AL amyloidosis

*Study of epidemiology, diagnosis and treatment with
emphasis on heart involvement*

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Abstract
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AL (immunoglobulin light chain) amyloidosis is often associated with delayed diagnosis and thereby high early mortality that is not overcome by contemporary treatment. There is a need for diagnostic methods promoting earlier diagnosis, especially in patients with cardiac involvement. Progress has been made in the treatment of AL amyloidosis and prolonged survival has been reported from specialized referral centers. However, population-based reports are scarce regarding epidemiology as well as treatment outcomes. Aims of this thesis were to increase the knowledge of the epidemiology of AL amyloidosis, investigate new imaging methods for early diagnosis and prognostication in cardiac amyloidosis (CA), and evaluate treatment options with focus on patients with cardiac involvement.

In paper I we presented real-world long-term results of treatment with high dose chemotherapy for AL amyloidosis in Sweden. We could conclude that long overall survival (median 8.2 years, 95% CI 5.1-11.2) was reached with high dose chemotherapy, but with inferior outcomes in patients with cardiac involvement. Treatment related mortality was comparable to that reported from larger centers during this period and was decreasing from 23.8% to 7.8% during the studied time period.

In paper II we studied the accuracy of PET with the amyloid binding tracer ^{11}C-PIB for the diagnosis of CA. ^{11}C-PIB PET showed high accuracy in detecting CA, and affinity was higher for AL compared to transthyretin amyloidosis. We concluded that ^{11}C-PIB PET can be a useful method to rule in or out amyloidosis in patients with unexplained diastolic heart failure. Our results also indicated that ^{11}C-PIB PET can detect CA at an earlier stage than echocardiography and might be a useful tool for early diagnosis.

In paper III we studied the prognostic value of cardiac function parameters from ^{11}C-acetate PET in CA. We found that reduced myocardial external efficiency was associated with inferior survival in CA patients. However, the strongest prognostic parameter was lowered ratio of forward stroke volume and left ventricular mass, which was the only independently prognostic parameter in multivariable analysis.

Paper IV was a population-based epidemiological study in which we could determine the standardized incidence of systemic AL amyloidosis to 12.0 (95% CI 9.3-14.7) per million person-years for Uppsala County, without significant change during the period 2000-2020. The 5-year limited duration prevalence increased numerically, but without statistical significance. Prolonged overall survival was observed over time, and there was also a decrease in early mortality, indicating earlier diagnosis of especially patients with cardiac involvement.

Keywords: immunoglobulin light chain (AL) amyloidosis, high-dose melphalan, cardiac amyloidosis, positron emission tomography (PET), incidence, population-based

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The only true wisdom is in knowing you know nothing.
Socrates

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Rosengren, S**., Mellqvist, U-H., Nahi, H., Forsberg, K., Lenhoff, S., Strömberg, O., Ahlberg, L., Linder, O., Carlson, K. (2016) Outcome of AL amyloidosis after high-dose melphalan and autologous stem cell transplantation in Sweden, long-term results from all patients treated in 1994-2009. *Bone Marrow Transplantation,* 51(12):1569-1572.

II. **Rosengren, S**.*, Skibsted Clemmensen, T.*, Tolbod, L., Granstam, S-O., Eiskjaer, H., Wikström, G., Vedin, O., Kero, T., Lubberink, M., Harms, H. J., Flachskampf, F. A., Baron, T., Carlson, K., Mikkelsen, F., Antoni, G., Frost Andersen, N., Hvitfeldt Poulsen, S., Sörensen, J. (2020) Diagnostic accuracy of ^{11}C-PIB positron emission tomography for detection of cardiac amyloidosis. *JACC Cardiovascular Imaging,* 13(6): 1337-1347. * Equal first authors.

III. **Rosengren, S**.*, Skibsted Clemmensen, T.*, Hvitfeldt Poulsen, S., Tolbod, L., Harms, H. J., Wikström, G., Kero, T., Thyrsted Ladefoged, B., Sörensen, J. (2023) Outcome prediction by myocardial external efficiency from ^{11}C-acetate positron emission tomography in cardiac amyloidosis. *ESC Heart Failure,* 11(1): 44-53. * Equal first authors.

