Secondary amyloidosis in Crohn’s disease

S. Seijo Ríos, M. Barreiro de Acosta, B. Vieites Pérez-Quintela, J. Iglesias Canle, J. Forteza and J. E. Domínguez Muñoz

Departments of Gastroenterology, and 1Pathology. University Hospital of Santiago de Compostela. A Coruña, Spain

ABSTRACT

Amyloidosis is a clinical entity that results from the deposition of an extracellular protein material that causes disruption in the normal architecture of multiple organs and tissues, and impairs their function. Secondary amyloidosis is a rare but serious complication that may worsen the prognosis of patients with cancer, infection or chronic inflammatory disease, including inflammatory bowel disease, particularly Crohn’s disease. We report two cases of Crohn’s disease associated with secondary amyloidosis.


INTRODUCTION

Amyloidosis occurs as a result of the deposition of an extracellular protein. This intractable and amorphous fibrillar structure (amyloid), which has a β folded feature, causes a disruption of the normal architecture of multiple organs and tissues (mainly the kidneys and liver) with consequent functional impairment (1). Secondary amyloidosis (AA) is a rare but serious complication that appears in the context of cancer, chronic inflammation, and chronic infectious diseases, such as inflammatory bowel disease (IBD), mainly long-standing Crohn’s disease (CD) (2,3); however, it has been reported in patients with CD for barely 2 years (4). There is controversy on whether it should be considered an extraintestinal manifestation or a consequence of a chronic disorder such as anemia. Renal failure is the most common clinical presentation of AA, ranging from nephrotic syndrome and impaired renal function – as is the case with the two patients reported below – to renal failure, with a potential for high morbidity (5,6). In the diagnosis of this disease histological confirmation (Congo red staining) is essential, which shows a characteristic green birefringence with the use of polarized light, as is immunohistochemistry.

So far there is no effective treatment for AA. Multiple therapeutic strategies have been employed with discour-
aging results, including colchicine and immunosuppressive drugs such as tumor necrosis factor alpha (TNF-α) inhibitors

CLINICAL CASE 1

We present the case of a 40-year Caucasian female who was diagnosed with Crohn’s disease at the age of 33 years, with presence of inflammatory and incomplete bowel strictures (A1L3B2 of Vienna Classification, A2L3B2 of Montreal Classification). She was an active smoker but reported no allergies or other illnesses. Her mother had been diagnosed with systemic necrotizing vasculitis with renal and splenic involvement. The patient was admitted to the emergency room in 2005 for acute watery diarrhea, more than five stools per day without pathological products. The patient also reported vomiting and epigastric pain radiating in a constant belt shape, which was not modified by food intake or defecation, with no fever or other clinical events. On the physical examination she had arterial hypotension (78/46 mmHg) and edema with fovea in the lower limbs as well as eyelid edema, without other major findings.

The patient was admitted with the clinical suspicion of a moderate flare of her CD so we began treatment with intravenous steroids (1 mg/kg/24 h) to induce remission, associated with low-molecular-weight heparin, metronidazole, and ciprofloxacin. Endoscopic and radiological intestinal transit revealed several areas of incomplete strictures in the jejunum and distal ileum, and showed no cecum or right colon involvement. Overinfection with CMV or Clostridium difficile was ruled out. During her hospital stay she developed marked and progressive malnutrition with alteration in all nutritional parameters (2.6 total protein, albumin 1.1, cholesterol 107, triglycerides 98, RBP 5.1, prealbumin 15, 25-OH vitamin D < 1, calcitriol < 5 pg/mL, TSH 3.43 mIU/L, FT4 1.08 ng/dL, FT3 2.23 pg/mL, B12 168 pg/mL, folic acid 1 ng/mL, iron 7 µg/dL, transferrin 58 mg/dL, ferritin 167 ng/mL), forcing the introduction of enteral nutrition and vitamin supplements. The patient had a torpid progression of her peripheral and eyelid edema to anasarca, with sustained hypotension, and no cardiac changes to justify her symptoms (echocardiogram done ad hoc). Severe hypoalbuminemia and hypoproteinemia was accompanied by progressive deterioration of renal function (creatinine 1.0 to 2.4 mg/dL in a few days), and overt proteinuria (24-hour urine: protein: 13.19 g/L, 26.38 g/24h, urine sodium 30 mm/L, urine potassium 19.0 mM/L, protein in urine: total protein 1600 mg/dL, creatinine 32.7, IgG 197.0 mg/dL, α: 14%, β: 6%, γ:19%, A/G 0.91). Upon the diagnosis of acute renal failure as the cause of hypotension and anasarca we initiated treatment with furosemide and hydrochlorothiazide following instructions from the Nephrology Division. Despite these therapeutic measures no clinical or renal improvement was obtained, so a rectal biopsy was decided upon with the suspicion of secondary amyloidosis in the context of CD. Congo red staining showed red-positive material resistant to permanganate, highly suggestive of AA. Immunohistochemistry showed the expression of amyloid protein A (AA) surrounding submucosal vessels, which demonstrates the secondary nature of this deposition (Fig. 1). With the diagnosis of secondary amyloidosis with nephrotic syndrome and renal failure we prescribed treatment with colchicine and noted a decline in anasarca and proteinuria, and a gradual improvement in renal function. Maintenance treatment for CD was done using azathioprine after measuring thiopurine methyltransferase levels. Thereafter, the patient had her renal function normalized, and has not been admitted to our hospital again for her CD.

