AMYLOIDOSIS IN RHEUMATOID ARTHRITIS
INVESTIGATED BY MEANS OF RECTAL BIOPSY

BY

G. ARAPAKIS* AND C. R. TRIBE

From the Rheumatic Diseases Research Centre and the Department of Morbid Anatomy,
Stoke Mandeville Hospital, Aylesbury, Bucks

The types of disease associated with secondary amyloidosis have undergone considerable change in recent years. Largely because of modern antibiotic treatment, amyloidosis associated with tuberculosis, osteomyelitis, chronic lung disease, and syphilis is now rarely seen. For this reason rheumatoid arthritis is now one of the commonest causes of secondary amyloidosis.

Although amyloidosis may be suspected on clinical or biochemical grounds, a definite diagnosis can only be made by tissue biopsy or post mortem. Most investigations of the incidence of amyloidosis in patients with rheumatoid arthritis have been based on post mortem studies. Missen and Taylor (1956), in reviewing the literature, found a wide variation in the reported incidence. The highest incidence—60 per cent.—was reported by Teilm and Lindahl (1954), whereas Rosenberg, Baggenstoss, and Hench (1943) had found only one case of amyloidosis in thirty necropsies on patients with rheumatoid arthritis (3-3 per cent.). This marked discrepancy reveals the limitations inherent in deriving the incidence of a disease from “selected” necropsies.

Rectal biopsy has recently been advocated by several authors as the best and most convenient method for the diagnosis of amyloidosis. Blum and Sohar (1962) obtained positive results in 75 per cent. of a series of 62 patients with amyloidosis already diagnosed by other methods.

In this paper we describe the results of rectal biopsy used as a screening test for amyloidosis in a random population of patients with rheumatoid arthritis, the majority of whom were currently under treatment at the Oxford Regional Rheumatism Centre. In addition to the rectal biopsy, other clinical and laboratory data were collected from each patient included in the study.

Material and Methods

During a period of 4 months rectal biopsy was performed on 115 patients who satisfied the criteria of the American Rheumatism Association for classical or definite rheumatoid arthritis (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959). The patients, 24 men and 91 women, were picked at random from the large numbers attending the Rheumatism Centre. They had all suffered from rheumatoid arthritis for more than 3 years, but were otherwise unselected.

A modified Truelove-Salt colonic biopsy instrument (Truelove, Horler, and Richards, 1955) was used to obtain specimens of rectal tissue. No preparation of the bowel was needed. Since most of the patients had arthritis of the knee or elbow joints, sigmoidoscopy was performed with the patient in the left lateral position. A short Strauss sigmoidoscope was inserted 10 to 15 cm. and the biopsy specimen was taken under direct inspection from the anterior wall of the rectum at that level. In 63 cases the examination was carried out in the outpatient department. The whole procedure took only 10 to 15 minutes and the patient was able to go home immediately afterwards.

Besides the rectal biopsy a full clinical examination was carried out, and the stage and activity of the disease, the co-existence of other major diseases, and details of current and past treatment were recorded.

Blood was taken for the following laboratory investigations:

1. Haemoglobin estimation.
2. Erythrocyte sedimentation rate (E.S.R.) (Wester-gren).
5. Sheep cell agglutination test (S.C.A.T.) (Greenbury, 1957) and latex-fixation test.

Finally, a specimen of urine was examined for the presence of protein and the nature of any deposit.

Histological Methods.—The biopsy specimens were fixed in 10 per cent. formol saline, routinely processed, and embedded in paraffin. One section from each biopsy was stained with haematoxylin and eosin and further sections were stained for amyloid by the following methods:

(b) The congo red method.
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It was soon found that the methyl violet method was superior to congo red for revealing the presence of small amounts of amyloid and the latter stain was omitted when examining the later biopsy specimens.

Vassar and Culling originally described the thioflavine T method for detecting amyloid by fluorescence microscopy in 1959 and claimed it as specific. In the present investigation this method was used both to serve as a check on positive reactions with methyl violet and as a possible way of revealing amyloid deposition at an earlier stage. As it happened amyloid material was revealed in equal amounts at identical sites by both methods in all the positive biopsy specimens.

Results

In six patients the tissue obtained by rectal biopsy showed histological evidence of amyloid. We consider that rheumatoid arthritis was the sole causative factor since none of these patients had at any time suffered from any other diseases likely to produce amyloidosis.

Table I shows the age and sex distribution of the 115 patients included in this study. Although the numbers are too small to allow definite conclusions to be drawn, they suggest that amyloidosis in rheumatoid arthritis is more likely to occur in men than in women and in patients over 50 years old.

