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Real-life evaluation of an algorithm for the diagnosis of cardiac amyloidosis

Short title: Diagnostic algorithm for cardiac amyloidosis

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Abstract

Objective: To evaluate the real-life use of a modified Gillmore algorithm with a "one-stop shop" approach, bone scintigraphy (BS), a monoclonal gammopathy test (GT), a salivary gland biopsy (SGB), and genetic testing performed at the same time, for the diagnosis of cardiac amyloidosis (CA) at the French National Reference Centre for Cardiac Amyloidosis (Henri Mondor Hospital, Créteil, France).

Method and Results: This retrospective cohort study included a total of 1222 patients with a suspected amyloidosis who underwent BS and GT between June 2008 and May 2019 : 349 had no cardiac uptake on BS and negative GT (BS-/GT-), 276 were BS-/GT positive (GT+), 420 patients were BS+/GT-, and 177 were BS+/GT+. Our "one-stop shop" check-up enabled us to diagnose 892 (73%) patients; 330 (27%) patients required additional examinations, such as mass spectrometry and/or a cardiac biopsy. This subset notably included 112 patients with amyloid light chain (AL) amyloidosis. Over 64% of the patients with transthyretin amyloidosis (ATTR) or another type of amyloidosis were diagnosed during the "one-stop shop" visit. Sensitivity/specificity of BS for ATTR diagnosis was 99%/96%. For AL diagnosis, they were 100%/76% for GT and 54%/100% for SGB. 205 (17%) of the 910 TTR genetic tests detected TTR mutations.

Conclusion: The results of our real-life cohort study confirmed the ability of a "one-stop shop" approach with a modified Gillmore algorithm to diagnose CA and the interest of simultaneous testing for earlier diagnosis. The SGB has diagnostic value because it is easy, quick, and less invasive than a cardiac biopsy.

Introduction

Amyloidosis is caused by misfolded proteins that form amyloid fibrils in various organs [1,2]; when the heart is affected, the condition can progress to restrictive cardiomyopathy [3,4] and ultimately death [5]. Three types of systemic amyloidosis are frequently associated with cardiac involvement: (i) amyloid light chain (AL) amyloidosis caused by excess monoclonal light chain production by a plasma cell clone; (ii) the hereditary form of transthyretin (TTR) amyloidosis (ATTRv), caused by the deposition of mutant TTR protein, and (iii) wild-type (non-hereditary) TTR amyloidosis (ATTRwt)[1,6–8].

Cardiac amyloidosis (CA) is often challenging to diagnose [9]. Furthermore, physician- and disease-related factors often lead to delay in the diagnosis of CA. Indeed, patients with the same type of amyloidosis show clinical heterogeneity, which makes early diagnosis even more difficult for clinicians. Gillmore *et al.* developed a typing algorithm for patients with suspected amyloidosis [6]. The algorithm took account of the presence of monoclonal gammopathy, cardiac uptake on bone scintigraphy (BS) [10,11], biopsy results, and TTR genetic testing. Gillmore *et al.*'s data showed that ATTR can be diagnosed by a negative monoclonal gammopathy test (GT) and positive cardiac uptake on BS [6], and that histological confirmation and amyloid typing should be performed in all cases of suspected CA not meeting these criteria.

The tools used to diagnose amyloidosis depend on the centre, i.e., organizational restrictions, availability, the time needed to obtain a result (e.g., 1 week for monoclonal GT and histology assessments, and 3 months for TTR genetic testing), and the need to reschedule appointments based on initial results. Patients with suspected CA from all over France are referred to our reference centre (Supplemental Figure 1). In order to obtain a rapid diagnosis, limit the patients' travel, and avoid delays and multiple appointments, we try to perform as many tests as possible during a "one-stop shop" visit, with BS, monoclonal GT, TTR genetic testing, and

a salivary gland biopsy (SGB). The main objective of the present study was to demonstrate the relevance of this time-sparing “one-stop shop” strategy based on Gillmore’s algorithm.

Methods

Patient referral and the diagnostic algorithm for CA

We retrospectively assessed a cohort of patients with suspected amyloidosis who were referred to the French National Referral Centre for Cardiac Amyloidosis (Henri Mondor Hospital, Creteil, France) between June 2008 and May 2019. Our centre uses a modified version of the diagnostic algorithm developed by Gillmore *et al.* (Supplemental Figure 2) [6]. Patients with suspected amyloidosis were invited for a “one-stop shop” check-up that included BS, GT, an SGB (or other biopsy, if available) and TTR genetic testing (Supplemental Figure 2).

Study population

All patients with suspected CA and having undergone BS and GT in our centre were included in the study. Suspected CA was defined as one or more signs of heart failure: a hospital admission for heart failure in the previous 12 months, treatment with a diuretic, clinical signs of heart failure (leg oedema or elevated jugular venous pressure), and diastolic dysfunction on echocardiography. Patients having undergone BS and GT at other centres or with missing data for BS and GT were excluded from the analysis (Figure 1).

The diagnostic tools included in the CA algorithm

Four diagnostic tools were included in the CA algorithm.

