

Cardiac amyloidosis in a heart transplant patient - A case report and retrospective analysis of amyloidosis evolution

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Summary

Cardiac amyloidosis is a very rare cause of heart failure in heart transplant recipients but an important differential diagnosis in cases of progressive cardiac failure. We report a 72-year-old male patient with the diagnosis of senile systemic amyloidosis (SSA) in a transplanted heart 15 years after transplantation by the initial diagnosis of the dilated cardiomyopathy. Additionally performed immunohistochemical analysis with anti-transthyretin antibody of the cardiac biopsies of the last 15 years enabled the possibility to show the evolution of this disease with characteristic biphasic pattern.

Keywords: ATTR amyloid, senile systemic amyloidosis, heart transplantation, cardiac biopsy

1. Introduction

Amyloidosis results from a systemic or localized accumulation of polypeptides and proteins, which are typically arranged in an anti-parallel β -sheet conformation, rendering them insolubly. Those amyloid deposits can affect diverse tissues and organs. Amyloidosis of the heart eventually leads to heart failure. Cardiac amyloidosis is most commonly of either ATTR- (SSA and hereditary), AL- or AANP-type. Other forms are exceptionally rare. The common types of cardiac amyloidosis have variability in their precursor protein, age of manifestation, extracardiac organ involvement, treatment and prognosis (1). Depending on the type of amyloid, supportive therapy and ultimately heart transplantation as therapeutic approach can be performed (AL amyloid with combined heart/ bone marrow transplant, hereditary ATTR amyloid with orthotopic heart transplantation or combined heart/

liver transplantation and SSA with orthotopic heart transplantation (2)).

Herein we report a case of a 72-year-old male patient with the diagnosis of isolated cardiac amyloidosis in a transplanted heart 15 years after transplantation. Subsequently performed retrospective immunohistochemical analysis of myocardial biopsies provided an insight in the time progression of this disease.

2. Case report

The now 72-year-old male Caucasian patient underwent heart transplantation in 1998 due to severe, idiopathic dilated cardiomyopathy (DCM). Following transplantation the patient was treated with immune suppressive therapy and underwent repeated diagnostic tests *i.e.* echocardiography, cardiac magnetic resonance imaging and a total of 42 repeated biopsies of the heart transplant (Figure 1) to evaluate signs of graft rejection. The post-transplant visits took place in the outpatient transplantation unit of the Aachen University Hospital RWTH. Due to low-grade graft vasculopathy the patient underwent percutaneous coronary intervention (PCI) of the left anterior descending (LAD) with implantation of a bare metal stent in the year 2000. In April 2001 a severe graft rejection (type A3) was diagnosed by biopsy. Consequently

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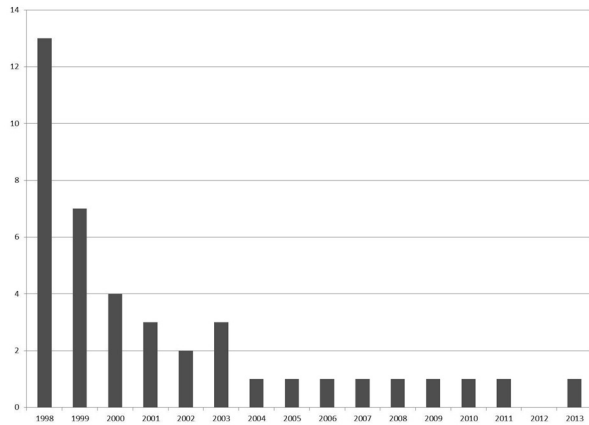


Figure 1. Numbers of biopsies per annum since 1998 (year of the heart transplantation).

the patient received a glucocorticoid pulse therapy of 1000 mg of methylprednisolone for three consecutive days. There were no signs of progression of the graft vasculopathy in follow-up angiographies. Two subsequent MRI studies (2011 and 2013) showed signs of intracardiac fibrotic remodelling mainly at the base of the interventricular septum and the inferolateral ventricle – which was clinically attributed to averted graft rejection - without overt aggravation. Repeated echocardiography revealed progressive lateral and septal wall thickening of the left ventricle and increasing diastolic dysfunction (Figure 2). To rule out storage diseases like amyloid deposition, a rectal biopsy was done in January 2013, which returned negative results. Furthermore, no λ - or κ - light chain proteins were detected by serum electrophoresis as possible sign of monoclonal gammopathy.