Table II shows that there was no correlation between the occurrence of amyloidosis and the duration of the rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Duration (yrs)</th>
<th>Men</th>
<th>Women</th>
<th>Both Sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-9</td>
<td>16</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>10-19</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Over 20</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

With the same reservations as regards the limited numbers, there was a trend towards a higher incidence of amyloidosis in patients with severe destruction of cartilage and juxta-articular bone revealed radiographically (Steinbrocker, Traeger, and Batterman, 1949). All six cases occurred amongst patients with Stage III or IV arthritis (Table III).

In contrast the degree of clinical activity, judged by tenderness and swelling of the joints at the time of biopsy, showed no such correlation. Three of the cases with amyloidosis were found in a group of forty patients with inactive arthritis.

Enlargement of the liver or spleen was not found to be a reliable clinical sign of the presence of amyloidosis. Among the 115 patients hepatomegaly was present in 31 and splenomegaly in nine. Amyloidosis was diagnosed in two of the former and one of the latter, and in three of the six patients with amyloidosis neither organ was enlarged.

No correlation was found between the blood pressure, haemoglobin level, or E.S.R. and the occurrence of amyloidosis. In three of the patients with amyloid a moderate to severe anaemia could be attributed to renal failure.

Table IV shows that three of the six patients with amyloidosis had neither proteinuria nor raised blood urea levels. The significance of these findings will be discussed later.

The plasma proteins are often altered in rheumatoid arthritis with a decrease in albumin and an increase in the α₂ and γ globulin fractions. The three patients with amyloidosis and signs of renal failure all showed a marked decrease in the albumin fraction, which was presumably due to their proteinuria. No difference in the electrophoretic patterns of the plasma proteins was noted between the cases with and without amyloidosis.

Five of the six patients with amyloidosis had positive serological tests at the time of biopsy and the sixth had had a positive test in the past. Since only fifteen out of the 115 patients had negative S.C.A. and latex-fixation tests, only a tentative conclusion can be drawn that amyloidosis does not occur in sero-negative cases.

<table>
<thead>
<tr>
<th>Stage of Disease</th>
<th>No. of Cases</th>
<th>Men</th>
<th>Women</th>
<th>Both Sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-II</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>11</td>
<td>1</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood Urea Level</th>
<th>Proteinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 49 mg./100 ml.</td>
<td>Absent</td>
</tr>
<tr>
<td>Greater than 50 mg./100 ml.</td>
<td>103*</td>
</tr>
</tbody>
</table>

* From a single specimen of urine in most patients.
The majority of patients were under treatment with salicylates or other analgesics and three-fifths of them were, or had been, taking a maintenance dose of 5 to 10 mg. prednisolone. Gold therapy had been used in the past in 49 patients. The details of past and present treatment in the six cases of amyloidosis are given in Table V, from which it appears that there is no correlation between the type of treatment and the occurrence of amyloidosis.

The principle clinical, laboratory, and pathological findings in the six patients with amyloidosis are summarized in Table V.

**Histological Findings**

The rectal biopsy specimens measured 3 to 5 mm. in diameter. Nearly all contained a strip of mucosa and underlying submucosa, and more than half also contained portions of inner muscle coat.

Four specimens contained only mucosa, but since amyloid material was demonstrated in this layer in five of the six positive cases, these have been included in the total 115 biopsy results.

Amyloid material was demonstrated in six cases both by methyl violet metachromasia and thioflavine T fluorescence. A detailed description of the sites of amyloid deposition in each case is included in Table V. Since the variations of amyloid deposition will be the subject of further discussion they can be summarized as follows:

(A) *Parenchymal Type.*—Two cases (Nos 2 and 4 in Table V). In these cases amyloid was demonstrated only in the stroma lying directly beneath the superficial layer of the mucosa (Fig. 1). Two cases of the mixed type also showed amyloid in this site.

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**Fig. 1.** Rectal mucosa, showing amyloid deposits in the stroma beneath the superficial epithelium—Parenchymal type. Thioflavine T fluorescent stain. × 300.

