

Step-by-step typing for the accurate diagnosis of concurrent light chain and transthyretin cardiac amyloidosis

Hidenori Moriyama¹, Hiroki Kitakata¹, Jin Endo^{1*} , Hidehiko Ikura¹, Motoaki Sano¹, Masayoshi Tasaki^{2,3}, Shunta Sakai⁴, Mitsuharu Ueda² and Keiichi Fukuda¹

¹Department of Cardiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan; ²Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; ³Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan; and ⁴Department of Cardiology, Showa General Hospital, Tokyo, Japan

Abstract

While ^{99m}Tc-pyrophosphate scintigraphy is clearly useful in diagnosing transthyretin amyloid cardiomyopathy (ATTR-CM), it is necessary to know the pitfalls of this test for proper use. We present a rare case of concurrent ATTR-CM and amyloid light chain (AL) cardiomyopathy. The patient showed congestive heart failure with left ventricular hypertrophy. ^{99m}Tc-pyrophosphate scintigraphy revealed abnormal cardiac uptake of Grade 3, a typical feature for ATTR-CM. However, the patient showed renal impairment with proteinuria and the presence of monoclonal gammopathy, which rather suggested AL amyloidosis. Endomyocardial biopsy, immunohistochemistry, and proteomic analysis by laser microdissection with liquid chromatography-coupled tandem mass spectrometry were performed, which finally confirmed both ATTR-CM and AL cardiomyopathy. This case implicates the importance of combining examinations and precisely interpreting the results to diagnose cardiac amyloidosis accurately.

Keywords Amyloidosis; Transthyretin; Amyloid light chain; Pyrophosphate scintigraphy

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*Correspondence to: Jin Endo, Department of Cardiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Email: jinendo@keio.jp

Introduction

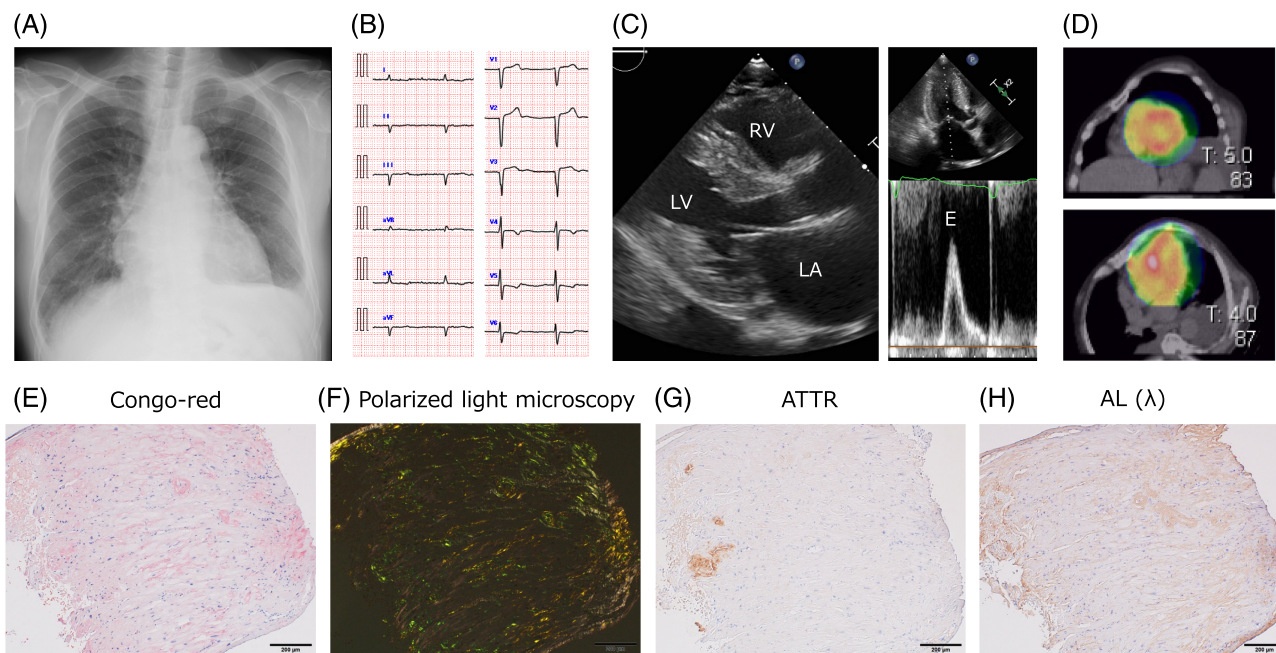
Cardiac amyloidosis is a progressive and fatal cardiomyopathy involving excessive deposition of amyloid fibrils in the heart. Nearly all cases are caused by the aggregation of transthyretin or immunoglobulin light chains.¹ Accurate diagnosis of these two subtypes is crucial, otherwise resulting in missed opportunities to provide efficient treatments. The recent advances in imaging modalities have enabled cardiologists to detect cardiac amyloidosis earlier and more accurately. In particular, bone scintigraphy is essential for transthyretin amyloid cardiomyopathy (ATTR-CM) diagnosis noninvasively.^{1,2} However, recent statements emphasized the danger of relying entirely on bone scintigraphy to differentiate ATTR-CM and light chain (AL) amyloidosis.^{2,3} We present a specific case involving multiple precursor proteins for cardiac amyloidosis, wherein

