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CASE REPORT

Advanced isolated light chain amyloid cardiomyopathy with negative immunofixation and normal free light chain ratio

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Abstract

Amyloid light chain (AL) cardiomyopathy is the most malignant specific cardiomyopathy. According to international recommendations, it should be ruled out non-invasively using the serum free light chain (FLC) ratio and immunofixation electrophoresis in both serum and urine. Here, we report on a 69-year-old female patient with new-onset heart failure with mid-range ejection fraction. Cardiac imaging was highly suggestive of cardiac amyloidosis. Amyloid scintigraphy showed faint myocardial tracer uptake according to Perugini Score 1, but immunofixation was negative and the FLC ratio was normal, despite a slight increase in lambda FLCs. Endomyocardial biopsy revealed advanced myocardial lambda immunoglobulin light chain deposition. Clinically relevant extracardiac amyloid organ infiltration could not be detected. Conclusively, non-invasive testing can in rare cases fail to exclude isolated AL amyloid cardiomyopathy. We suggest that even slight increases in serum lambda or kappa FLCs should be considered abnormal in suspected cardiac amyloidosis if non-invasive testing delivers discrepant results.

Keywords Cardiac amyloidosis; Monoclonal gammopathy; Endomyocardial biopsy; Light chain amyloidosis; Free light chain

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Introduction

Amyloid light chain (AL) cardiomyopathy affects more than 50% of patients with systemic AL amyloidosis and carries a dismal prognosis if untreated.[1,2] Isolated cardiac manifestation is present if no other target organ shows clinically relevant involvement. With the advent of effective treatment options for both AL and transthyretin (TTR) amyloidosis, awareness regarding the entity of cardiac amyloidoses rises.[3,4] As a consequence, cardiologists are increasingly confronted with patients with suspected cardiac amyloidosis and should be aware of potential pitfalls in the diagnostic work-up. Rule out of AL amyloidosis is of high importance because only early treatment improves outcomes. While its diagnosis requires histopathological confirmation, current recommendations suggest ruling out AL amyloidosis using serum free light chain (FLC) ratio assessment and immunofixation electrophoresis of both serum and urine to detect monoclonal proteins.[5,6] The present case, however, demonstrates that isolated AL amyloid cardiomyopathy can occur without significant increase in circulating amyloidogenic FLCs and can therefore be missed non-invasively.

Case report

A 69-year-old female patient was referred for congestive heart failure with progressive exertional dyspnoea according

to New York Heart Association (NYHA) Class III and angina pectoris according to Canadian Cardiac Society Class I during the last months. She presented with congested jugular veins, a third heart sound, and mild leg oedema. Cardiovascular risk factors comprised smoking (50 pack years) and arterial hypertension with an office blood pressure of 135/86 mmHg. Levels of both N-terminal pro brain natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin T were elevated (1199 and 26 pg/mL, respectively), while serum creatinine was normal (0.78 mg/dL) and estimated glomerular filtration rate (eGFR) was only slightly impaired with 78 mL/min/1.73 m^2 (normal $>$90 mL/min/1.73 m^2). Transthoracic echocardiography showed a left ventricular mid-range ejection fraction of 47% and typical features of infiltrative cardiomyopathy (*Figure 1*), and cardiac magnetic resonance imaging was strongly suggestive of cardiac amyloidosis (*Figure 2*).

Following the non-invasive diagnostic algorithm suggested by Gillmore *et al*., we performed amyloid scintigraphy and searched for monoclonal paraproteins.[7] ^{99m}Tc-DPD bone scintigraphy revealed a faint myocardial radiotracer uptake according to Perugini Score 1. Despite slightly increased levels of serum lambda FLCs (30.20 mg/L, upper normal range 26.3 mg/L) in concomitance with normal kappa FLCs (16.5 mg/L, upper normal range 19.4), the serum FLC kappa to lambda ratio was not altered (0.55; normal range 0.26–1.65). Neither serum nor urine immunofixation electrophoresis was positive for monoclonal paraproteins. These results were confirmed in a repeated assessment.