Firstly, cardiac uptake on BS was considered to be present if the heart/mediastinum (H/M) ratio was ≥ 1.21 (in early-phase BS) or if the Perugini score was 1, 2 or 3 (in late-phase BS) [12]. If no cardiac uptake was observed (Perugini score=0) or the H/M ratio was < 1.21 , the

patient was BS negative (BS-). 403 patients had cardiac uptake (according to the H/M ratio) during early-phase BS [12]. The Perugini score was estimated during the late-phase BS in 112 cases (16 with a score of 1, 58 with a score of 2, and 35 with a score of 3, the remaining patients had a score of 0). To facilitate the analysis, we pooled patients with cardiac uptake ($H/M \geq 1.21$) and those with a Perugini score of 1 or more.

Secondly, patients were screened for monoclonal gammopathy. A positive GT was defined as at least one abnormal kappa/lambda ratio in a serum free light chain (sFLC) assay by nephelometry N-Latex FLC Siemens assay or using Freelight assay (The Binding Site) or in an immunofixation electrophoresis (IFE) assay of a serum or urine sample [13,14]. The reference ratios were 0.31-1.56 for the N-Latex assay and 0.26-1.65 for Freelight assay [13,14].

Thirdly, non-cardiac biopsies (including the SGB) were positive if amyloid deposits were observed using Congo red and Sirius red staining, with typical “apple-green” birefringence in polarized light. Immunostaining was also used to identify the type of amyloidosis. AL was defined as positive kappa- or lambda-positive immunostaining (using anti-kappa clone A21-Y and anti-lambda clone K22-Y, Clinisciences/DB Biotech, and a Bond Leica automat). ATTR was diagnosed if the immunostained biopsy was positive for the TTR (polyclonal rabbit anti-TTR, Dako, and a Bond Leica automat) and negative for kappa or lambda antibodies. In cases of strongly suspected CA with monoclonal gammopathy but a negative SGB, a cardiac biopsy was taken during a subsequent visit. A small number of patients with ATTR described here had to undergo a cardiac biopsy because it was mandatory for inclusion in a therapeutic trial. In the event of doubt about a false positive, the sample was always typed with proteomics. Lastly, all the patients who provided their written, informed consent underwent TTR genetic testing.

Patient subgroups

In the present analysis, the patients were first divided into four groups according to their BS/GT status: BS-/GT-, BS-/GT+, BS+/GT+, and BS+/GT-. Next, the patients were classified by the type of amyloidosis or disease: (i) AL-CA, (ii) ATTRv cardiac and mixed (ATTRv patients with cardiomyopathy with or without neuropathy), (iii) ATTRwt, (iii) non-typed ATTR (including patients lacking TTR genetic test results), (iv) ATTRv with neuropathy but no cardiomyopathy, (v) non-ATTR or non-AL, and (vi) other cardiopathies.

Data collection

The study data were collected by the investigators during routine clinical practice at the first consultation and during follow-up. The recorded variables included clinical, cardiovascular, laboratory and echocardiography characteristics, disease history, cardiovascular risk factors, cardiac decompensation (hospital admission for the intravenous administration of a diuretic), heart transplantation, death, and treatment-related variables (Supplemental Table 1). The Reference Centre for Cardiac Amyloidosis' database was registered with the French National Data Protection Commission (*Commission National de l'Informatique et des Libertés*, Paris, France) and the study was approved by an independent ethics committee (authorization number: #1431858), and all study participants gave their written, informed consent.

Statistical analyses

All statistical analyses were performed using SAS software (version 9.4, SAS Institute, Inc., Cary, NC, USA). Continuous variables were described as the mean \pm standard deviation (SD) or (if not normally distributed) the median [interquartile range (IQR)], and categorical variables were described as the frequency (percentage). We used a Kaplan-Meier survival

analysis with a log-rank test to compare the different amyloidosis subgroups regarding the following outcomes: death, heart transplantation and death, or heart transplantation and cardiac decompensation. The same type of analysis was used to assess outcomes for patients with ATTR as a function of the GT results and for patients with AL as a function of the BS results.

Results

Study population

Of the 2084 individuals referred for suspected amyloidosis to the French National Reference Centre for Cardiac Amyloidosis, 1222 had undergone BS and GT at the centre and so were included in the present study. We excluded 862 patients having undergone BS or GT at other centres or with missing data for BS or GT (Figure 1).

The mean \pm SD age was 71.6 ± 12.8 , 867 (71%) of the patients were men, and 42% were NYHA class II. 22.9% of the patients had atrial fibrillation, the median [IQR] serum level of N-terminal fraction of pro-B-type natriuretic peptide (NT-proBNP) was 2403.0 pg/mL [844.0–5785.0 pg/mL] (Supplemental Figure 2 and Supplemental Table 1).

Description of the algorithm for diagnostic work-up

As described above, our diagnostic work-up was organized into a “one-stop shop” (an amyloidosis check-up with BS, GT, SGB and genetic testing) and additional tests : cardiac and other biopsies, or mass spectrometry when these biopsies were not possible or when the “one-stop shop” procedure had not provided a firm diagnosis. There were 349 BS-/GT- patients, 276 BS-/GT+ patients, 177 BS+/GT+ patients, and 420 BS+/GT- patients (Table 1). Relative to BS- patients, BS+ patients were older, more likely to be male, and had a greater

incidence of atrial fibrillation, pacemaker implantation, carpal tunnel surgery or symptoms of deafness (Supplemental Table 2).