bone scintigraphy and other evaluation tests were performed to confirm the presence of ATTR-CM and AL amyloidosis.

Case report

A 77-year-old man presented with dyspnoea, peripheral oedema, and an elevated serum BNP level of 393 pg/mL a few months ago. His chest radiograph revealed cardiomegaly and slight bilateral pleural effusion (Figure 1A). He underwent surgery for lumbar spinal stenosis at the age of 67 years and was diagnosed with carpal tunnel syndrome at the age of 75 years. He also had renal dysfunction with an eGFR of 30 mL/min/1.73 m² and proteinuria. His electrocardiogram showed atrial fibrillation, poor R progression, and

Figure 1 (A) Chest X-ray. (B) Twelve-lead electrocardiogram. (C) Transthoracic echocardiography in parasternal long axis view (left) and transmitral flow with early transmitral flow velocity of 114 cm/s and deceleration time of 157 ms (right). (D) ^{99m}Tc-pyrophosphate scintigraphy. (E–H) Histopathological findings of the myocardium. Congo-red staining (E). Apple-green birefringence on polarized light microscopy (F). Immunostaining with an antibody against transthyretin (G). Immunostaining with an antibody against free light chain λ (H). (I) Results of proteomic analyses by LMD-LC-MS/MS. LA, left atrium; LV, left ventricle; LMD-LC-MS/MS, laser microdissection with liquid chromatography-coupled tandem mass spectrometry; RV, right ventricle.



(I) LMD-LC-MS/MS proteomic analysis

ATTR				AL (λ)			
Accession	Description	# PSMs	NSAF (%)	Accession	Description	# PSMs	NSAF (%)
P02766	Transthyretin	49	77.80883	J00254	IGLC3*01	22	25.69122
Q8IYA6-2	Cytoskeleton-associated protein 2-like	5	3.494409	P02649	Apolipoprotein E	19	7.279303
P68871	Hemoglobin subunit beta	2	3.175871	P68871	Hemoglobin subunit beta	7	5.783306
P02649	Apolipoprotein E	3	2.209084	P06727	Apolipoprotein A-IV	17	5.213738
P02743	Serum amyloid P-component	2	2.093511	P08590	Myosin light chain 3	7	4.359723
P07477	Trypsin-1	2	1.890093	P04004	Vitronectin	14	3.557096
P06727	Apolipoprotein A-IV	3	1.768383	P10909-3	Clusterin	8	3.545968
P12883	Myosin-7	10	1.206338	P69905	Hemoglobin subunit alpha	4	3.42111
P08590	Myosin light chain 3	1	1.197059	P17661	Desmin	13	3.359239
P35030-3	Trypsin-3	1	0.945047	P12883	Myosin-7	52	3.263757
P10909-3	Clusterin	1	0.851922	P63267	Actin, gamma-enteric smooth muscle	9	2.907034
P12235	ADP/ATP translocase 1	1	0.78331	P68133	Actin, alpha skeletal muscle	9	2.899323
Q01353	Protein U7	1	0.682534	Q8IYA6-2	Cytoskeleton-associated protein 2-like	5	1.818105
P60709	Actin, cytoplasmic 1	1	0.622471	P19429	Troponin I, cardiac muscle	3	1.734992
P01024	Complement C3	4	0.561459	P02743	Serum amyloid P-component	3	1.633849
Q62TA4-2	Tripartite motif-containing protein 67	1	0.323754	P10916	Myosin regulatory light chain 2	2	1.463246
Q6ZU11	Uncharacterized protein FLJ44066	1	0.25208	P01834	Ig kappa chain C region	1	1.145749
P0C0L4	Complement C4-A	1	0.133845				