IV. **Rosengren, S**., Thelander, U., Mattsson, M., Carlson K. Population-based incidence, prevalence and survival of systemic AL amyloidosis in Sweden. *Manuscript.*

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Contents

Abbreviations

AL	amyloid light chain
ATTR	amyloid transthyretin
ATP	adenosine triphosphate
AA	amyloid serum A
ASCT	autologous stem cell transplantation
AUC	area under the curve
BCL2	B-cell lymphoma 2
BMPC	bone marrow plasma cells
CA	cardiac amyloidosis
CI	confidence interval
CMR	cardiac magnetic resonance imaging
CO	cardiac output
CR	complete response
CRAB	hypercalcemia, renal dysfunction, anaemia, bone disease
CT	computer tomography
dFLC	difference between involved and uninvolved free light chain
iFLC	involved free light chain
ECG	electrocardiogram
ECOG	eastern cooperative oncology group
ECV	extra cellular volume
EW	external work
FISH	fluorescence in situ hybridization
FLC	free light chain
FSV	forward stroke volume
FSVI	forward stroke volume index
HDM	high dose melphalan
ICD	international classification of diseases
IGVL	immunoglobulin light chain variable region
IMiD	immunomodulatory drug
IMWG	International Myeloma Working Group
IQR	interquartile range
IVS	interventricular septum
LGE	late gadolinium enhancement
LS	longitudinal strain
LV	left ventricle

LVM	left ventricular mass
EF	ejection fraction
MAPK	mitogen-activated protein kinase
MBF	myocardial blood flow
MCF	myocardial contraction fraction
MEE	myocardial external efficiency
MGUS	monoclonal gammopathy of undetermined significance
MRD	measurable residual disease
MVO_2	myocardial oxygen consumption
Nt-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
OS	overall survival
PET	positron emission tomography
PFS	progression free survival
PIB	Pittsburgh compound B
PR	partial response
RI	retention index
ROC	receiver operating curve
RV	right ventricle
SAP	serum amyloid P
SUVR	standardized uptake value ratio
SVI	stroke volume index
TAPSE	tricuspid annular plane systolic excursion
TRM	treatment related mortality
T1	longitudinal relaxation time
T2	transverse relaxation time
VCd	bortezomib, cyclophosphamide, dexamethasone
VGPR	very good partial response

Introduction

Systemic immunoglobulin light chain (AL) amyloidosis is a rare disease caused by a, usually small, bone marrow plasma cell clone which produces free light chains with the ability to aggregate and form amyloid plaques. Amyloid deposition is systemic and can affect various organs such as the heart, kidney, liver, gastrointestinal tract, peripheral and autonomous nervous system. Patients can present with a wide range of symptoms depending on the type of organ involvement, these include oedema, shortness of breath, gastrointestinal symptoms, weight loss, skin changes, peripheral neuropathy and orthostatic hypotension. Symptoms are unspecific for amyloidosis, and more typical signs such as macroglossia, periorbital purpura and bilateral carpal tunnel syndrome are in fact relatively uncommon. Many clinical findings such as increased cardiac wall thickness, proteinuria, hepatomegaly and neuropathy can have various other causes. This requires for the physician to actively think of amyloidosis as a differential diagnosis in order to proceed with more directed investigations. Historically, the awareness of the diagnosis has been low, and the general perception was that of an untreatable disease with no chance of long-term survival for the patient. Furthermore, the lack of reliable methods for amyloid typing caused uncertainty regarding classification of the disease and the possibility of treatment.

Early diagnosis of AL amyloidosis is one of the most important factors for survival. Delay in diagnosis causes organ failure to progress, which limits treatment possibilities and the chance of organ recovery after treatment. With higher awareness of the disease and improved diagnostic methods, earlier diagnosis has been promoted. In combination with more effective treatments, the prognosis of patients is improving. Progress is also seen in ATTR (transthyretin amyloidosis), the other major amyloidosis subtype involving the heart, with the introduction of new treatments where there previously were no treatment possibilities. This has led to an increased awareness of amyloidosis among cardiologists and to more patients with heart symptoms being evaluated for amyloidosis in general, which likely has had a positive impact on the diagnosis of AL patients as well.