930 SGBs were performed during the “one-stop shop” visit, 319 cardiac or non-cardiac biopsies were performed (i.e. when the “one-stop shop” procedure had not provided a firm diagnosis) and 910 TTR genetic tests were performed (Table 1).

BS had a sensitivity of 99% and a specificity of 96% for the diagnosis of ATTR. For the diagnosis of AL, GT and SGB had respectively a sensitivity of 100 and 54%, and a specificity of 76 and 100% (Supplemental Table 3).

Genetic tests were performed on 910 (74%) patients, and in 205 (17%) of them, a *TTR* mutation was identified (Table 1).

Final diagnosis

Of the 1222 patients included in the study, 934 (76%) had cardiac signs that were compatible with a diagnosis of CA. These signs included heart failure, syncope, bradyarrhythmia or suggestive echocardiogram and/or cardiac MRI findings. The proportion of patients with one or more of these signs ranged from 51.3% in the BS-/GT- group to 92.1% in the BS+/GT+ group (Table 1).

The “one-stop shop” visit (BS, GT, SGB and TTR genotyping) enabled us to diagnose 73% of our patients (Figure 1). 330 (27%) patients needed an additional test visit with cardiac or non-cardiac biopsies and/or mass spectrometry to obtain a final diagnosis. The additional tests concerned 53% of the AL patients, 35% of the ATTR_v patients, 29% of the ATTR_{wt} patients and only 6% of the patients with no amyloidosis or the presence of another cardiopathy (Figure 1).

Only 39 of the 330 patients needing an additional test were BS-/GT- concerned. Around a third (27%) of the patients needed an additional test (Table 1).

The details of the patients' final diagnosis by BS and GT status are shown in Table 2. All BS+/GT+ patients were diagnosed with CA (including 26 (15%) with AL and 114 (64%) with ATTRwt). All BS+/GT- patients had ATTR-CA. Only 14% of the 349 BS-/GT- patients were diagnosed with amyloidosis, and the great majority (83.7%) had other cardiopathies. 66.7% of the BS-/GT+ patients had AL. Regarding the type of cardiac disease, BS-/GT- status was more commonly associated with other cardiopathies (83.7%). Respectively 4.3%, 4.6% and 3.4% of the cases of amyloidosis corresponded to ATTR with neuropathy, non-ATTR and non-AL, and asymptomatic (carrier) ATTR. None of the GT-patients was diagnosed with AL.

As described in Supplemental Table 4, 910 patients were tested for TTR gene mutations. A total of 205 mutations were identified, with the greatest frequency in patients aged between 45 to 84 and especially in the 66–74 age group. Among patients over 85 years of age, less than 3% of the genetic tests were positive (Figure 2). Very few patients were aged under 45 and so this age group was not been included in the analysis. The most frequently observed mutations were ATTRV122I and ATTRV30M, especially among the BS+ patients with CA. Mutations identified in BS- patients were generally associated with ATTRv with neuropathy only or asymptomatic carrier status (Supplemental Table 4). It is noteworthy that of the 179 ATTRv patients included in our study, 114 (63.7%) were diagnosed as ATTRv at the one-stop shop visit. 67 (59%) of the 114 patients had the ATTRv Val122Ile (pVal142Ile) mutation. 18 (10.1%) were referred with a known mutation: 11 (61%) with ATTR Val30Met (pVal50Met) and 3 (17%) with ATTR Ser77Tyr (p.Ser97Tyr).

Despite the overall effectiveness of the modified Gillmore algorithm, some diagnoses were unusual regarding the medical findings (e.g., BS-/GT- patients with ATTRv). Eight patients with an atypical clinical presentation are described in Table 3.

Survival rate

The median follow-up time was 2.3 years for patients with AL or ATTRV with cardiomyopathy (with or without neuropathy), 3 years for patients with neuropathy only, 1.2 years for patients with ATTRwt, and less than 1 year for patients without ATTR or AL or with another diagnosis. In Kaplan-Meier analyses (Supplemental Figure 3), the time to death/heart transplantation or cardiac decompensation was shorter for patients with AL than for other patients with amyloidosis. The analyses also showed that the survival time for patients with ATTR did not depend on the GT result and that the survival time for patients with AL did not depend on the BS result (Supplemental Figure 4).

Discussion

The diagnostic algorithm used in the French National Reference Centre for Cardiac Amyloidosis is based on that developed by Gillmore *et al.* [6]. The diagnostic workflow implements BS, monoclonal GT, SGB, and TTR genetic testing during a single “one-stop shop” visit. If a firm diagnostic is not obtained during this first visit, additional test (including cardiac or non-cardiac biopsies and, if required, mass spectrometry) can be arranged. This workflow limits delays, reduces the number of visits, and ensures the early diagnosis of amyloidosis.

The present results show that the modified Gillmore algorithm [6] is accurate for the diagnosis of CA in our centre. We confirmed that (i) if the monoclonal GT is negative, AL can be ruled out (i.e., GT has a sensitivity of 100% in this respect), (ii) asymptomatic TTR-

mutation carriers are always BS-, and (iii) 14% of BS-/GT- patients have amyloidosis and 6% have CA. In fact, few patients presented an unexpected diagnosis regarding the various tests. The SGB has diagnostic value because it is easy, quick and less invasive than a cardiac biopsy; it helped us to diagnose 73% of our patients in a “one-stop shop” approach. Our results also highlight the value of systematic genetic testing for patients with suspected amyloidosis below the age of 85.