Given the inconclusive constellation after non-invasive diagnostic work-up, we performed endomyocardial biopsy (EMB). Histopathological analysis of Congo-red stained biopsy specimens revealed myocardial amyloid infiltration on 40% of the cutting surface (*Figure 3*). Unexpectedly, immunohistochemical characterization of amyloid depositions identified lambda immunoglobulin light chains. Bone marrow aspiration unveiled a normocellular bone marrow without evidence of an underlying multiple myeloma. Diagnostic work-up also included karyotyping as well as fluorescent *in situ* hybridization analysis of the bone marrow aspirate. Cytogenetics displayed a normal karyotype, and despite magnetic-activated cell sorting-based enrichment for plasma cells, fluorescent *in situ*

Figure 1 Transthoracic echocardiography showed a left ventricular ejection fraction of 47% and (A) pronounced concentric bi-ventricular hypertrophy; (B) left ventricular global longitudinal strain of 9.1% with significant relative apical sparing; and (C) a restrictive transmitral filling pattern with an E/A ratio of 2.18.

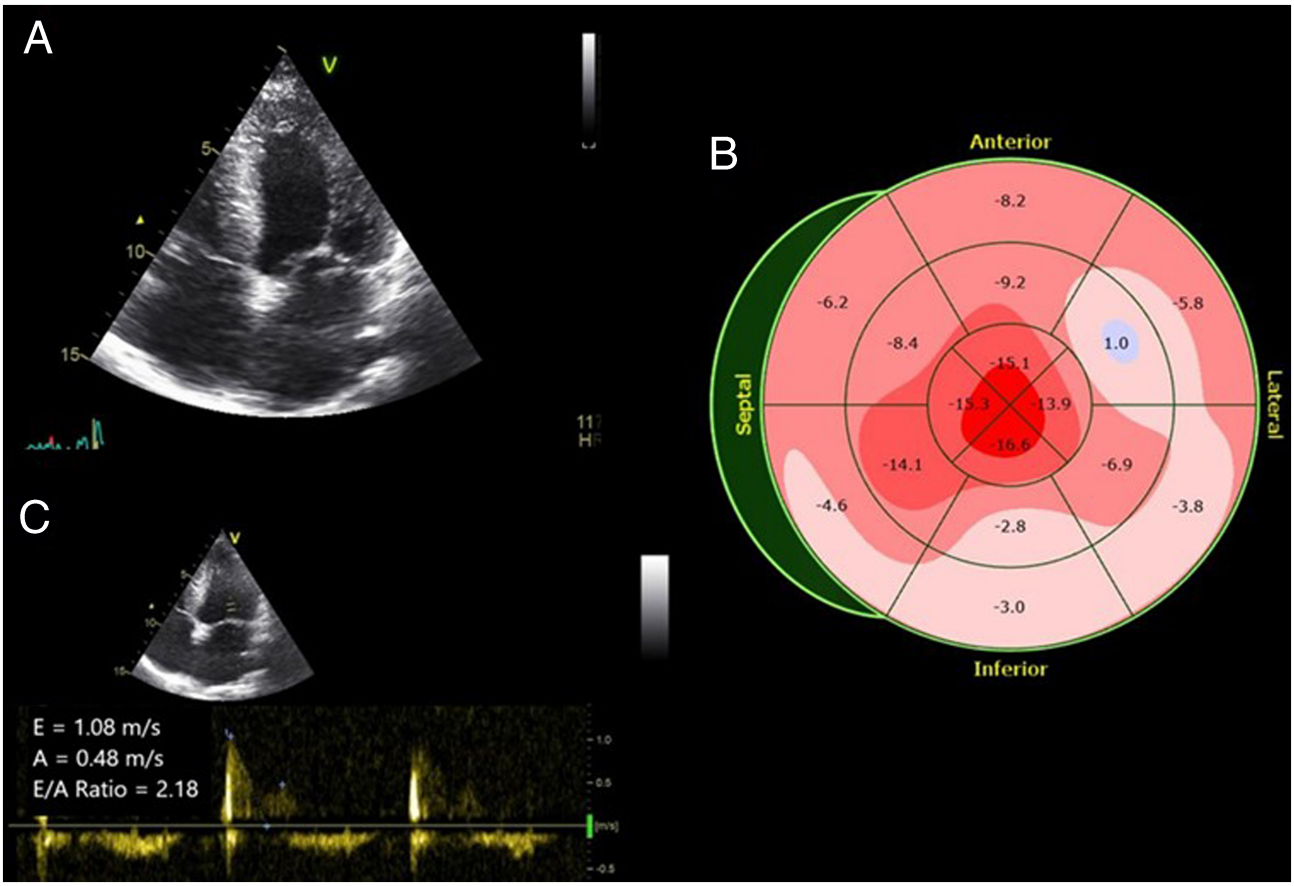

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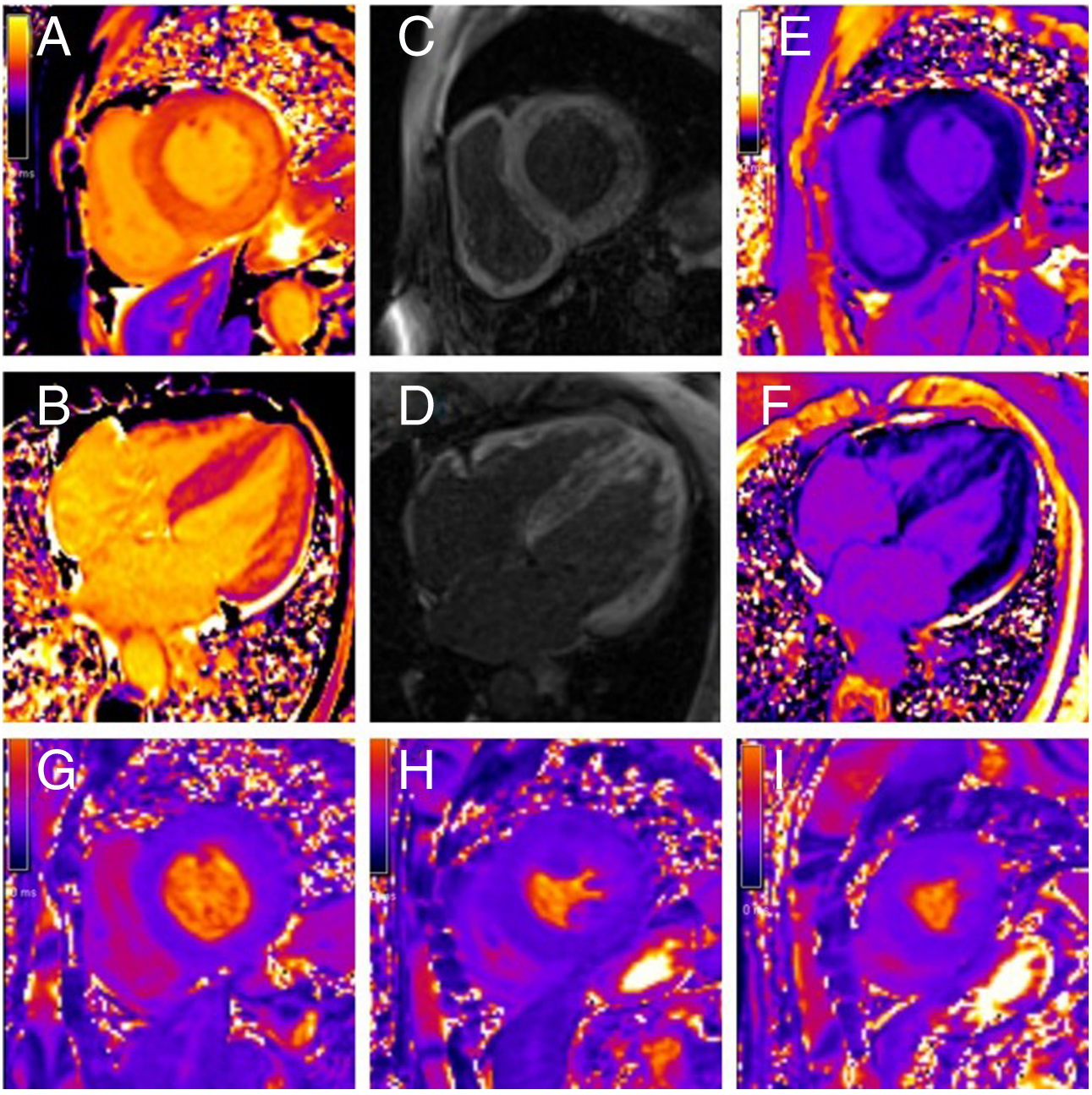
Figure 2 Cardiac magnetic resonance imaging. (A,B) Native myocardial T1 and extracellular volume mapping revealed increased values for both parameters (1411 ± 83 ms, *z*-score = 7; and 69 ± 7%, *z*-score = 15, respectively); (C,D) left ventricular (LV) mid and apical segments portrayed subendocardial late gadolinium enhancement, while the basal segments showed a transmural pattern. In the right ventricle, global late gadolinium enhancement was present; (E,F) Post-contrast T1 maps confirmed transmural accumulation of the contrast agent at basal LV and right ventricular myocardial segments, as well as global subendocardial accumulation of the contrast agent at mid-ventricular to apical LV and right ventricular myocardial segments; (G–I) global LV myocardial T2 times were within the normal range excluding myocardial oedema.