However, in a survey of 533 amyloidosis patients from 2015, 37.1% of patients reported that the diagnosis was delayed more than a year after the initial symptoms (1). And in a recently published European observational study, time from symptoms to the diagnosis of AL amyloidosis had not

improved significantly for patients starting treatment after compared to before 2010 (2). Early mortality in AL amyloidosis is high with approximately ¼ of patients dying within the first 6 months after diagnosis (3). A reduction in early mortality in the later years has been reported from highly specialized centres (3, 4) but not from more real-world conditions (2), suggesting that patients remain to be diagnosed in advanced disease stages. Hence, there is still an unmet need for earlier diagnosis, especially when it comes to cardiac AL amyloidosis, and improved treatment strategies for patients diagnosed with advanced disease.

This thesis sought to increase the knowledge of the epidemiology of AL amyloidosis, investigate new imaging methods for early diagnosis and prognostication in cardiac amyloidosis, and evaluate treatment options with focus on patients with cardiac involvement.

Background

Amyloid formation

There are more than 40 different proteins identified, with the ability of amyloid fibril formation (5), which is characterized by a cross-beta-sheet structure, and fluorescence when stained with Congo red (6). The origin of the amyloid forming proteins, as well as the organ affinity, varies widely. The pattern of distribution of amyloid deposits also differs between amyloidosis subtypes. In AL amyloidosis deposition is usually diffuse extracellular and arterial, as opposed to the nodular deposition more often seen in ATTR amyloidosis (7). Therapy can be directed against several stages in the amyloid formation process and differs markedly depending on the type of amyloidosis.

The origin of AL amyloidosis is a plasma cell clone producing amyloidogenic monoclonal immunoglobulin light chains. The plasma cell clone is usually small and indolent, although AL amyloidosis can also be associated with malignant disease (mainly myeloma or lymphoma) (8). When analysed with whole exome sequencing the plasma cells in AL amyloidosis do not present most of the driver mutations defined in myeloma, and generally display fewer mutations, but with an increase correlated with higher plasma cell count (9, 10). Studies have not been able to identify a common set of mutations for AL amyloidosis, instead there seems to be a greater clonal heterogeneity compared to myeloma.

Mutations in the variable domain of the light chain (IGVL) genes are frequent and can cause destabilization of the domain making the light chains prone to aggregation. Mutational hotspots are more frequent in lambda compared to kappa light chain genes (11). Specific IGVL gene mutations have been associated with systemic compared to localized AL amyloidosis, as well as with type and grade of organ involvement (12). For example, the mutation LV1-44 has been associated with heart, LV6-57 with kidney and KV1-33 with liver involvement.

Aggregation is partially dependent on the level of the precursor protein. But in some patients, low FLC levels can still be associated with advanced stage amyloidosis. It has been shown that in these patients, the location of the IGVL gene mutations, rather than the amount of the precursor protein determine the propensity for amyloid formation (13). Cryogenic electron microscopy studies have been able to localize mutational changes as well as post-

translational modifications such as disulphide bond and N-glycosylation in the fibril protein, and connect these to fibril morphology (14).

When analysing gene expression, there are differences between plasma cells in AL amyloidosis compared to plasma cells in myeloma or MGUS (monoclonal gammopathy of undetermined significance). Genes overexpressed in AL amyloidosis are related to for example chromatin organization and N-linked protein glycosylation (15). It has been shown that N-glycosylation of light chains is increased in AL amyloidosis (especially of kappa subtype) compared to other plasma cell diseases, and associated with the development of AL amyloidosis from MGUS (16). The N-glycosylation shows a pattern that differs from what is seen in non-amyloidogenic clonal light chains (17), suggesting that it plays a pathogenic role at least in a subset of AL patients.