BS: false-negative and false-positive results

In our study, the sensitivity and specificity of BS for the diagnosis of ATTR (99% and 96%, respectively) were in line with the values presented by Gillmore et al.[6]. Despite the effectiveness of Gillmore *et al.*'s algorithm [6], some patients with ATTRv and ATTRwt had atypical, diagnostically challenging clinical presentations – as previously highlighted by Hanna *et al.* [15]. Physicians must be aware of these atypical presentations. BS has been widely used as a means of diagnosing ATTR-CA in the absence of a cardiac biopsy. However, our study demonstrated that false-negative BS results can occur in patients with ATTR-CA (one with ATTRwt and two with ATTRv (V122I and Pro113Thr)), despite the proven presence of amyloidosis in a cardiac biopsy. Similar findings have been described in patients with ATTRv Phe64Leu and Val30Met [15,16]. Therefore, the diagnosis of CA in patients with ATTRv can be challenging when they carry the mutation and have other conditions that cause an increase in LV wall thickness (e.g., hypertension, aortic stenosis, or sarcomeric cardiomyopathy). Two of our four patients without CA were carriers (BS-) and two were diagnosed with non-cardiac amyloidosis. It is difficult to identify patients with a false-positive BS because a cardiac biopsy is not performed systematically in BS+ patients who lack GT results. Thus, it is important for the cardiologist to recognize a false-positive BS prompted by recent myocardial infarction or hydroxychloroquine-induced cardiomyopathy

[17]; the latter has become more frequent since the onset of the coronavirus disease 2019 pandemic. A false-positive BS result might also indicate that bone tracer cardiac uptake can occur in AL or in rare amyloidoses like ApoA1 and AA [18]. It is therefore essential to compare the BS results with the monoclonal GT results, as proposed by Gillmore *et al.* [6]. One novel feature of the modified algorithm is its pooling of all BS+ patients. In Gillmore *et al.*'s algorithm, all patients with a score of 1 must have a biopsy to prove the amyloidosis and its type - independently of the GT result. As the diagnosis of CA improves, patients are now diagnosed at an earlier stage in the disease and so have fewer amyloid deposits and lower cardiac uptake of bone tracer. According to Gillmore *et al.*'s algorithm, these early-stage ATTR patients might need a cardiac biopsy to prove presence of ATTR-CA if a non-cardiac biopsy is inconclusive. Thus, our modified algorithm pools the BS+ patients and might avoid a cardiac biopsy in GT- patients.

Monoclonal gammopathy

Untreated AL is a rapidly progressive, fatal disease, and so early diagnosis is essential. Most cases of AL will have an abnormal result in a serum or urine IFE assays or a serum free light chain assay [19]. As reported by Gillmore *et al.* [6], AL is characterized by the presence of monoclonal protein. At present, suspected AL can be effectively ruled out on the basis of simple laboratory tests: IFE of the serum and urine, and the sFLC assay. The absence of monoclonal protein in any of the tests gives a negative predictive value of ~99% for AL [13,14]. In Gillmore *et al.*'s study at the UK National Amyloidosis Centre, n (98.9%) of the 714 patients with systemic AL had evidence of monoclonal protein [6]. Of 1465 patients studied in a collaboration between the Amyloidosis Research and Treatment Centre, Pavia, and the Mayo Clinic, 1462 (99.8%) had detectable monoclonal protein (unpublished data). In line with these results, all our patients with AL were GT+. Hence, the sensitivity and

specificity of monoclonal GT (100% and 76%, respectively) were in line with the literature data [6] on the diagnosis of AL

Biopsies

Gillmore *et al.* [6] recommended histological assessments and amyloid typing in patients with CA. In contrast, monoclonal protein was detected in a surprisingly high proportion (19%) of the patients with ATTR in Gillmore *et al.*'s cohort (probably due to referral bias, at least in part), on radionuclide scan for diagnosing cardiac ATTR; this finding highlighted the need for immunohistochemical or proteomic amyloid typing on (typically) an endomyocardial biopsy.

In our cohort, 152 patients with ATTR-CA were GT+. Systematic analysis of a SGB or another non-cardiac biopsy can identify the type of amyloidosis and determine whether it is AL or ATTR. Here, 122 patients with ATTR-CA underwent a cardiac biopsy. . BS-/GT- patients were less likely than other patients to need an additional test with cardiac or non-cardiac biopsy and (in some cases) mass spectrometry. Thus, each of the BS+/GT+, BS-/GT+ and BS+/GT- groups accounted for 30% of the patients attending the additional test.

Genetic testing

According to Gopal *et al.*, TTR gene sequencing is necessary when imaging suggests a diagnosis of ATTR and when AL has been ruled out by serum/urine testing [20]. Genetic testing can indeed confirm the diagnosis of patients with ATTR. As described by Brown *et al.* [21], genetic testing should be performed in most cases of ATTR. Accurate recognition of hereditary ATTR is important for estimating the prognosis, arranging appropriate family counselling, and guiding the choice of treatments (e.g., liver transplantation). 910 (74%) of the patients in our study underwent TTR gene sequencing (Table 1) [21]. A mutation was found in more than 16% of the patients aged between 45 and 84 but only 3% of the over-85

patients, and so did not appear to of value in this older age group. Restricting genetic testing to patients under the age of 85 might decrease the cost of this procedure and improve its cost-effectiveness.

