Crush and Smear Technique for Rapid Detection and Semiquantitation of Amyloid Deposition

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ABSTRACT. A method using Congo red to rapidly identify and semiquantitate amyloid deposits in tissues for experimental research and clinical medicine is described. Examination by polarization microscopy revealed amyloid deposits as bright green birefringent clumps on a dark red background. On semiquantitative evaluation, good correlation was found between this technique and the conventional histological one, the present technique being more sensitive. The method described saves time and expense.

Key words: amyloid, amyloid detection, smear technique, Congo-red

A critical step in the research of amyloidosis and in its diagnosis in clinical medicine is identification and quantitation of amyloid. Today this is accomplished by fixation in formalin, dehydration and paraffinization, embedding and sectioning of the tissues, and finally staining with Congo red (Puchtler et al. 1962, Elghetany and Saleem 1988). The process takes 24 hr and requires expensive instruments and experienced histotechnologists. Frozen sections also require expensive instrumentation and experienced histotechnologists.

We have developed a quick, inexpensive and simple technique. The aim of this study is to evaluate its accuracy and sensitivity in experimental animals and in human amyloid-laden organs.

MATERIALS AND METHODS

Experimental Animals

Seventy-five male Swiss mice, six to ten weeks old, were given daily subcutaneous injections of 0.5 ml Vit-free casein (ICN, 12% in 0.05 N NaOH) for different periods of time, and sacrificed at days 7–21. Ten untreated mice served as controls. One half of each spleen was fixed in 10% formalin and was subjected to the conventional Congo red staining method (Puchtler et al. 1962). The other half was prepared by the crush and smear (C & S) technique.

Human Tissues

Twenty-four tissue samples from different organs of human amyloidotics (liver, spleen, kidney, brain, heart, lung and thyroid), were stored at -32 C to -20 C for several months up to ten years. The control group consisted of matching organs from seven consecutive nonamyloidotic post mortem cases. From each case a 10 x 10 x 2 mm sized tissue sample was processed routinely and an adjacent tissue piece of about 30–60 mg was used for the C & S technique.

Crush and Smear Technique

The unfixed tissue sample was crushed between two glass slides and smeared homogeneously over the inferior slide. The slide was dried for 10 min at 65 C, then...
stained for 1 min in 0.5% Congo red solution (Gurr's stains and Reagents, England) in 50% ethanol/H₂O, blotted with filter paper, dipped one to two times (1 sec each) in 0.2% KOH in 80% ethanol/H₂O and blotted again.

**Evaluation of the Smears**

The smears were examined with a Leitz polarization microscope. The amyloid appeared as bright green birefringent clumps on a dark red background (Fig. 1). Without polarization the amyloid exhibited a cherry red color. A false positive birefringence could sometimes be seen in areas where the tissue was not homogeneously smeared and on the edges of the smeared material. In normal light they appeared as holes. By rotating the microscope table, these areas changed homogeneously from green to red, while amyloid deposits retained their bright green color.

**Grading of Amyloid Deposition in Experimental Animals**

1) Crush & Smear Technique:
Grade 0: Negative for amyloid deposits.
Grade 1 (traces): Up to five small deposits (not exceeding 1 mm) or one larger deposit per slide (Fig. 1).
Grade 2: Six to ten small deposits or two to five larger deposits.
Grade 3: More than grade 2 and up to 20% of the total area of the smear.
Grade 4: More than 20% of the total area of the smear (Fig. 2).

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**Fig. 1.** Grade 1 smear of spleen of a casein-treated amyloidotic Swiss mouse, C & S technique, Congo red stained, photographed under polarized light. The amyloid appears as the green birefringent clump. × 35.

**Fig. 2.** Grade 4 smear of a spleen of a casein-treated amyloidotic Swiss mouse, C & S technique, Congo red stained, photographed under polarized light. × 35.

**Fig. 3.** Grade 4 histological section of perifollicular amyloid deposits in a casein-treated amyloidotic Swiss mouse spleen, Congo red stained, photographed under polarized light. × 35.