non-specific ST-T change (Figure 1B). At the same time, echocardiography revealed concentric left ventricular hypertrophy (the thicknesses of the intraventricular septum and posterior

wall were 14 and 12 mm, respectively) with a preserved ejection fraction of 58.5% but elevated E/e' of 23.8 (Figure 1C). The serum-free light chain concentration was κ 80.3 mg/dL

and λ 169.8 mg/dL (κ/λ ratio 0.47). Serum and urine immunofixation electrophoresis revealed IgA λ monoclonal protein, which was suggestive of AL amyloidosis. However, ^{99m}Tc -pyrophosphate scintigraphy showed abnormal cardiac uptake (Grade 3), a typical feature for ATTR-CM (Figure 1D). Genetic testing for transthyretin revealed no mutations.

Because the non-invasive test results suggested both types, an endomyocardial biopsy from the right ventricle was performed. The sample tested positive on Congo-red staining (Figure 1E) and exhibited an apple-green birefringence on polarized light microscopy (Figure 1F), indicating amyloid fibril deposition. We performed immunohistochemistry using highly specific diagnostic antibodies possessed by the Group of Surveys and Research of Amyloidosis in Japan (GSRA-J)⁴ and found that the different sites of the specimen tested positive for both transthyretin and immunoglobulin light chain λ (Figure 1G and 1H). To resolve the confusing evidence for simultaneous findings of ATTR-CM and AL amyloidosis, proteomic analysis by laser microdissection with liquid chromatography-coupled tandem mass spectrometry (LMD-LC-MS/MS) was performed to identify the protein from the positive sites. The patient was finally histologically diagnosed with concurrent ATTR-CM and AL amyloidosis (Figure 1I). In the kidney biopsy sample, Congo red-positive amyloid deposits were also confirmed, although the λ chain alone was positive on immunostaining, indicating that his renal amyloidosis was AL. He has received treatment with tafamidis for ATTR-CM. Chemotherapy was also being considered for AL amyloidosis, but he died of advanced heart failure and renal failure 5 months after diagnosis.

Discussion

This case of concurrent ATTR-CM and AL cardiomyopathy demonstrated the importance of combining examinations and precisely interpreting the results to diagnose cardiac amyloidosis accurately. While bone scintigraphy has been established as an essential tool for ATTR-CM diagnosis, positive findings cannot exclude the presence of AL amyloidosis.^{5,6} Myocardial uptake of bone tracer can diagnose ATTR-CM without histological confirmation only when monoclonal gammopathy was not present due to the false-positive result for bone scintigraphy in AL amyloidosis. When the bone scintigraphy is positive and monoclonal protein is detected, the patient may have ATTR with monoclonal gammopathy of undetermined significance (MGUS). However, because this case also presented with nephrotic syndrome, which is rarely observed in ATTR, the presence of AL amyloidosis should be considered. Furthermore, because there have been recent reports on two-type cardiac amyloidosis in a single patient,^{7–9} it became necessary to consider the possibility of concurrent ATTR and AL amyloidosis.

The present case also demonstrated that mass spectrometry is a potential tool for differential diagnosis of amyloid subtypes. LMD-LC-MS/MS is a novel technique that enables specific and sensitive typing of amyloidosis in biopsy specimens and is useful for identifying rare amyloid species for which diagnostic antibodies are not available.¹⁰ Immunostaining is the gold standard for amyloid typing, but sometimes it does not give definitive results. Therefore, cases with an uncertain diagnosis, such as double-amyloidosis or other uncommon types, must be analysed by mass spectrometry.

It is difficult to determine whether AL or ATTR is the main cause of cardiac lesions in the present case. The patient primarily shows characteristics of AL except for bone scintigraphy, and it is easy to understand the hypothesis that wild-type ATTR accumulated with aging incidentally coexisted. However, it is also necessary to consider that ATTR deposition contributed to some degree of cardiac dysfunction, as strong myocardial uptake was observed throughout the left ventricle with bone scintigraphy.

Recent advances in treatment options for amyloidosis allow the combination of administration with transthyretin tetramer stabilizer for ATTR and chemotherapy (or autologous stem cell transplantation) for AL if an early diagnosis is made. In particular, because AL shows a rapid progression, in such cases it is necessary to perform endomyocardial biopsy, Congo red staining, and immunohistochemistry for amyloidosis subtyping and, if required, mass spectrometry analysis without hesitation. A comprehensive workup and optimizing the diagnostic tools may contribute to accurate amyloid typing, resulting in appropriate treatment.

Declaration of interest

None.

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Conflict of interest

None declared.

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