Survival

In contrast to the report by Malka *et al.* [22], we found that the survival rate (as defined by cardiac decompensation, heart transplantation, or death) was not significantly lower among patients with monoclonal gammopathy and ATTRwt. This disparity was probably due to the small population size in Malka *et al.*'s study, which focused on non-cardiac uptake in BS. Likewise, we did not find an association between survival and BS status among patients with AL; this contrasts with the report by Zheng *et al.* [23].

Limitations

This algorithm was used in only one cohort composed by the population referred to our centre for suspected amyloidosis that limit the study in terms of external validation. This limitation must be balanced against the large size of the cohort used here. Moreover, this population was particular with a prevalence of 76% of CA suggested that the clinical presentation given the referring physicians strong clinical suspicion. A total of 862 patients with suspected CA were excluded because of missing BS or GT data or because these data were collected in another centre. 218 of the 862 excluded patients were diagnosed with amyloidosis (120 with AL , 62 with ATTRv, and 36 with ATTRwt) and so could have increased the total population size in our study. Furthermore, we used only one type of bone tracer (hydroxymethylene diphosphonate (HMDP)); this prevented us from comparing our results with those obtained using other tracers. However, we previously reported that HMDP and DPD were equally sensitive for the diagnosis of ATTR-CA [19].

Conclusion

Our present results validated Gillmore *et al.*'s findings [6] by demonstrating the ability of a modified diagnostic algorithm and a “one-stop shop” approach to diagnose CA in a real-life population attending a reference centre. A few cases of atypical amyloidosis were difficult to diagnose. The “one-stop shop” approach enabled the earlier diagnosis of CA and avoids the need for patients to travel to several appointments. To support our results, an external validation with another cohort could be interesting.

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Declaration of Helsinki: The study complied with the tenets of the Declaration of Helsinki and was approved by an independent ethics committee. All participants or their legally authorized representatives gave their written, informed consent.

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Table legends

Table 1: The tests performed as part of our “one-stop shop” approach.

Table 2: Final diagnosis, according to the modified algorithm

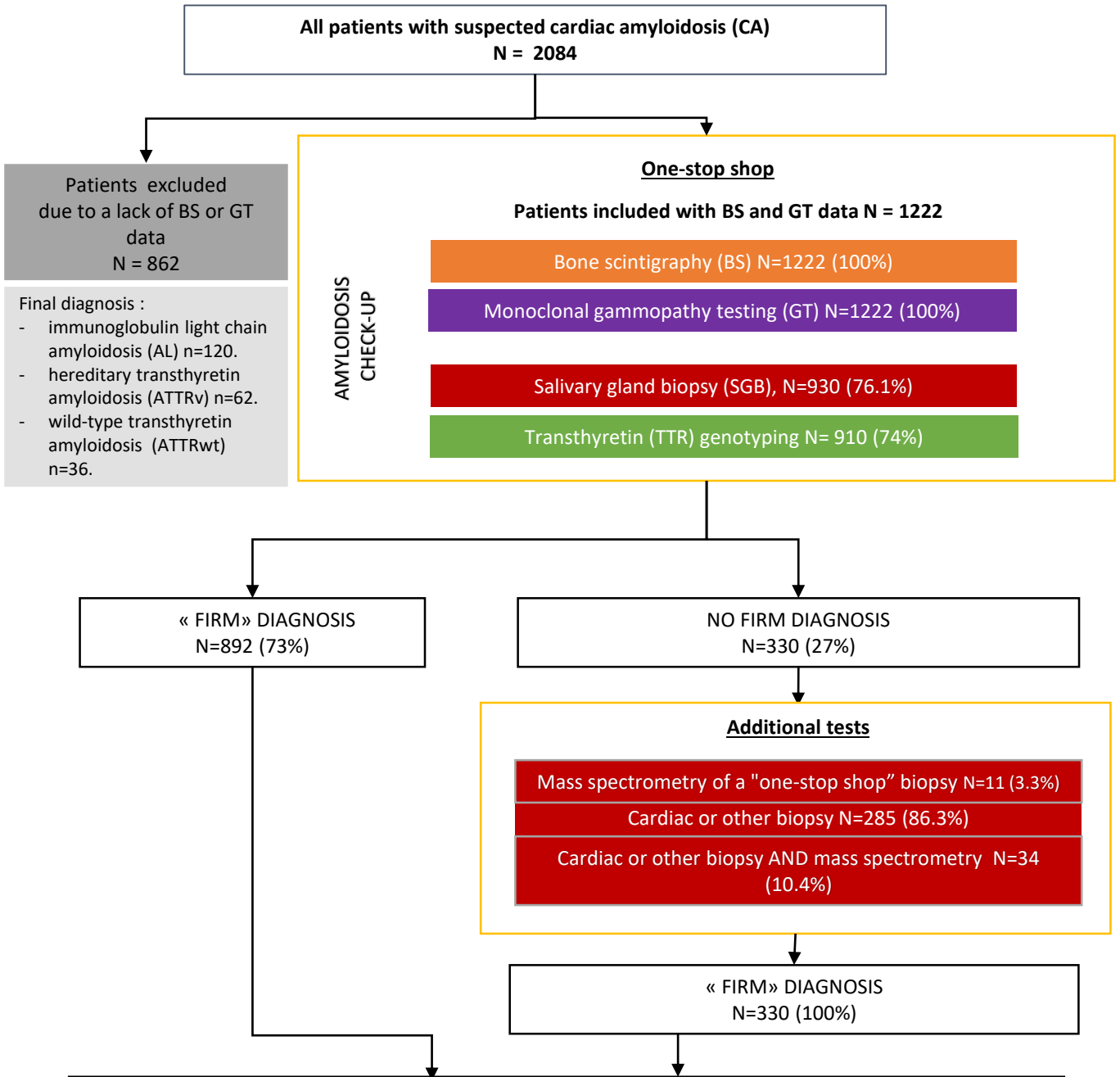
Table 3: Baseline characteristics of patients with an atypical clinical presentation