hybridization analysis did not reveal chromosomal changes recurrently found in multiple myeloma. Minimal residual disease measurements were not performed. Subsequent assessment revealed the presence of clinically non-relevant perivascular amyloid depositions in the bone marrow. Amyloid was not detectable in abdominal fat pad aspiration.

Systemic AL amyloidosis with isolated amyloid cardiomyopathy Stage II was confirmed. Interdisciplinary disease management included symptomatic heart failure treatment and chemotherapy with eight cycles of bortezomib/dexamethasone followed by 4 cycles of melphalan/dexamethasone. Heart transplant followed by autologous

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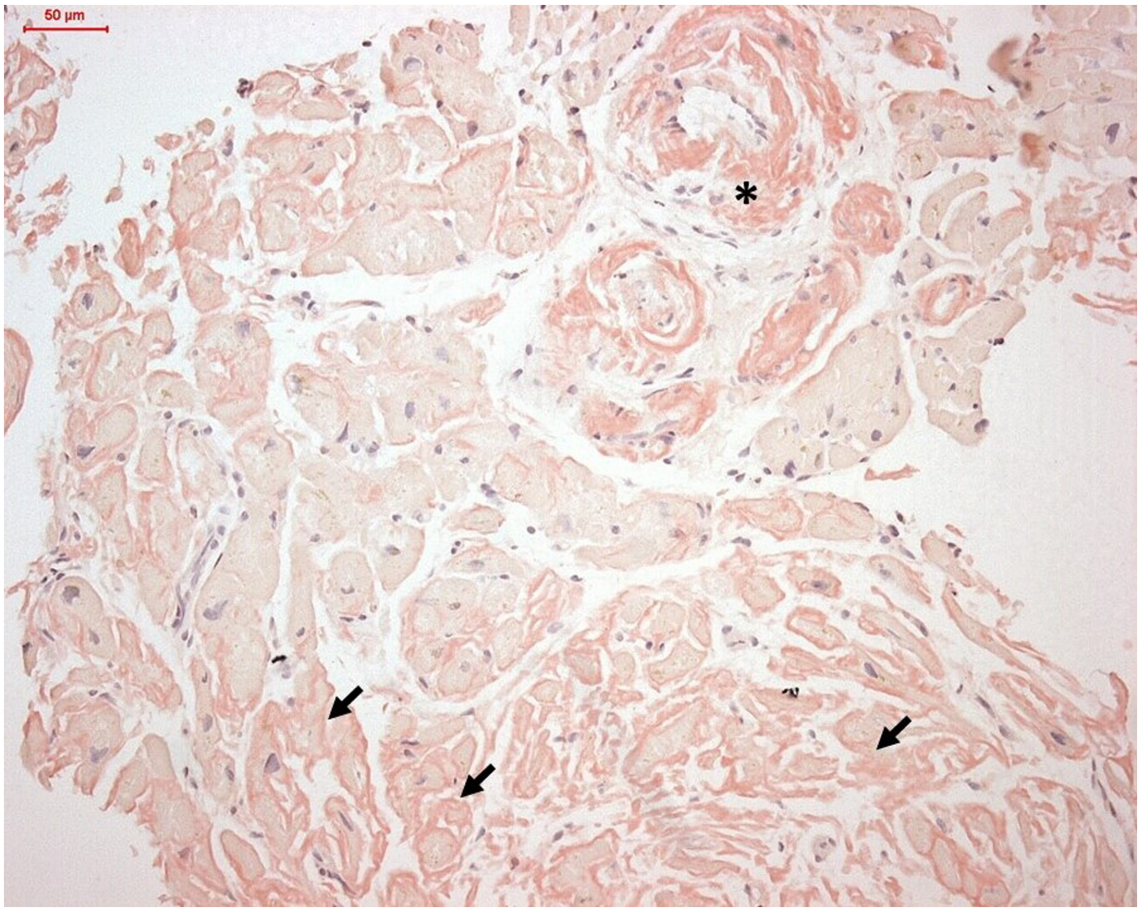
Figure 3 Histopathological analysis of Congo-red stained endomyocardial biopsy specimen demonstrating perivascular (*) and interstitial ($Calibri(TrueType)"$2000(TrueType)}2794) myocardial amyloid depositions (Congo-red stain, ×200).