In spring 2013 the patient presented with an onset of new symptoms of dyspnoea on exertion (New York heart association, NYHA grade II) and paroxysmal palpitations especially during night time. NT-proBNP serum levels progressively increased. Electrocardiography revealed new onset of intermittent atrial fibrillation. Due to his history of graft vasculopathy, the patient underwent repeated angiography, this time revealing a severe vasculopathy with a three-vessel disease needing PCI with implantation of two drug-eluting stents into the circumflex artery. Immune suppression was changed from cyclosporine and mucofenolat-mofetil to cyclosporine and everolimus. In 2013 the state of health progressively deteriorated and dyspnoea worsened. The patient developed ankle oedema and signs of pulmonary venous congestion upon chest X-ray. Another graft biopsy was finally taken and revealed the presence of homogeneous eosinophilic material in a routine haematoxylin eosin (H&E) stain, suggestive of amyloidosis. This was confirmed by light microscopy on Congo red stain with characteristic green birefringence under polarized light (Figure 2). The immunohistochemical classification of the amyloid

deposits revealed ATTR amyloidosis resulting in two differential diagnoses: *i*) hereditary ATTR amyloidosis and *ii*) senile systemic amyloidosis (SSA). Genetic testing unravelled wild type-*TTR*. Despite one single episode of syncope in 2013 as a possible sign of involvement of autonomic nervous system, clinically no other evidence of amyloid deposition was found. *E.g.* urinary protein excretion was minimal. Finally, a diagnosis of SSA was obtained. Typical for this type of amyloidosis are cardiac manifestations and involvement of the peripheral nervous system with symptoms such as carpal tunnel syndrome (3). The cardiac involvement - as seen in our patient - manifests itself with cardiac failure, due to disorders of transmission of electrical impulses and atrial fibrillation (4), whereas a carpal tunnel syndrome was not found.

Based on frequently performed cardiac biopsies during the 15 years after the heart transplantation we had an opportunity to analyse the evolution of amyloidosis with immunohistochemical methods and subsequent quantification of amyloid load.

3. Materials and Methods

For histological analysis H&E stained slides and paraffin blocks from the heart biopsies were retrieved from the archive of the Institute of Pathology of the University Hospital RWTH Aachen. 4 μ m thick paraffin sections were stained with H&E. Amyloid was detected in Congo red-stained sections viewed under cross-polarized light. Immunostaining was carried out as described in detail elsewhere (5).

The immunohistochemically (anti-transthyretin-antibody) stained slides were scanned using a Leica SCN400 whole slide scanner (Leica Biosystems, Nussloch, Germany) with 40 times magnification. For image analysis the slide images were exported as overview and with 9 times magnification, corresponding to a pixel width of about 1.2 μ m. The percentage of the amyloid area of each specimen was evaluated using ImageJ version 1.47v (National Institute of Health, USA) by counting the immunohistologically stained and non-stained pixels. In a prior step artifacts surrounding the specimen within the original image were removed manually using Adobe Photoshop CS4 Extended Version 11.0.2 (Adobe Systems Incorporated, San Jose, USA). Processing in ImageJ was done using the "Color Threshold" function to filter pixels based on ranges of hue, saturation and brightness values in the HSB color model. Background was detected by filtering pixels of high brightness and low saturation. The specimen's pixel count was calculated by subtracting the background's pixel count from the total pixel count of the image. Stained areas (red pixels) were detected by filtering the corresponding range of hue values in combination with a lower threshold of saturation. The threshold values were adjusted individually for each