Fig. 1. A: Immunohistochemical expression of amyloid A protein in the material deposited around submucosal vessels. B: Congo red staining without permanganate, which shows the presence of characteristic amyloid deposition.
CLINICAL CASE 2

We next present the case of a 45-year male with 10-year CD of rectum-sigmoid location, with penetrating behavior, perianal disease, and vesicosigmoidal fistula (A1L2B3 of Vienna Classification, A2L2B3p of Montreal Classification). The patient reported moderate ethanol intake and was a former smoker. He had metronidazole intolerance, and was diagnosed with peripheral arthritis as an extraintestinal manifestation of his CD. The patient had developed corticosteroid dependence, so he was switched to immunosuppressive therapy, initially with infliximab (5 mg/kg) that was suspended after the second dose due to severe skin reaction and glottal swelling. Subsequently, he presented with severe flared disease with colonic fistulas, for which we prescribed azathioprine (AZA) plus a full steroid cycle to induce remission. During this flare-up he had a urinary septic shock and hemorrhagic cystitis related to severe obstructive uropathy with renal impairment. The patient required surgery (cystostomy and placement of a temporary J catheter). As a result we decided to remove immunosuppression with AZA, and started treatment with methotrexate at doses of 15 mg every 4 weeks (MTX). Twelve months later we also removed MTX due to hypertransaminasemia. In 2006, the patient started treatment with adalimumab with good tolerance and positive clinical evolution. After 5 months of treatment with adalimumab the patient presented to the emergency room complaining of a two-month constitutional syndrome, arthromyalgia, dysthermia without fever, and downward trend in the pace of defecation, with no findings in the physical examination. Endoscopic and barium enema studies demonstrated colonic strictures in the sigma, important mucosal inflammation in the rectum and sigma, as well as the presence of perianal fistulas. Lab workup only showed moderate renal failure, hypoproteinemia, hypoalbuminemia (urea 78 mg/dL, creatinine 1.9 mg/dL, total protein 5.3 g/dL, albumin 2.8 g/dL) and nephrotic proteinuria (3.8 g protein, with creatinine 67.4 mg, sodium 54, and potassium 34 in 24-hour urine). During hospitalization the patient had several episodes of orthostatic hypotension, which coupled with renal impairment prompted our suspicion for secondary amyloidosis. The diagnosis was confirmed with a rectal biopsy and Congo red staining, which showed the presence of an amorphous eosinophilic material around submucosal vessels, with birefringence without potassium permanganate (Fig. 2). The patient was evaluated by the Nephrology Division, treatment with furosemide 40 mg daily was started, and we decided to continue with adalimumab as maintenance therapy for his CD. Unfortunately, he had an intestinal occlusion that required left hemicolectomy with resection of the upper rectum and colostomy. During follow-up the patient had a gradual deterioration of his renal function with hypotension, and died 5 months later from amyloidosis. The main features of both patients are summarized in table I.

DISCUSSION

The term amyloidosis refers to a group of diseases characterized by the deposition of an extracellular, amorphous, intractable, rigid, low-molecular-weight protein called amyloid. This protein is resistant to proteolysis, and causes a disruption of the normal architecture of multiple organs and tissues, with their consequent functional alteration (7).