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**CLINICAL PARTICULARS OF SIX CASES OF RHEUMATOID ARTHRITIS**

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Duration (yrs)</th>
<th>Stage</th>
<th>Activity</th>
<th>Palpable Liver</th>
<th>Palpable Spleen</th>
<th>Blood Pressure (mm.)</th>
<th>Hb (per cent.)</th>
<th>E.S.R. (Westergren) (mm./l.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>F</td>
<td>10</td>
<td>IV</td>
<td>+</td>
<td>No</td>
<td>No</td>
<td>110/80</td>
<td>94</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>M</td>
<td>4</td>
<td>III</td>
<td>-</td>
<td>+</td>
<td>No</td>
<td>150/90</td>
<td>99</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>M</td>
<td>38</td>
<td>IV</td>
<td>+</td>
<td>No</td>
<td>+</td>
<td>120/80</td>
<td>110</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>M</td>
<td>7</td>
<td>III</td>
<td>++</td>
<td>++</td>
<td>No</td>
<td>130/80</td>
<td>68</td>
<td>57</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>F</td>
<td>25</td>
<td>IV</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>220/110</td>
<td>41</td>
<td>85</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>F</td>
<td>17</td>
<td>IV</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>240/140</td>
<td>73</td>
<td>60</td>
</tr>
</tbody>
</table>
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(B) Vascular Type.—One case (No. 3 in Table V). In this case amyloid was only demonstrated in the walls of the submucosal vessels (Fig. 2).

(C) Mixed Type.—Three cases (Nos. 1, 5, and 6 in Table V).

Other histological findings of no specific significance and probably unrelated to the presence of amyloid included:

(a) The presence of lymph follicles in 65 per cent. of the biopsy specimens. Occasionally the lymph follicles showed "reactive" germinal centres resembling those seen in lymph nodes from patients with rheumatoid arthritis.

(b) In many of the sections stained with thioflavine T, we observed groups of strongly fluorescent foamy histiocytes usually situated in the stroma at the base of the mucosa (Fig. 3, overleaf). On further investigation these cells were found to contain an acid mucopolysaccharide. The nature, distribution, and significance of these cells are being further investigated.

![Fig. 2.—Amyloid in the walls of the submucosal vessels of the rectum—Vascular type. Thioflavine T fluorescent stain. × 300.](image-url)

IN WHICH RECTAL BIOPSY SHOWED AMYLOID DEPOSITS

<table>
<thead>
<tr>
<th>Proteinuria (mg./100 ml.)</th>
<th>Blood Urea (mg./100 ml.)</th>
<th>Blood Proteins (g./100 ml.)</th>
<th>S.C.A.T. Titre</th>
<th>Treatment</th>
<th>Sites of Amyloid Deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>21</td>
<td>Alb. 2-45 Glob. 2-55</td>
<td>512</td>
<td>Steroids</td>
<td>Amyloid found in submucosal vessels and in stroma around basal mucosal glands</td>
</tr>
<tr>
<td>Nil</td>
<td>34</td>
<td>Alb. 3-5 Glob. 3-0</td>
<td>1.024</td>
<td>Nil</td>
<td>Amyloid confined to stroma directly beneath the surface epithelial layer of mucosa (Fig. 1)</td>
</tr>
<tr>
<td>Nil</td>
<td>21</td>
<td>Alb. 4-2 Glob. 2-0</td>
<td>4.096</td>
<td>Salicylates</td>
<td>Amyloid only in walls of submucosal vessels (Fig. 2)</td>
</tr>
<tr>
<td>410</td>
<td>88</td>
<td>Alb. 0-7 Glob. 4-2</td>
<td>4.096</td>
<td>Phenylbutazone</td>
<td>Similar to Case 2</td>
</tr>
<tr>
<td>224</td>
<td>201</td>
<td>Alb. 1-9 Glob. 3-1</td>
<td>Negative (Had been positive in the past)</td>
<td>Phenylbutazone</td>
<td>Amyloid in sites similar to Cases 2 and 3 and also within submucosal lymph follicle</td>
</tr>
<tr>
<td>150</td>
<td>50</td>
<td>Alb. 1-5 Glob. 4-05</td>
<td>128</td>
<td>Steroids</td>
<td>Amyloid in sites similar to Cases 2 and 3, also within submucosal lymph follicle and between smooth muscle fibres of the inner muscle coat</td>
</tr>
</tbody>
</table>

Nil

Steroids
Salicylates
Phenylbutazone
Gold

Nil
Salicylates

Phenylbutazone
Gold

Phenylbutazone
Steroids
Salicylates

Phenylbutazone
Steroids
Salicylates
Discussion

Incidence

As mentioned in the introduction, the incidence of amyloidosis in rheumatoid arthritis estimated from post mortem studies has ranged from 3 to 60 per cent. There are several reasons why the material included in such studies should fail to be representative of the disease as it is encountered in unselected individuals in life. Death from a well-recognized complication of rheumatoid arthritis is liable to occur in hospital rather than at home and the interrelationship is unlikely to be overlooked at necropsy. Patients with uncomplicated and often mild rheumatoid arthritis who die from unrelated conditions may, on the other hand, be poorly represented in post mortem records, either because they die at home or because the pathologist, preoccupied with the major cause of death, fails to mention the presence of arthritis. Together these two factors would load a post mortem study with a disproportionate number of severe and complicated cases of rheumatoid arthritis, among which the incidence of amyloidosis might be expected to exceed that for unselected material. The degree of selection has presumably varied from one study to another, but it is a reasonable assumption that the highest estimates for the incidence of amyloidosis far exceed the figure for unselected living patients. At this hospital 36 necropsies have been performed in cases of rheumatoid arthritis in the 5-year period from 1957 and histological evidence of amyloidosis has been found in four.