**Fig. 4.** Grade 5 smear of a spleen of an amyloid-laden patient, C & S technique, Congo red stained, photographed under polarized light. × 35.
2) Conventional Histologic Sections:
Grading was based on the percentage of amyloid-positive follicles.
Grade 0: Negative for amyloid deposition.
Grade 1: Up to 5% of the follicles were surrounded by amyloid (usually one to two Malpighian follicles per section).
Grade 2: 5–25% of the follicles were surrounded by amyloid.
Grade 3: 26–50% of the follicles were surrounded by amyloid.
Grade 4: more than 50% of the follicles were surrounded by amyloid (Fig. 3).

**Grading of Amyloid Deposition in Human Tissues**

1) Crush and Smear Technique:
   Grades 1–4: As in experimental animals.
   Grade 5: More than 90% of the total area of the smear was positive (Fig. 4).
2) Conventional Histologic Sections:
   This was based on the percentage of positive tissue area.
   Grade 1: Less than 2% of the tissue contained amyloid.
   Grade 2: 2–5% of the tissue contained amyloid.
   Grade 3: 6–10% of the tissue contained amyloid.
   Grade 4: 11–90% of the tissue contained amyloid.
   Grade 5: More than 90% of the tissue contained amyloid.

The smears were evaluated independently by one author and the histologic slides by another. Whenever there was a significant difference between the histologic sections and the smears, deeper serial sections were cut, stained, and evaluated.

**RESULTS**

**Experimental Animals**

The findings from the slides from the casein induced amyloidotic mice are summarized in Table 1.
1) All the positive histologic sections were also positive with C & S technique.
2) All the negative smears were also negative on histology.
3) All the smears that were grade 2 and above were also positive on histology.
4) The difference between the histology and smears was never more than one grade in any given case.
5) In grade 1 there were 11 positive C & S cases. Of these, five were grade 1, one was grade 2 and five were negative on histology. On serial sections in one of the negative cases, a small deposit of amyloid could be detected. The other four cases remained negative also on recuts.
   All untreated mice of the control group were negative for amyloidosis, both in smears and slides.

**Human Tissues (Table 2)**

1) All the 24 positive smears were also positive on histology.
2) In 17 cases the grade of the smears corresponded to the grade of the histological slide, in six cases the difference was one grade between the smears and the slides, and only in one case did it amount to two grades.
3) All the 49 negative control smears were also negative on histology.
DISCUSSION

Our results show that there were no false negatives in any of the smears. There were also no false positives in the smears of untreated experimental animals and in the control human tissues. Since in a semiquantitative method a difference of one grade is not significant, there is good correlation between C & S technique and histology.

We found the smear technique to be more sensitive than histology. Small amounts of amyloid that are not discernible on histology can be detected in the smears. This is supported by the case that became positive on recuts. Furthermore, in the C & S technique in experimental animals, half of the spleen is examined and not just one section as in histology. Clearly the chances of finding deposits of amyloid are higher. This explains why five cases were positive in the C & S technique and negative on histology. Since false positive cases were found neither in the controls nor in the higher grades, we consider these cases as positive and as false negative on histology.


The advantages of the C & S technique are obvious. In traditional histoprocessing, a fully equipped histopathologic laboratory is needed and 48 hr are needed to obtain results. The C & S technique produces results within 15 min without the use of expensive equipment and labor.

The smear technique is also applicable in clinical pathology of human amyloid-laden tissues. Similar techniques are used for diagnosis of several diseases (Chan et al. 1988, Gharib and Goellner 1981, Klemi et al. 1987, Pasternack 1974, Westermark and Stenkvist 1973, Westermark et al. 1982). Rapid results are important in amyloid-containing tumors like medullary carcinoma of the thyroid. The C & S technique can help in the preliminary diagnosis of amyloid-containing tumors and of generalized amyloidosis in post mortem cases so that tissues can be frozen for further research of amyloidosis.

In conclusion, the C & S technique, both in experimental research and in clinical pathology, is more sensitive and much quicker than the histological method. It is cheap, simple, easy to learn and apply and, if generally accepted, will contribute to uniformity of evaluating and presenting findings.

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