In fibril formation, the amyloidogenic immunoglobulin light chains first aggregate into soluble oligomers/protofibrils, which then misfold and aggregate further into the cross-beta-sheet structure of amyloid fibrils (6). Interactions with the extracellular environment may cause proteolytic cleavage and binding of components such as glycosaminoglycans and collagen that facilitate aggregation (11). In vitro, factors such as high temperature and extreme pH values have been shown to destabilize proteins facilitating amyloid formation. The glycoprotein serum amyloid P (SAP) binds to amyloid fibrils independent of the origin protein, stabilizing the amyloid fibrils and preventing degradation. Apart from the type of IGVL gene mutations, the organ affinity can also be influenced by local conditions such as pH, presence of salts and interaction with various tissue constituents (18).

All amyloid fibrils have the ability to self-propagate through recruitment of more precursor proteins from the surroundings, referred to as "seeding"(11). This process proceeds slowly in the beginning and accelerates as the number of amyloid fibrils increases. If the amyloid burden is reduced, the self-replication rate decreases.

Epidemiology

AL amyloidosis was previously referred to as the most common type of systemic amyloidosis, and studies (19-21) have reported a higher incidence for AL than for ATTR and AA amyloidosis. However, a population-based autopsy study from Finland (22) showed that wild-type ATTR was present in the myocardium of 25% of subjects from 85 years of age. Furthermore, when screening patients with heart failure and preserved ejection fraction with bone scintigraphy, 13-18% has been found to have wild-type ATTR amyloidosis (23, 24), and 16% screened positive among patients undergoing aortic valve replacement (25). Thus, the incidence of wild-type ATTR has likely been underestimated and is probably higher than for AL amyloidosis.

The first published incidence number of AL amyloidosis is that from Kyle et al (19) who reported on the incidence of systemic AL amyloidosis in Olmsted County, Minnesota between 1950 and 1989. For residents in this region, almost all medical diagnoses were made at the Mayo Clinic and one more medical institute and were recorded in a centralized registry. Review of patient records with amyloidosis diagnosis in this registry, as well as autopsy reports resulted in 21 patients that fulfilled criteria set out for AL amyloidosis (typing of biopsy or autopsy material, or the presence of amyloid plus a monoclonal protein). The age- and sex-adjusted incidence was calculated to 8.9 (95% CI 5.1-12.8) per million person-years. The same authors published a similar incidence study from the same region during the time period 1990 to 2015 (26). 35 patients with AL amyloidosis were identified, and the age- and sex-adjusted incidence was calculated to 12 (95% CI 8-16) per million person-years. No significant change in the incidence was observed during the studied time period. When including the incidence reported in the previous study, there was a trend towards an increasing incidence, but without statistical significance. In the two studies, the reported median age at diagnosis of AL amyloidosis was 73.5 and 76 years respectively, and there was a male dominance with 62 and 54% males.

One recently published incidence report from Taiwan used their National Health Insurance Database, described as a population-based claims database, and identified all patients who received the international classification of diseases (ICD-10) code of amyloidosis (E85.4 "organ limited amyloidosis", E85.8 "other amyloidosis" or E85.9 "amyloidosis unspecified") and had an adjacent record of biopsy taken from a site possibly involved by AL amyloidosis (27). 841 cases were identified and the age-adjusted incidence was calculated to 5.26-6.55 cases per million person-years. A study from Sweden used similar methodology based on diagnose codes from the National Patient Register (28). The same ICD-10 codes as in the Taiwanese study were used, and inclusion also demanded one of the following 1) prescription of an anti-plasma cell therapy, 2) diagnosis made on a haematology or oncology clinic or 3) the specific code for AL amyloidosis E85.8A was used. The study identified 846 cases, and the age-adjusted incidence ranged from 5.4 to 6.8 per million person-years during the studied time period, with significant increase over time. However, in both of these studies, cases were not confirmed as having AL amyloidosis, and especially since there previously was no specific diagnose code for AL amyloidosis in the ICD system, there is a risk of misclassification. Other epidemiological studies in AL amyloidosis are not population-based or present merely an estimation of the incidence (20, 21, 29, 30).