Figures based on studies of unselected cases in life depend for their accuracy on the reliability of the test or tests used in screening and diagnosis. Many authors have pointed out that the first clinical sign of amyloidosis is usually albuminuria. Fearnley and Lackner (1955) screened 183 patients with rheumatoid arthritis and found 24 with proteinuria. Seven of these had amyloidosis, proved either by liver biopsy or the congo red test. It is recognized, however, that amyloidosis may occur without albuminuria and Teilum and Lindahl (1954), in a series of 28 cases of amyloidosis with rheumatoid arthritis, found no record of albuminuria in 55 per cent. Our series included twelve patients with proteinuria, of whom three proved to have amyloidosis on rectal biopsy. In the remaining three patients with amyloidosis the urine was free from protein and no other signs of renal involvement were evident. Clearly the diagnosis may be missed if albuminuria is used as the sole screening test for amyloidosis.

From its introduction by Bennhold (1923), the congo red test remained the only method of diagnosing amyloidosis during life until tissue biopsy became common practice. Its chief disadvantage is the frequency of false negative results (Stemerman and Auerbach, 1944; Calkins and Cohen, 1960). Blum and Sohar (1962) reported the results of rectal
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biopsy on 62 cases of amyloidosis. Of these 27 had secondary amyloidosis and the rectal biopsy was positive in nineteen (70 per cent.). Rectal biopsy studies have also been reported by Gafni and Sohar (1960), Ducrot, Montera, Méry, and Rueff (1961), and Fentem, Turnberg, and Wormsley (1962), all of whom obtained good results and advocated rectal biopsy as the first method of investigation in patients suspected on clinical evidence of having amyloidosis.

In our experience rectal biopsy has proved to be an excellent method for the diagnosis of amyloidosis. The procedure is painless, causes minimal discomfort, and can be performed on out-patients. In practice it takes much less time and causes less discomfort than the congo red test which requires accurately timed serial venepunctures. Complications of the rectal biopsy are very rare; only one patient in the present series had excessive bleeding from the biopsy site, and the haemorrhage in this case ceased soon after the patient was admitted to hospital and blood transfusion was not required. We suggest that rectal biopsy, performed by the method described, can be used, not only as a means of diagnosing amyloidosis, but also as a screening test in patients suffering from diseases, such as rheumatoid arthritis, in which amyloidosis may occur.

Despite its convenience rectal biopsy has its limitations since the gastro-intestinal tract is not invariably involved in secondary amyloidosis. Some reference to gastro-intestinal involvement is made in most post mortem studies in cases of secondary amyloidosis, but the incidence varies considerably and in many papers the amount of histological material examined is not always clear. One of us (C.R.T.) has found evidence of amyloidosis post mortem in 49 paraplegic patients. In this series (Tribe, 1963) histological material from different levels of the gastro-intestinal tract was available in only eighteen cases, but in every instance amyloid material was demonstrable. These findings suggest that involvement of the gastro-intestinal tract in secondary amyloidosis is higher than previously stated, and that a correspondingly high percentage of positive rectal biopsies can be predicted. They also suggest that amyloidosis of the kidney severe enough to cause signs of renal failure is always associated with simultaneous involvement of the gastro-intestinal tract.

It has, therefore, been difficult to establish the overall incidence of amyloidosis in rheumatoid arthritis, because the absence of a reliable screening test has made it difficult to establish the diagnosis in life, and post mortem studies have necessarily embraced selected material. Rectal biopsy established the presence of amyloidosis in 5 per cent. of our series, which was unselected except for the exclusion of early cases and patients with disease so mild as not to require treatment. If liberal allowance is made for the limitation of the method of diagnosis, the true incidence in this series would be most unlikely to exceed 10 per cent.

Pathological Aspects
The most interesting finding was the wide variation in the sites of amyloid deposition in the rectal biopsy specimens. This was in marked contrast to the reports of other authors who have always found amyloid material in the walls of the submucosal arterioles and occasionally lying between the smooth muscle fibres of the muscle coats, but never exclusively in the mucosa (Gafni and Sohar, 1960; Ducrot and others, 1961; Ducrot, 1962; Fentem and others, 1962).