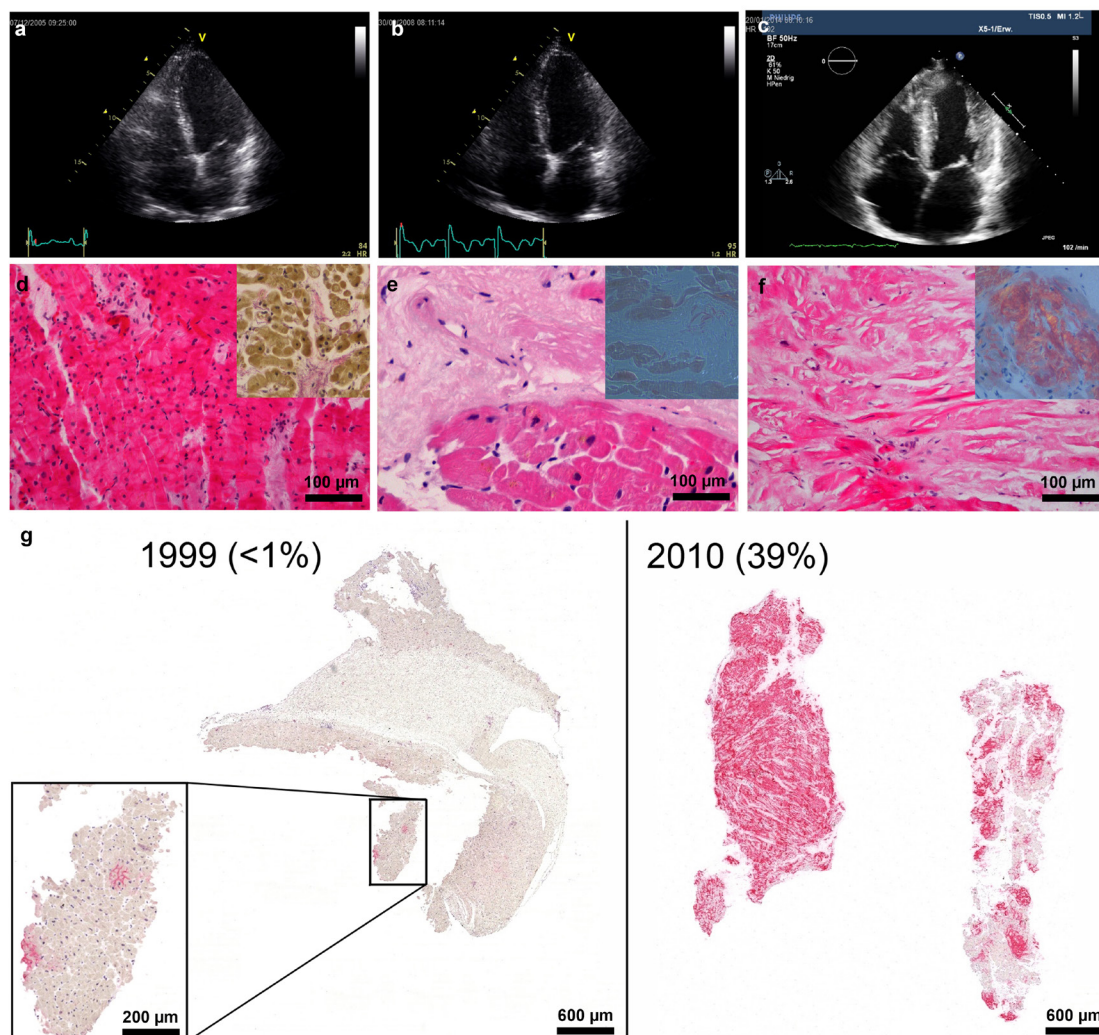


Figure 2. First row: Echocardiography: **a:** 4-chamber view from 2005 showing normal size of all four chambers without signs of left- or right ventricular hypertrophy, **b:** 4-chamber view from 2008 showing light left ventricular hypertrophy, **c:** 4-chamber view from 2014 now showing severe concentric left- and right ventricular hypertrophy as well as the typical sparkling pattern of storage diseases with myocardial affection. **Second row: Histology of heart biopsies:** **d** initial biopsy after heart transplantation without fibrosis or signs of eosinophilic deposits, hematoxylin and eosin-stain (H&E) and Elastica van Giesson (EvG) stain, **e** focal marked fibrosis 8 years after transplantation, H&E and Congo red stain under polarized light (insert) without green birefringence, **f** current biopsy, H&E and Congo red stain (insert) under polarized light with green birefringence indicating amyloid deposits. **Third row: Immunohistochemical staining with anti-transferrin antibody:** **e** comparison shows the remarkable contrast of the amyloid load in 1999 and 2010.

image under supervision of a pathologist in order to compensate for variations of the staining process.

4. Results