Little is known about the incidence of AL amyloidosis in different ethnicities. In other plasma cell dyscrasias such as MGUS and myeloma, there are marked variations between ethnicities, with an increased incidence in African Americans and Hispanics, and a lowered incidence in Asians and Pacific Islanders compared to Whites (31, 32). In the incidence studies from Olmstead

County, almost all cases were Whites, which represented the composition of the studied population. The incidence number from the Taiwanese study was lower, which could reflect differences between ethnicities. In a report from Boston amyloidosis centre, ethnic minorities with AL amyloidosis were underrepresented, compared to what would be expected from the general population, especially the presence of Hispanics were unexpectedly low (33). However, this could also be influenced by disparities when it comes to referral to a specialized amyloidosis centre.

There are few studies of the prevalence of AL amyloidosis. One study based on cases from United States insurance claims data (not truly population-based) found an increase in the standardized prevalence from 20.1 cases per million in 2007 to 50.1 per million in 2015 (30). The previously described Swedish study (28) reported a significant increase in 5-year limited duration prevalence from 32 per million in 2011 to 47 per million in 2019. In a study of the global epidemiology of AL amyloidosis (34), estimations of the prevalence were performed based on previously published incidence and survival data. An increase in the estimated prevalence was found in all of the analysed countries. The estimated 5-year prevalence in 2018 for Sweden was 32.3 cases per million and 20-year prevalence was estimated to 56.7 per million.

Diagnosis

The presence of a monoclonal protein together with unexplained cardiac hypertrophy, proteinuria, hepatomegaly, peripheral neuropathy or hypotension should lead to the suspicion of AL amyloidosis. In around 90% of the cases, amyloid can be found in abdominal fat or bone marrow aspirate/biopsy (35). In cases where both fat and bone marrow are amyloid negative, and there is still a suspicion of AL amyloidosis, biopsy should be performed from the affected organ. Bone marrow sample is also indicated to evaluate the size of the plasma cell clone, which is a prognostic factor in AL amyloidosis (36). FISH (fluorescence in situ hybridization) analysis of bone marrow plasma cells can also provide prognostic information, which is described further on.

The beta-pleated-sheet configuration of amyloid causes the characteristic positive Congo red staining when viewed under polarized light, which is a requirement of amyloidosis diagnosis. According to the IMWG (International Myeloma Working Group) criteria (37), the diagnosis of AL amyloidosis requires all of the following:

1. Prescence of amyloid related systemic disease (cardiac, renal, hepatic, gastrointestinal or nervous system involvement)
2. Positive Congo red staining in any tissue
3. Evidence that the amyloid is light chain related
4. Evidence of a monoclonal plasma cell proliferative disorder (monoclonal protein in blood or urine, abnormal FLC ratio or clonal plasma cells in the bone marrow)