All these authors emphasize the importance of identifying a portion of submucosa in the biopsy before it can be reported as negative. Without denying this, we would stress that five of our six positive biopsies could have been diagnosed from examination of a portion of mucosa only.

In comparison with primary amyloidosis (Symmers, 1956), the pattern of amyloid distribution within individual organs has received little attention in the literature on secondary amyloidosis. Only Levine (1962) has attempted to differentiate between the parenchymal and vascular types of amyloid deposition.

Whether these different histological types of amyloid deposition in the rectum have any relation to the frequency and degree of renal involvement, and therefore to the prognosis of this disease, is not yet clear. Correlation of the different patterns of amyloid distribution with the stage of renal involvement in a greater number of cases may provide an answer to this problem.

Summary
(1) Rectal biopsy was performed in 115 patients with rheumatoid arthritis of more than 3 years' duration.
(2) In six cases amyloid was detected in the rectum. Only three of these had proteinuria and raised blood urea levels at the time of biopsy.
(3) Our results suggest that amyloidosis in rheumatoid arthritis occurs more frequently in men, in the late stages of the disease, and usually in association with positive serological tests.
(4) The frequency of gastro-intestinal involvement in secondary amyloidosis is discussed, and
it is suggested that the probable incidence of amyloidosis in rheumatoid arthritis lies between 5 and 10 per cent.

(5) The positive rectal biopsies showed different sites of amyloid deposition. The possible significance of these findings are discussed.

(6) The authors believe that rectal biopsy is the method of choice for the diagnosis of amyloidosis and can also be used as a screening test for this disease.

We wish to thank Dr. A. G. S. Hill, Dr. S. C. Truelove, and Dr. H. J. Harris for their encouragement, criticism, and helpful advice. We are also grateful to Dr. M. Saxty Good and Dr. I. Meanock for permission to include some of their patients in our series, to Mr. D. G. Standen for the photographs, to Mr. W. A. Kears and his colleagues for histological work, and to Miss K. Smith for secretarial assistance. This work was aided by a grant from the Empire Rheumatism Council.

REFERENCES


AMYLIDOSE DANS L'ARTHRITE RHUMATISMALE RECHERCHÉE PAR LA BIOPSIE RECTALE

RÉSUMÉ

1. On procéda à des biopsies rectales chez 115 malades atteints d'arthrite rhumatismale présente depuis plus de 3 ans.
2. Dans six cas la dégénérescence amyloïde fut décelée dans le rectum. Seulement trois d'entre eux eurent de la protéinurie et le taux sanguin de l'urée augmenté au temps de la biopsie.
3. Nos résultats indiquent que l'amyloidose dans l'arthrite rhumatismale survient plus souvent chez des hommes, aux états avancés de la maladie, et habituellement en association avec des réactions sérologiques positives.
4. On discute la fréquence de l'implication gastro-intestinale et on suggère que la fréquence probable de l'amyloidose dans l'arthrite rhumatismale est de 5 à 10 pour cent.
5. Les biopsies rectales positives montrèrent des différents endroits de dépôt amyloïde. On discute la possible importance de ces résultats.
6. Les auteurs croient que la biopsie rectale est la méthode de choix dans le diagnostic de l'amyloidose et peut-être utile pour dépister cette maladie.

AMILIOIDEN ES IN LA ARTRITIS REUMATOIDE INVESTIGADA POR LA BIOPSIAS RECTAL

SUMARIO

1. Se efectuaron biopsias rectales en 115 enfermos con artritis reumatoide presente desde más de 3 años.
2. En seis casos amiloídes fue detectada en el recto. Solamente tres de estos casos acusaban proteinuria y el nivel de urea sanguínea aumentada al tiempo de la biopsia.
3. Nuestros hallazgos sugieren que la amiloíden en la artritis reumatoide sobreviene más a menudo en hombres, en el período adelantado de la enfermedad y, generalmente, en asociación con reacciones serológicas positivas.
4. Se discute la frecuencia de la complicación gastrointestinal y se sugiere que la frecuencia probable de la amiloíden en la artritis reumatoide es de 5 a 10 por ciento.
5. Las biopsias rectales positivas revelaron la existencia de varios sitios con depósitos amiloídes. Se discute el significado posible de estos resultados.
6. Los autores creen que la biopsia rectal es el método de elección en el diagnóstico de amiloíden y se la puede usar como test en la búsqueda de esta enfermedad.