Figure legends

Figure 1: Study flow chart

Figure 2: The number of TTR genetic tests and the proportions of mutations identified by age.

Figure 1: Study flow chart



	Patients diagnosed after the "one-stop shop"	Patients diagnosed after additional tests	All diagnoses
AL	98 (46.7%)	112 (53.3%)	210 (100%)
ATTRv	106 (64.6%)	58 (35.4%)	164 (100%)
ATTRwt	286 (71.1%)	116 (28.8%)	402 (100%)
Untyped ATTR and other types of amyloidosis	38 (64.4%)	21 (35.6%)	59 (100%)
No amyloidosis or another cardiopathy	364 (94%)	23 (6%)	387 (100%)

Figure 2: The number of TTR genetic tests performed and the proportions of mutations identified by age.

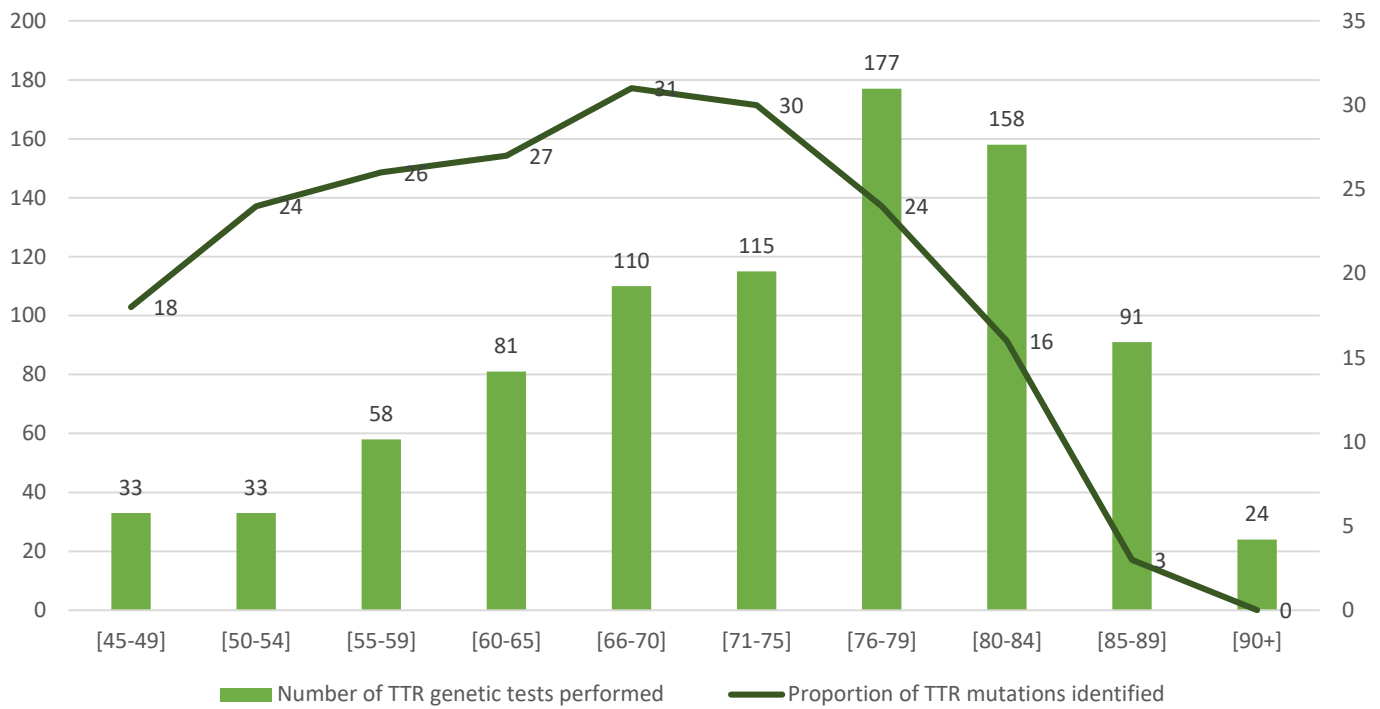


Table 1: The tests performed as part of our “one-stop shop” approach.

