3%) of 93 patients with negative results of fat tissue analysis had amyloid in another tissue. Therefore, similar to the results in the standard group, the sensitivity of fat tissue aspiration in the screening group was higher than the sensitivity of biopsy of the rectum and bone marrow biopsy.

The high accuracy of fat tissue aspiration, along with the other advantages of being inexpensive, simple, fast, safe, and more convenient for the patient, makes fat aspiration the preferred method to use when searching for amyloid. Early diagnosis may have a favorable effect on clinical outcome, whereas both patient and clinician benefit from the reduction of uncertainty. Currently, the only disadvantage of fat tissue aspiration is its unsuitability for using immunohistologic analysis to establish the type of amyloid involved. This disadvantage can be overcome by using other approaches, such as biochemistry, immunochemistry, and immunoelectron microscopy (21,27–29). However, these other techniques are not yet generally available. In most cases, typing of amyloid is not strictly necessary, because the clinical data indicate the type of amyloid. In the rare cases in which typing is obligatory, our current policy is to perform another biopsy (generally of the rectum) that permits immunohistologic characterization.

As Andrews et al already reported, "the yield of a subcutaneous fat aspirate in patients with isolated peripheral neuropathy and no other associated family history, signs, or symptoms of amyloidosis is low" (30). The results of the current study confirm this observation, because amyloid was detected in none of the 20 patients for whom there was no suspicion of a specific type of systemic amyloidosis (no chronic underlying disease, no plasma cell dyscrasia, no positive family history). In most of these patients, the indication for fat aspiration was neuropathy.

In the screening group, the percentage of subjects for whom proteinuria was an indication for biopsy was

higher in the subgroup with AL amyloidosis than in the subgroups without AL amyloidosis. In contrast, among patients with chronic (rheumatic) disease, proteinuria was not strongly indicative of AA amyloidosis.

In conclusion, the results of our study show that subcutaneous fat aspiration is the method of choice for routine screening for systemic amyloidosis, because it is a simple, convenient, inexpensive, fast, safe, bedside procedure with high diagnostic accuracy. If the results for properly examined fat tissue are positive, the diagnosis is established, and there is no need for another invasive biopsy. If the results of fat tissue aspiration are negative and the clinical suspicion of systemic amyloidosis remains high, unstained fat tissue may be sent to a tertiary center for thorough evaluation. If results are still negative, the additional value of subsequent biopsy of the rectum or bone marrow biopsy is low.

ACKNOWLEDGMENTS

We thank Elizabeth Haagsma, Edo Vellenga, and Pieter Limburg for their support in obtaining patient data and for providing the opportunity to analyze the fat tissue samples.

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