Varying Levels of Small Microcalcifications and Macrophages in ATTR and AL Cardiac Amyloidosis: Implications for Utilizing Nuclear Medicine Studies to Subtype Amyloidosis

Miriam A. Stats, James R. Stone

Background

The amyloidoses are a group of systemic disorders characterized by the deposition of proteinaceous amyloid within tissues throughout the body. Amyloidosis is caused by the misfolding of a specific protein from its native conformation to an extended beta-sheet structure. Clinically significant cardiac amyloidosis is most often due to amyloid resulting from immunoglobulin light chain (AL amyloidosis) or amyloid resulting from Transthyretin (ATTR amyloidosis). Both the treatment and prognosis for AL amyloidosis differ substantially from those for ATTR amyloidosis. In the clinical setting, the type of amyloid present is most often discerned from the clinical presentation and direct typing of amyloid in tissue biopsies. For patients with cardiac amyloidosis, endomyocardial biopsy can yield tissue for direct amyloid subtyping, but this procedure can in rare cases be associated with serious complications. While patients with AL amyloidosis often have involvement of non-cardiac tissues more amenable to biopsy, for ATTR amyloid patients, biopsies of non-cardiac tissues such as skin and gastrointestinal tract are often negative for amyloid, despite the presence of cardiac amyloidosis.

Introduction

Recently there has been much interest in using nuclear medicine studies to noninvasively identify and subtype cardiac amyloidosis. In particular, modified bone scans using 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) and 99mTc-pyrophosphate (99mTc-PPy) are being used to selectively identify patients with ATTR amyloidosis rather than AL amyloidosis. However, the mechanism by which these agents selectively bind to ATTR amyloid deposits versus AL amyloid deposits has not been elucidated. 99mTc-DPD and 99mTc-PPy are known to bind to calcium, and amyloid deposits have previously been shown to contain calcium deposits in some settings. Thus, the increased binding of these agents to amyloid could result from microcalcifications within the amyloid deposits. Alternatively, the increased uptake of these agents in amyloidosis might theoretically result from enhanced metabolic activity in the tissue due to infiltration of macrophages. Previously, macrophages have also been identified in amyloid deposits in pathologic specimens. However, it is unclear if the levels of either microcalcifications or macrophages differ when comparing ATTR with AL amyloidosis.

Goal

This study sought to determine if there were in fact differing levels of microcalcifications or macrophages in ATTR vs. AL cardiac amyloidosis to help determine if either of these tissue alterations could be causing the enhanced binding of 99mTc-DPD and 99mTc-PPy to ATTR amyloid observed in imaging studies.

Methods

The cases comprised endomyocardial biopsies obtained at Massachusetts General Hospital between 2013 and 2015, which were positive for amyloid by Congo red staining. The amyloid was directly subtyped as either ATTR or AL amyloid by immunofluorescence or mass spectrometry. The biopsies were stained with von Kossa calcium stains and with immunohistochemistry for the macrophage marker CD68.

Data Analysis and Statistical Methods

The number of CD68+ macrophages and the number of microcalcifications on von Kossa manually counted at a magnification of 200×. The mean number of macrophages and microcalcifications per 200× field was determined. The differences in the numbers of macrophages and microcalcifications between ATTR and AL amyloidosis cases were compared by t test after log transformation of the data. Patient characteristics were compared using Wilcoxon test. Correlations between the macrophage and microcalcification densities and patient characteristics were assessed using linear regression.

Patient Characteristics

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<tr>
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<th>ATTR</th>
<th>AL</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>70 (59-80)</td>
<td>61 (45-70)</td>
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<tr>
<td>Sex (female [%])</td>
<td>51 (28-65)</td>
<td>30 (24-67)</td>
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<td>Posterior wall thickness (mm)</td>
<td>15 (12-21)</td>
<td>15 (12-21)</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>7.0 (4.0-9.0)</td>
<td>11 (10-14)</td>
<td>0.21</td>
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<tr>
<td>Serum calcium (mg/dl)</td>
<td>9.1 (8.5-9.9)</td>
<td>9.3 (9.0-10.4)</td>
<td>0.10</td>
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Evaluation of the hematoxylin and eosin (H&E)-stained sections revealed a readily apparent confluent area of calcification in only one of the ATTR cases and none of the AL cases (Figure 1). However, on von Kossa stains, numerous small microcalcifications measuring 1–20 μm were visible in all of the cases (Figures 2 and 3). Comparison between the ATTR and AL cases revealed a significantly greater number of microcalcifications in the ATTR cases compared with the AL cases (mean=16.8 vs. 6.5 per 200× field, P=0.008, Figure 4A). Comparison of ATTR and AL cases revealed a significantly greater degree of macrophage infiltration in the AL cases (mean=2.5 vs. 11.7 per 200× field, P=0.004, Fig. 4B).

Discussion

Importantly, the degree of macrophage infiltration was significantly lower in ATTR amyloid compared with AL amyloid. Thus, increased tissue metabolic activity due to macrophage infiltration appears to not explain the enhanced binding of 99mTc-DPD and 99mTc-PPy to ATTR amyloid. However, these agents are known to bind to calcium, and the increased level of microcalcifications in ATTR amyloid cases likely explains the enhanced affinity of these agents for this amyloid subtype.

Conclusion & Impact

In summary, ATTR amyloidosis contains greater numbers of microcalcifications but fewer macrophages compared with AL amyloidosis. This enhanced degree of cardiac microcalcifications likely explains the selective binding of imaging agents such as 99mTc-DPD and 99mTc-PP to ATTR amyloid over AL amyloid. The greater degree of macrophages in AL amyloid compared with ATTR amyloid suggests that macrophage-targeted imaging may also help discriminate between these two disorders noninvasively.