stem cell therapy was considered a possible treatment option, but was declined by the patient. Two years after the initial diagnosis, she has remained in a clinically stable condition, with exertional dyspnoea according to NYHA Class II, NT-proBNP levels of 1408 pg/mL, and normalized levels of serum lambda FLCs of 11.4 mg/L.

Discussion

The present case illustrates that immunofixation electrophoresis and FLC ratio assessment according to international recommendations can fail to detect AL amyloidosis complicated by isolated AL amyloid cardiomyopathy. Normal or borderline concentrations of FLCs resulting in a normal FLC ratio have also been reported by others, suggesting that this phenomenon may affect a relevant proportion of patients with isolated AL cardiomyopathy.[8] Together, these cases raise doubts on whether the current definition of normal FLCs yields sufficient sensitivity for the detection of isolated AL cardiomyopathy.[6] Moreover, it emphasizes that EMB should be performed in any patient with a high likelihood of cardiac amyloidosis where a plasma cell clone cannot be definitely excluded.

AL amyloid cardiomyopathy carries the worst prognosis within the spectrum of specific cardiomyopathies.[1] In the vast majority of amyloid cardiomyopathies, the underlying cause is either TTR or AL amyloid fibril deposition.[9] Both AL and TTR amyloid infiltration can be associated with scintigraphic myocardial tracer uptake. In fact, 20% of patients with AL amyloid cardiomyopathy had a Perugini Score $\geq$2 in the landmark study by Gillmore *et al*. Vice versa, 6% of patients with TTR amyloid cardiomyopathy presented with Perugini Score $<$2.[7] International recommendations therefore suggest that EMB can be avoided if non-invasive amyloid subtyping is specific of TTR amyloidosis. Amyloid scintigraphy with Perugini score $\geq$2 is considered diagnostic of TTR amyloidosis if AL can be excluded.[5,6] Determination of serum FLC ratio in addition to immunofixation electrophoresis of both serum and urine is considered to have at least 99% sensitivity for monoclonal gammopathy and is therefore

recommended as the gold-standard method to exclude AL amyloidosis.[10,11] Consequently, international recommendations rate the presence of AL amyloidosis as very unlikely if the FLC ratio is normal and amyloid scintigraphy results are normal or indeterminate.[6] Our case, however, demonstrates that isolated AL amyloid cardiomyopathy can be present even if the assessment of the FLC ratio is unremarkable and serum as well as urine immunofixation are negative.