Cardiac uptake on bone scintigraphy	+/-	-	-	+	+
Gammopathy	+/-	-	+	+	-
N	1222	349	276	177	420
Cardiac phenotype					
Cardiac morphology compatible with CA ^{ab} , n (%)	934 (76)	179 (51)	209 (76)	163 (92)	383 (91)
unspecified, n (%)	96 (8)	47 (13)	21 (8)	8 (4)	20 (5)
CA defined by IVST, LVPWED and NT-proBNP, n (%)	809 (66)	126 (36)	178 (64)	151 (85)	354 (84)
unspecified, n (%)	199 (16)	93 (27)	46 (17)	18 (10)	42 (10)
CA with positive LGE on MRI ^c , n (%)	276 (23)	29 (8)	69 (25)	52 (29)	126 (30)
unspecified, n (%)	874 (71)	286 (82)	189 (68)	118 (67)	281 (67)
CA defined by thickened wall on MRI, n (%)	400 (33)	84 (24)	95 (34)	60 (34)	161 (38)
unspecified, n (%)	695 (57)	205 (59)	138 (50)	108 (61)	244 (58)
“One-stop shop” diagnosis n(%)	892 (73)	310 (89)	173 (63)	84 (47)	325 (77)
Gammopathy tests performed					
Serum electrophoresis, n(%)	1205 (99)	323 (93)	265 (96)	173 (98)	411 (98)
Serum immunofixation assay, n(%)	1172 (96)	323 (93)	265 (96)	173 (98)	411 (98)
Dosage of light chains performed n(%)	908 (74)	181 (52)	197 (71)	152 (86)	378 (90)
Using N-Latex FLC (Siemens), n(%) ^d	880 (97)	177 (98)	186 (94)	142 (93)	375 (99)
Urine electrophoresis performed, n(%)	966 (79)	233 (67)	231 (84)	161 (91)	341 (81)
Biopsies performed					
Salivary biopsy at the “one-stop shop”, n(%)	930 (76)	199 (57)	220 (80)	146 (82)	365 (87)
Genetic tests performed					
TTR genetic test, n(%)	910 (74)	220 (63)	148 (54)	151 (85)	391 (93)
Pathogenic TTR mutation identified, n(%)	205 (17)	36 (10)	7 (2)	35 (20)	127 (30)
Diagnosis with additional tests n(%)	330 (27)	39 (11)	103 (37)	93 (52)	95 (23)
Biopsies performed					
Cardiac biopsies, n(%)	215 (65)	17 (44)	68 (66)	77 (83)	53 (56)
Biopsies from other sites n(%)	166 (50)	26 (67)	65 (63)	28 (30)	47 (50)

^a Abbreviations: CA: cardiac amyloidosis; IVST: interventricular septal wall thickness; LGE: late gadolinium enhancement; LVPWED: left ventricular posterior wall at end-diastole; MRI: magnetic resonance imaging

^b defined as: an increase in left ventricular wall thickness of >12 mm, alteration in overall strain with an apical sparing pattern and preserved LVEF with a serum NT-proBNP level >332ng/L or a serum BNP level >81ng/L or late gadolinium enhancement or increase in T1 mapping at the cardiac MRI.

^c 877 patients underwent cardiac MRI.

^d Serum and FLC concentrations were measured in 97% by nephelometry using N-Latex FLC (Siemens) on a BN-ProSpec from Siemens

Table 2: Final diagnosis^a, according to the modified algorithm

Cardiac uptake on bone scintigraphy	+/-	-	-	+	+
Gammopathy testing	+/-	-	+	+	-
N	1222	349	276	177	420
Amyloidosis, n (%)	835 (68)	49 (14)	189 (68)	177 (100)*	420 (100) ^b
Other cardiopathy, n (%)	362 (30)	292 (84)	70 (25)	0	0
Other diagnosis, n (%)	25 (2)	8 (2)	17 (6)	0	0
Cardiac amyloidosis, n (%)	803 (66)	21 (6)	185 (67)	177 (100)*	420 (100)*
AL, n (%)	210 (17)	0	184 (66.7)	26 (15)	0
ATTRwt, n (%)	402 (33)	0	1 (0.4)	114 (64)	287 (68)
ATTR untyped, n (%)	12 (1)	3 (0.9)	0	3 (2)	6 (1)
ATTRv with cardiomyopathy, n (%)	164 (13)	3 (1)	0	34 (19)	127 (30)
ATTRv with neuropathy only, n (%)	15 (1)	15 (4)	0	0	0
Non-ATTR and non-AL amyloidosis, n(%)	19 (2)	16 (5)	3 (1)	0	0
ATTR asymptomatic (carrier), n (%)	13 (1)	12 (3)	1 (0.4)	0	0


^a By definition as cardiac fixation at the scintigraphy defined the diagnostic of cardiac amyloidosis. None of the patients was considered to be a false negative.

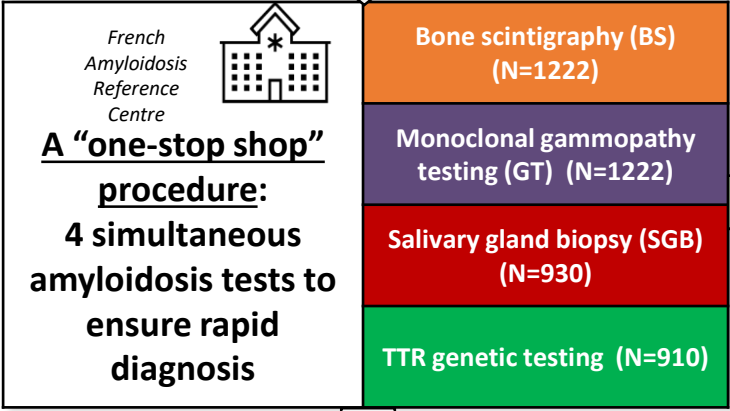
^b Positive definition of bone scintigraphy was considered as cardiac amyloidosis in the absence of other diagnostic (Acute Myocardial infarction, Hydroxylchloroquine treatment).