Reassessment of the stained slides of the heart biopsies showed that the patient's own myocardium did not contain any amyloid. During the first 7 years after the transplantation three graft biopsies enclosed no amyloid. Nine out of 12 biopsies obtained before 2006 enclosed tiny (< 2%) amyloid deposits, which were unnoticeable by routine H&E-staining. Starting in 2006 the amyloid load increased gradually from 4% to 43% (Figure 2 and 3). Interestingly, a biopsy obtained in 2011 harboured only 12% amyloid load, while a biopsy obtained in 2010 and 2013 enclosed 39% and 43%,

respectively, indicating that amyloid load is susceptible to sampling errors.

5. Discussion

Amyloidosis is a heterogeneous disease according to the diversity of proteins, which are able to form amyloid and the various different aetiologies. However, the clinical presentation of cardiac involvement is rather homogenous and may present as hypertrophy of the left ventricle, diastolic and/or systolic heart failure. Especially in SSA the heart involvement leads – with the exception of one reported case of a 77 years old patient with dilated cardiomyopathy and angina pectoris (6) – usually to congestive heart failure. Dyspnoea, oedema and reduced physical activity are typical

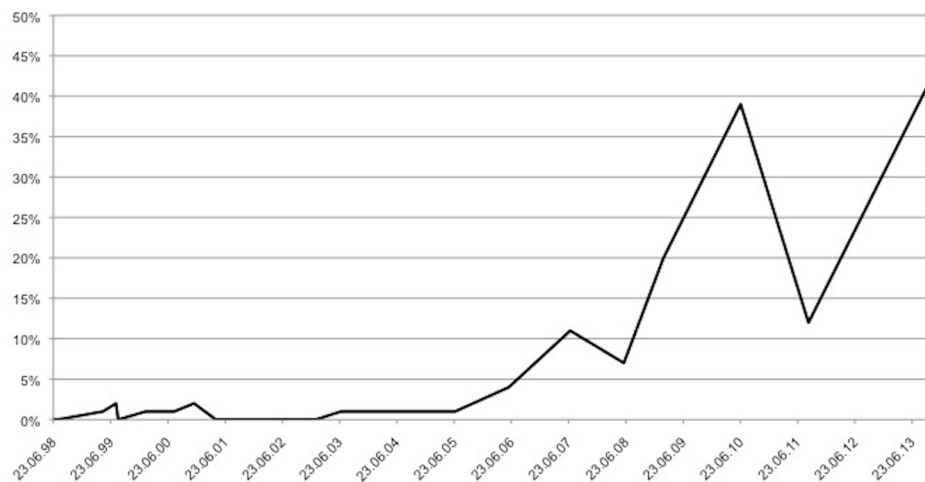


Figure 3. Changes of amyloid load in the heart biopsies during 15 years, starting in 1998, the year of transplantation. The first result from 23rd June of 1998 shows the amyloid load in patient's own heart, the other results are from the transplanted heart.

clinical signs.