It is a disorder of unknown etiology, with low incidence and prevalence, and only a few cases reported in the literature (2,5,7). The current classification of amyloidosis is based on the plasma protein precursor that is ab-

Fig. 2. A: Hematoxylin-eosin (HE) staining that demonstrates a submucosal vessel surrounded by amorphous eosinophilic material around its wall. B: Congo red-stained image without permanganate showing positivity in the material deposited around vessels.
normally deposited in numerous organs. There are several types of amyloid fibrils but all of them have a β pleated sheet, rather than the normal α configuration of physiological proteins (8-10). One common component to all types of amyloid is the amyloid P component, a glycoprotein that builds 10-15% of the deposits, brings stability to the complex, and makes it resistant to tissue degradation (11-13). Primary amyloidosis (AL) is the result of the deposition of part or all of monoclonal λ and κ light immunoglobulin chains in the context of hematological diseases (11). In contrast, secondary amyloidosis (AA) occurs in the context of neoplastic, infectious or chronic inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis, tuberculosis, bronchiectasis, inflammatory bowel disease, or familial Mediterranean fever (8). AA is a very stable molecule that stems from the N-terminal fragments of the protein serum amyloid A, SAA, an acute phase reactant. It is produced in response to maintained inflammation under the regulation of TNFα and multiple interleukins such as IL-1 and IL-6 (11,11). The different types of amyloidosis are summarized in table II. The cases presented herein are two patients with secondary amyloidosis (AA) associated with chronic inflammatory diseases, probably responsible for the production of SAA and amyloid deposition in tissues.

The association between secondary amyloidosis and inflammatory bowel disease is well known, and although rare, it is still a serious complication with very few cases reported. The largest series was reported by Greenstein et al. at Mount Sinai Hospital in New York and Wester et al. in Oslo, with 25 and 18 patients, respectively (2,5). The incidence of AA is 0.9-6% for CD, and is exceptional in ulcerative colitis (UC) (with an incidence close to 0.07%) (14); prevalence is higher in males (1-3,5,15). The series previously described shows an enrichment of ileocecal locations, although in our patients the location of CD was uneven (one of them showed the typical ileocolonic location whereas the second one presented with colonic disease). AA is characteristic of long-standing inflammatory diseases with penetrating disease and suppurrative complications (2,5). Our patients have a latency in the diagnosis of AA of 7 and 10 years respectively, similar to those in the series previously mentioned (15 and 4 years). Only one of them presented with a penetrating form of CD, and none had suppurrative abscesses or other complications. So far, there is no consensus on whether this complication is more common in patients with extraintestinal manifestations (2,5,6).

The death of one of the patients confirms the high mortality described for this subgroup of patients. Amyloidosis is frequently described as a major cause of death in patients with CD (16), with a long-term mortality between 40 and 60% (2,5). In fact, a relevant number of diagnoses of amyloidosis are made in autopsies (5). It has also been described an extremely high percentage of deaths in the two months following surgery by CD (17), a situation that has occurred in our patient.

Clinical manifestations are multiple, which is often the cause of delayed diagnosis. Renal involvement in AA, as well as in AL, occurs in 90% of cases: renal impairment with nephrotic proteinuria, generally intense, evolving to renal failure (7,8,11). A fact that helps in the differential diagnosis with other entities of renal failure is the normal size and morphology of the kidneys, and the presence of arterial hypotension instead of hypertension (11). Likewise, the liver and spleen are often other target organs affected in this type of amyloidosis. In AA, cardiac involvement is exceptional and macroglossia is absent, both facts that differentiate it from AL (7,8). Our patients have the typical course of secondary amyloidosis with renal failure, marked hypotension and proteinuria.

### Table I. Main features of two patients with Crohn’s disease and amyloidosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age at onset of CD</th>
<th>CD location</th>
<th>Behavior</th>
<th>CD complications</th>
<th>Extraintestinal manifestations</th>
<th>Smoking</th>
<th>Immunosuppressant</th>
<th>Age at diagnosis of amyloidosis</th>
<th>Diagnosis latency between CD and amyloidosis</th>
<th>Amyloidosis main manifestation</th>
<th>Amyloid biopsy location</th>
<th>Other chronic inflammatory diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>33 years</td>
<td>Distal ileum</td>
<td>Inflammatory stricturing</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>40 years</td>
<td>7 years</td>
<td>Renal</td>
<td>Rectum</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>35 years</td>
<td>Rectum, sigmoid</td>
<td>Penetrating stricturing</td>
<td>Intestinal occlusion</td>
<td>No</td>
<td>Yes</td>
<td>Ex-smoker</td>
<td>45 years</td>
<td>10 years</td>
<td>Renal</td>
<td>Rectum</td>
<td>No</td>
</tr>
</tbody>
</table>

CD: Crohn’s disease.