Accurate classification of the amyloid is crucial. Presence of a monoclonal protein together with unclassified amyloid in biopsy material is not enough, since MGUS is present in about 20% of patients with wild-type ATTR amyloidosis (38). Typing of the amyloid can be performed by immunohistochemistry, mass spectroscopy, immune-electron microscopy, western-blot or a combination of methods (39). In a study of 117 amyloidosis patients, immunohistochemistry performed by specialized pathologists was able to classify the amyloid in 94% of cases (40). Mass spectroscopy has high accuracy in detecting and classifying amyloidosis (41), the method also has the ability to detect peptide sequences from the mutated IGVL genes, thereby revealing the pathogenesis of the disease (42). Immuno-electron microscopy is used at some centres and can also characterize amyloid with high accuracy (43). The specialized amyloid laboratory in Uppsala uses western-blot in combination with immunohistochemistry, and if needed mass spectroscopy for amyloid typing. 84% of their samples could be typed with western-blot and immunohistochemistry, and the majority of cases not typed with this method were due to minimal amyloid deposits usually seen in ATTR (44).
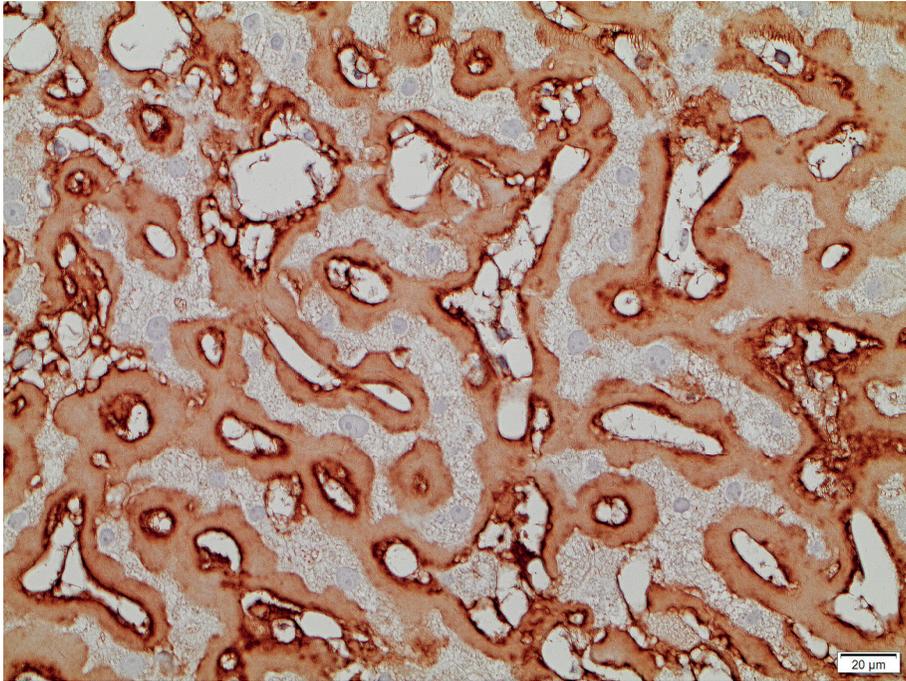
Figure 1. Liver biopsy with AL kappa amyloidosis. Pronounced amyloid deposition (in brown) is demonstrated with immunohistochemistry and a monoclonal antibody against AL kappa. Courtesy of Professor Per Westermark.

In patients with clinical suspicion of amyloidosis, serum and urine immunofixation together with serum FLC measurement can be used as a screening method for AL. In a study of 121 AL amyloidosis cases, the serum FLC ratio was pathologic in 76% of the cases, whereas serum and urine immunofixation together with FLC ratio captured 100% of AL cases (45).

Except from evaluation of the plasma cell clone, investigations are performed to evaluate the extent of organ involvement. Because of the increased risk of bleeding in amyloidosis patients, biopsies to determine organ involvement generally should be avoided, and non-invasive methods are recommended. The original consensus criteria published 2005 from the 10th International Symposium of Amyloidosis were established to define organ involvement (46). When assessing heart involvement, measurement of cardiac biomarkers (Nt-proBNP and troponins), as well as ECG (electrocardiogram) and echocardiography are indicated. Cardiac magnetic resonance imaging (CMR) or positron emission tomography (PET) with amyloid binding tracers can also be used in the evaluation of suspected amyloidosis. Elevated Nt-proBNP or troponins without other possible causes as well as typical imaging features on CMR or PET have been included as criteria for cardiac involvement in a newer consensus document (47).

Table 1. Criteria for organ involvement.

Organ involvement	Criterion (46, 47)
Heart	Mean wall thickness > 12 mm on echocardiography or Elevated Nt-proBNP or troponin without other cause or Typical features on CMR or amyloid specific PET
Kidney	24h-urine albumin > 0.5 g
Liver	Liver span > 15 cm or Alkaline phosphatase > 1.5 times the upper limit of normal
Gastrointestinal	Biopsy verification with symptoms
Nervous system	Symmetric sensory or motor peripheral neuropathy or Autonomous neuropathy
Lungs	Biopsy verification with symptoms Interstitial radiologic abnormalities

Cardiac imaging

ECG changes are common including low voltage (46%), pseudo infarct pattern (47%), criteria for left ventricular hypertrophy (16%) and atrial fibrillation (10%) (48). However, low voltage is reported to be a late finding in AL cardiac amyloidosis and is not useful for early detection of the disease (49).