It is important to note that our patient presented with slightly increased free lambda immunoglobulin light chains in the presence of mild chronic kidney disease (eGFR of 78 mL/min/1.73 m^2), although this did not lead to a significant alteration of the FLC ratio. Increases in both kappa and lambda FLC concentrations can occur in the presence of impaired kidney function. However, significant increases in FLC concentrations, particularly affecting kappa FLCs, and alterations of the FLC ratio are almost exclusively evident in patients with an eGFR $<$55 mL/min/1.73 m^2, where adaptations of the normal reference ranges for FLCs and the FLC ratio have been suggested.[12] Apart from minimal perivascular amyloid infiltration in bone marrow specimen, there was no evidence of clinically relevant extra-cardiac infiltration, allowing for the diagnosis of isolated AL amyloid cardiomyopathy. This imbalance between undetectable systemic monoclonal light chain burden and severe myocardial infiltration could potentially be explained by selective cardiac tropism of monoclonal lambda light chains expressed in our patient. Cardiac tropism is considered as the crucial mechanism leading to selective myocardial infiltration in AL amyloidosis. It is well accepted that certain monoclonal amyloidogenic light chains are more cardio-selective and cardio-toxic than others.[13] However, underlying mechanisms remain elusive in large parts. Other factors that both increased the likelihood of AL amyloidosis and made ATTR amyloidosis unlikely were the rapid progression of heart failure symptoms within months and the female gender.

The present case report underlines that non-invasive methods of amyloid subtyping, i.e. amyloid scintigraphy and FLC assessment, should be interpreted in the context of an individual patient's likelihood to have cardiac amyloidosis. Also the clinical course and the patient's gender should be taken into account. In fact, the diagnostic accuracy of these methods has been validated in a cohort of patients with suspected cardiac amyloidosis.[7] In experienced hands, echocardiographic assessment of myocardial thickening, diastolic function and filling pressures, and global longitudinal strain pattern remains at the forefront to raise suspicion of cardiac storage disease.[14] In addition, cardiac magnetic resonance imaging provides excellent diagnostic accuracy for the diagnosis of cardiac amyloidosis, particularly if T1 mapping and quantification of myocardial extracellular volume are applied.[15] Our case reaffirms, as suggested by Gillmore *et al*., that patients with suspected cardiac amyloidosis and Perugini Score of 1 should undergo EMB irrespective of results of the FLC assessment.[7] In experienced hands, EMB is a relatively safe procedure leading to life-threatening complications in less than 1% of performed procedures.[16] Given the substantial consequences of misdiagnosed AL amyloid cardiomyopathy for individual patients, actions to increase the sensitivity of FLC assessment are warranted. We therefore suggest that the definition of abnormal FLCs in a patient with suspected cardiac amyloidosis and discrepant non-invasive testing should be extended by evidence of unexplainable elevation of either lambda or kappa FLCs, even if the FLC ratio is normal and a monoclonal band cannot be detected using immunofixation electrophoresis.

Conclusion

We conclude that patients with isolated AL amyloid cardiomyopathy can in rare cases present with false negative FLC assessment if the current definition of normal FLC assessment is applied. Cardiologists should be aware that isolated AL amyloid cardiomyopathy cannot be excluded with absolute certainty using non-invasive testing. In patients with suspected cardiac amyloidosis and discrepant results following non-invasive testing, we suggest that the definition of abnormal FLC assessment should be extended so that unexplainable elevation of serum FLCs should be considered abnormal, even if the FLC ratio is normal and a monoclonal band cannot be objectified using immunofixation electrophoresis. Patients with a high likelihood of cardiac amyloidosis should undergo EMB if non-invasive amyloid subtyping is not specific of TTR amyloidosis.

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Conflict of interest

The authors declare no conflict of interest.