Our case provides several interesting and partially novel findings:

i) SSA can affect cardiac grafts and should be considered in the differential diagnosis of graft failure. Thus, using Congo red as a routine stain in graft biopsies of elderly patients may be sensitive in order to reach an early diagnosis. However, minimal amyloid deposits can be missed and sensitivity may be increased by using fluorescence microscopy and immunohistochemistry, as has been suggested before (7). In our case cardiac amyloidosis was eventually diagnosed 15 years after the heart transplantation, despite continuous close monitoring of graft function and repeated close-meshed biopsies (Figure 1), which previously did not show signs of amyloid deposition in H&E routine stains like homogenous eosinophilic low cellular areas, which would have led to additional histological investigations. Storage diseases were initially clinically considered when echocardiography showed increased thickening of the left ventricular wall and the septum (8). Usually, SSA becomes apparent beyond the age of 80 (9) despite isolated reports about early cardiac involvement in SSA as early as 67 years of age (10). Our patient was 72 years old at the time of the diagnosis.

ii) Interstitial deposits of cardiac ATTR-amyloidosis commonly show a patchy deposition pattern, which is different from the more uniform reticular deposition pattern of cardiac AL-amyloidosis (unpublished observation of Co-author Christoph Röcken). This carries the risk of a sampling error and a negative test results may not exclude the presence of amyloid. In our series, 3 out of 12 biopsies obtained before 2006 enclosed no amyloid, reaching a false negative rate of 25% at the early stage of the disease. Thus, amyloid should be sought even when previous graft biopsies

failed to demonstrate amyloid. After diagnosis of graft amyloidosis the important question to clarify remained whether the initial heart failure before the patient was transplanted was associated with amyloidosis? This might at that time have fundamentally altered the diagnostic and therapeutic approach (11). But reassessment of the heart biopsies before transplantation did not reveal signs of amyloidosis.

iii) This study provides for the first time biopsy-proven insights into the natural course of SSA. The progression of SSA appears to follow a biphasic pattern with a relatively long lag-time without any obvious disease progression and minimal amounts of amyloid deposition followed by an acceleration phase with a steady incline of the amyloid load finally leading to clinically overt SSA. SSA is a disease of the elderly and factors contributing to this age-dependency are slowly unravelled. Amyloidosis is caused by misfolding and aggregation of polypeptides in a β -pleated sheet confirmation. A lack of protective chaperones, which are essential for the physiological folding of proteins, facilitates amyloid formation. The age-dependent disease manifestation may depend on the activity of stress-responsive signalling pathways. Their activity decreases with increasing age and enable the occurrence of misfolded, potentially amyloidogenic peptides and proteins (12). Heat shock proteins (HSP) are involved in these pathways and processes (13). The expression of HSP27 and HSP70 correlate with the presence of ATTR amyloid. Furthermore, the heat shock transcription factor-1 (HSF-1) is up-regulated in ATTR amyloidosis. HSF-1-deficient mice rapidly develop an amyloidosis (Almeida *et al.* 2012). Thus, the mere detection of amyloid in heart biopsies currently allows no prediction of disease progression. Amyloid load can be relatively static for a long period of time.

How is the prognosis of this disease? The outcome

of SSA is generally better compared to cardiac light chain AL amyloidosis. Median survival time of SSA is 7 - 8 years with supportive therapy and estimated survival time of cardiac light chain AL-amyloidosis is 48 months (14). It is fundamental to identify the subtype of the amyloid depositions (15) and to rule out any underlying and possibly treatable disease such as plasmacytoma for AL amyloidosis although in the case presented even earlier diagnosis of amyloidosis probably would have not altered the performed supportive therapy since there is no causative clinical approach. Due to the patient's age and comorbidities the patient presented cannot be considered for re-transplantation, although, his disease will probably progress. Destination therapy with ventricular assist devices may be a therapeutic option for patients as him.

In summary, amyloidosis (16,17) has to be added to the list of differential diagnoses of graft failure in a heart transplant recipients. The diagnosis is difficult to obtain and needs to be verified *e.g.* by Congo red-staining in combination with fluorescence microscopy and immunohistochemistry in order to detect the disease at an early stage.

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