Table 3: Baseline characteristics of patients with an atypical clinical presentation

Reason for selection	ATTR-CA with BS ^a -	ATTR-CA with BS-	ATTR-CA with BS-	ATTR mutation with LVH and without CA	ATTR mutation with LVH and without CA	ATTR mutation with LVH and without CA	ATTR mutation with LVH and without CA	Non-typable amyloidosis
Age, years	81	66	78	81	38	51	49	89
Sex	W	M	M	W	M	M	W	M
NYHA Class	II	III	III	I	I	I	NA	III
Serum NT-proBNP, ng/L	416	313	2469	114	983	444	NA	13903
Serum troponin T HS, ng/L	20	33	27	NA	34	9	NA	178
LVEF, %	72	45	53	59	32	42	62	20
SGL, %	19.6	NA	14.7	24.0	9.4	13.0	18.7	NA
IVST, mm	12	11	14	10	25	16	15	13
MRI-LGE (CA)	NA	NA	+	-	-	-	+	+
BS	-	-	-	-	-	-	-	+
GT	-	-	+	-	-	-	-	+
Diagnosis of amyloidosis	ATTRv	ATTRv	ATTRwt	ATTRv	Carrier	Carrier	ATTRv	Amyloidosis untyped
ATTR-CA	Yes	Yes	Yes	No	No	No	Yes	Yes
ATTR mutation	V122I	P113T	-	V122I	V122I	V122I	V30M	-
SGB	CR-	CR+ TTR+	CR+. TTR +++	CR+ TTR +	NA	NA	CR+ other hospital	CR + TTR -
SGB mass spectrometry analysis	NA	ATTR	ATTR	NA	NA	NA	NA	NA
Heart biopsy	CR+. TTR+++.	CR+. TTR+++	CR+. TTR +++	Refused	CR -	NA	NA	Refused
Heart proteomic analysis	NA	ATTR	ATTR	NA	NA	NA	NA	NA
One-stop shop or additional tests before diagnosis	Additional tests	Additional tests	Additional tests	One-stop shop	Additional tests	One-stop shop	One-stop shop	One-stop shop
Final Diagnosis	ATTRv	Heart transplant in 2012. Recurrence of amyloidosis in the transplanted heart. ATTRv	ATTRwt	Aortic stenosis and amyloidosis: ATTRv	Sarcomeric hypertrophic cardiomyopathy + TTR mutation carrier	Hypertensive cardiopathy + TTR mutation carrier	ATTRv neurologic with other cardiomyopathy	The family refused a cardiac biopsy due to the patient's poor overall condition. Considered as ATTRwt

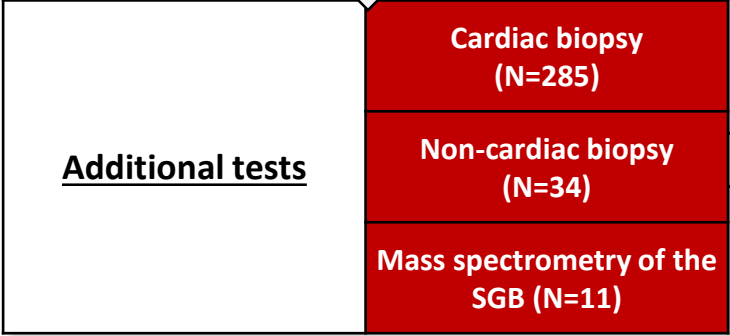
^a Abbreviations: BS: bone scintigraphy; CA: cardiac amyloidosis; CR: Congo Red; GT: Gammopathy testing; IVST: interventricular septal wall thickness; LVEF: left ventricular ejection fraction ; MRI LGE: cardiac magnetic resonance imaging with positive late gadolinium enhancement ; NYHA Class: New York Heart Association class; SGB: salivary gland biopsy.

 1222 patients with suspected cardiac amyloidosis (CA)



Firm diagnoses: N= 892 (73%)	N(%)
AL	98 (46.7%)
ATTRv	106 (64.6%)
ATTRwt	286 (71.1%)
Nontyped ATTR and other types of amyloidosis	38 (64.4%)
Another cardiopathy	364 (94%)

No firm diagnosis: N= 330 (27%)



Firm diagnoses: N = 330 (27%)	N(%)
AL	112 (53.3%)
ATTRv	58 (35.4%)
ATTRwt	116 (28.8%)
ATTR untyped and other type of amyloidosis	21 (35.6%)
Another cardiopathy	23 (6%)

Cardiac amyloidosis is diagnosed:
 - in 8 days with the "one-stop shop" procedure only
 - In 16 days with additional tests, if a new biopsy is performed

Time scale

- One day
- One week for the GT and SGB biopsy results
- One week needed to schedule a new biopsy
- One day if a new biopsy is performed
- One week for the new biopsy results

A simplified procedure for the diagnosis of CA: "One-stop shop" (in one day)