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References

1. Kocher F, Kaser A, Escher F, Doerler J, Zaruba M-M, Messner M, Mussner-Seeber C, Mayr A, Ulmer H, Schneiderbauer-Porod S, Ebner C, Poelzl G. Heart failure from ATTRwt amyloid cardiomyopathy is associated with poor prognosis. *ESC Heart Fail* 2020; **7**: 3919–3928.
2. Falk RH. Cardiac amyloidosis: a treatable disease, often overlooked. *Circulation* 2011; **124**: 1079–1085.
3. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, Kristen AV, Grogan M, Witteles R, Damy T, Drachman BM, Shah SJ, Hanna M, Judge DP, Barsdorf AI, Huber P, Patterson TA, Riley S, Schumacher J, Stewart M, Sultan MB, Rapezzi C. Tafamidis treatment for patients with transthyretin amyloid cardiomyopathy. *N Engl J Med* 2018; **379**: 1007–1016.
4. Roussel M, Merlini G, Chevret S, Arnulf B, Stoppa AM, Perrot A, Palladini G, Karlin L, Royer B, Huart A, Macro M, Morel P, Frenzel L, Touzeau C, Boyle E, Dorvaux V, Le Bras F, Lavergne D, Bridoux F, Jaccard A. A prospective phase 2 trial of daratumumab in patients with previously treated systemic light-chain amyloidosis. *Blood* 2020; **135**: 1531–1540.
5. Gertz MA. Immunoglobulin light chain amyloidosis: 2020 update on diagnosis, prognosis, and treatment. *Am J Hematol* 2020; **95**: 848–860.
6. Maurer MS, Bokhari S, Damy T, Dorbala S, Drachman BM, Fontana M, Grogan M, Kristen AV, Lousada I, Nativi-Nicolau J, Cristina Quarta C, Rapezzi C, Ruberg FL, Witteles R, Merlini G. Expert consensus recommendations for the suspicion and diagnosis of transthyretin cardiac amyloidosis. *Circ Heart Fail* 2019; **12**: e006075.
7. Gillmore JD, Maurer MS, Falk RH, Merlini G, Damy T, Dispenzieri A, Wechalekar AD, Berk JL, Quarta CC, Grogan M, Lachmann HJ, Bokhari S, Castano A, Dorbala S, Johnson GB, Glaudemans AWJM, Rezk T, Fontana M, Palladini G, Milani P, Guidalotti PL, Flatman K, Lane T, Vonberg FW, Whelan CJ, Moon JC, Ruberg FL, Miller EJ, Hutt DF, Hazenberg BP, Rapezzi C, Hawkins PN. Nonbiopsy diagnosis of cardiac transthyretin amyloidosis. *Circulation* 2016; **133**: 2404–2412.
8. Milani P, Valentini V, Ferraro G, Basset M, Russo F, Foli A, Palladini G, Merlini G. A patient with AL amyloidosis with negative free light chain results. *Clin Chem Lab Med* 2016; **54**: 1035–1037.
9. Ablasser K, Verheyen N, Glantschnig T, Agnetti G, Rainer PP. Unfolding cardiac amyloidosis—from pathophysiology to cure. *Curr Med Chem* 2019; **26**: 2865–2878.
10. Palladini G, Russo P, Bosoni T, Verga L, Sarais G, Lavatelli F, Nuvolone M, Obici L, Casarini S, Donadei S, Albertini R, Righetti G, Marini M, Graziani MS, Melzi D'Eril GV, Moratti R, Merlini G. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. *Clin Chem* 2009; **55**: 499–504.
11. Hanna M, Ruberg FL, Maurer MS, Dispenzieri A, Dorbala S, Falk RH, Hoffman J, Jaber W, Soman P, Witteles RM, Grogan M. Cardiac scintigraphy with Technetium-99m-labeled bone-seeking tracers for suspected amyloidosis: JACC review topic of the week. *J Am Coll Cardiol* 2020; **75**: 2851–2862.
12. Molina-Andújar A, Robles P, Cibeira MT, Montagud-Marrahi E, Guillen E, Xipell M, Blasco M, Poch E, Rosiñol L, Bladé J, Quintana LF. The renal range of the κ/λ sFLC ratio: best strategy to evaluate multiple myeloma in patients with chronic kidney disease. *BMC Nephrol* 2020; **21**: 111.
13. Merlini G, Palladini G. Light chain amyloidosis: the heart of the problem. *Haematologica* 2013; **98**: 1492–1495.
14. Pagourelias ED, Mirea O, Duchenne J, van Cleemput J, Delforge M, Bogaert J, Kuznetsova T, Voigt J-U. Echo parameters for differential diagnosis in cardiac amyloidosis: a head-to-head comparison of deformation and nondeformation parameters. *Circ Cardiovasc Imaging* 2017; **10**: e005588.
15. Zhao L, Tian Z, Fang Q. Diagnostic accuracy of cardiovascular magnetic resonance for patients with suspected cardiac amyloidosis: a systematic review and meta-analysis. *BMC Cardiovasc Disord* 2016; **16**: 129.
16. From AM, Maleszewski JJ, Rihal CS. Current status of endomyocardial biopsy. *Mayo Clin Proc* 2011; **86**: 1095–1102.