### Table II. Amyloidosis classification

<table>
<thead>
<tr>
<th>Precursor protein</th>
<th>Nomenclature</th>
<th>Associated features</th>
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</thead>
<tbody>
<tr>
<td>Primary amyloidosis</td>
<td>Immunoglobulin light chains (κ or λ)</td>
<td>AL</td>
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<tr>
<td>Secondary amyloidosis</td>
<td>Amyloid A protein</td>
<td>AA</td>
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<td></td>
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<tr>
<td>Familial amyloidosis</td>
<td>Transthyretin</td>
<td>ATTR</td>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other familial amyloidosis</td>
<td>Apolipoprotein A-1</td>
<td>AapoA-1</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen</td>
<td>AFib</td>
</tr>
<tr>
<td></td>
<td>Gelsolin</td>
<td>AGel</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>ALys</td>
</tr>
<tr>
<td>Senile systemic amyloidosis</td>
<td>β-protein</td>
<td>Aβ</td>
</tr>
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<td></td>
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<td></td>
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<tr>
<td>Dialysis-related amyloidosis</td>
<td>β2 microglobulin</td>
<td>Aβ2m</td>
</tr>
</tbody>
</table>

RA: Rheumatoid arthritis; TB: Tuberculosis; IBD: Inflammatory bowel disease; FMF: Familial Mediterranean fever.
Diagnosis requires clinical suspicion and histological confirmation. A biopsy of target organs like the liver or kidney provides a highly effective diagnostic (about 90%), but is not without significant risk. That is why other locations have been described for obtaining histological samples – these are less invasive, less risky procedures, but also have less diagnostic sensitivity, as is the case with abdominal fat (sensitivity of 60-80%) or rectum (50-70%) biopsy (7). Congo red staining is essential, and shows the typical green birefringence with polarized light microscopy. Immunohistochemistry will allow to differentiate between different types of amyloid, especially between AA and ATTR.

The therapeutic approach of these patients should be dual. On the one hand it aims at minimizing the activity of the underlying disease in order to mitigate or prevent SAA production, the precursor of plasma amyloid that is deposited in tissues and will be responsible for their malfunction. On the other hand it is aimed at treating the disease once it is established. The treatment of AA has two pillars: a) improvement of renal dysfunction; and b) reduction of inflammation, the constant source of SAA, thus trying to prevent the formation of amyloid, and hence curb the progression of disease (1,7). Colchicine has been widely used in the treatment of AA, achieving a decrease in proteinuria, stabilization of renal function, and significantly improved survival in different series (5,6,18). TNFα inhibitors have been used to treat the inflammation generated by the underlying disease. Multiple studies have evaluated these agents in rheumatologic diseases, specifically infliximab (IFX) and etanercept (19-23). The use of these drugs is based on the effect on TNFα over hepatocytes during acute-phase inflammation, stimulating them to produce SAA. Likewise, it favors the deposition of SAA in tissues in the form of amyloid, and causes a disruption of the normal architecture of organs. Studies with TNFα inhibitors have demonstrated a decrease in SAA circulating levels and proteinuria, as well as a stabilization of renal function (19-23). However, there are few studies evaluating the efficacy of IFX in patients with CD and AA. Results are not as good as in patients with rheumatic diseases, since all patients achieve an improvement in proteinuria but the effect on renal function is questionable (15,24-26). In our patient, and due to the infusional reaction he had previously shown to IFX, we were unable to continue this drug. However, in accordance to recent studies regarding actions in rheumatic diseases, we decided to maintain anti-TNFα therapy with adalimumab. To date, only the study published by Perry et al., one patient with inflammatory arthritis was switched to adalimumab due to a psoriasis-form rash to etanercept (22). However, strong evidence is still needed to support the use of these two drugs, IFX and adalimumab, in patients with CD and AA. Unfortunately, there is no approved treatment to eliminate already deposited amyloid (27). Preclinical data support the use of compounds that bind to a specific form of amyloid P component. This causes a reduction in serum levels, prevents binding to amyloid components, promotes dissociation from them, and favors removal of tissue deposits (10,12,13). Dialysis and renal transplantation are reserved for patients with end-stage renal failure. Survival rates for these patients have improved in recent years with the introduction of these drugs, but there is still no standardized treatment, and prognosis remains dismal.

REFERENCES
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