Echocardiography is still the basis of cardiac assessment in amyloidosis. According to the original definitions of organ involvement (46), cardiac AL amyloidosis is considered evident if amyloid is detected and typed on any localization, and echocardiography shows a left ventricular wall thickness > 12 mm. Except from increased cardiac wall thickness, echocardiography typically shows diastolic dysfunction and in around half of the patients a "granular sparkling" pattern of the myocardium (50). Decreased ejection fraction is usually only seen in late stages of cardiac amyloidosis. Tissue doppler and speckle tracking imaging provides quantification of the motion and deformation of regional myocardium, and allows for measurement of longitudinal strain. Typically, in cardiac amyloidosis, deformation is reduced in basal and middle segments, while preserved in apical segments until late stages (51).

Endomyocardial biopsy is considered the gold standard for diagnosis of cardiac amyloidosis. However, the method is invasive and not considered indicated if amyloid is detected and classified elsewhere. A complication rate of up to 6% has been reported, involving for example arrhythmia, pneumothorax, focal neurological complications and perforation (52).

Except from echocardiography, CMR is a commonly used imaging method in cardiac amyloidosis. Traditionally, gadolinium contrast has been used showing late gadolinium enhancement with a distinct pattern from subendocardial to transmural as the disease progresses. More recently non-contrast CMR with T1 (longitudinal relaxation time) mapping has showed high accuracy in detecting cardiac amyloidosis (53, 54). Measurement of extracellular volume (ECV) with CMR provides quantification of the amyloid containing extracellular space; however, this method requires the administration of contrast agent. Compared to T1 mapping, ECV gives a more specific measurement of amyloid burden, as opposed to T1 mapping that is based on the signal from both the myocytes and the extracellular space (54).

Several PET-agents have been investigated in cardiac amyloidosis, the most studied are ^{11}C-PIB (Pittsburgh compound B), ^{18}F-florbetapir and ^{18}F-florbetaben, all of which have shown increased tracer-uptake in both AL and ATTR cardiac amyloidosis (55-57). These compounds are thioflavin-T derivates that binds to the beta-pleated structure of amyloid fibrils of any type, but with higher affinity for AL than for ATTR amyloidosis (58). ^{11}C-PIB was first developed to image beta-amyloid in Alzheimer's disease (59). Later several small studies have shown high accuracy of ^{11}C-PIB PET in detecting cardiac amyloidosis (55, 60-62).

Table 2. Previous ^{11}C-PIB PET studies in cardiac amyloidosis.

Author, year	No of subjects	Measure	Sensitivity	Specificity	Accuracy
Antoni, 2013 (55)	10	Visual	100%	100%	100%
Minamimoto, 2020 (60)	9	Visual	100%	100%	100%
Lee, 2015 (62)	22	SUVR>2.0	87%	100%	91%
Ezawa, 2018 (61)	15	Visual	92%	83%	89%

SUVR, standardized uptake value ratio.

Amyloid PET detects both AL and ATTR cardiac amyloidosis, but can possibly be used to differentiate between the two subtypes, since a sustained tracer uptake is seen in AL, as opposed to ATTR where increased uptake is seen early after tracer injection (56). This might be an advantage with PET compared to CMR in amyloid diagnostics.

Both CMR and PET have shown positivity for amyloid in a proportion of patients who do not fulfil the echocardiographic criteria for cardiac involvement (63, 64) and thus seems to be more sensitive methods to detect cardiac amyloidosis. Even among patients without elevation of Nt-proBNP, a substantial proportion has cardiac involvement according to ECV measurement and

amyloid PET uptake (63). As mentioned earlier, typical features of cardiac amyloidosis on CMR or PET can, according to recent guidelines be used to define cardiac involvement in extracardiac biopsy proven AL or ATTR amyloidosis (47).

When it comes to radio nuclear methods, SAP-scintigraphy can visualize AL amyloid in the spleen, liver and kidneys, but the method is not able to detect cardiac amyloid deposition (65, 66). Moreover, the method is not approved in all countries due to the need for human plasma in tracer production.

Radionuclide bone scintigraphy with technetium-labelled bisphosphonates has shown high sensitivity and specificity in detecting cardiac ATTR amyloidosis but the method is only able to visualize cardiac amyloid in a small proportion of patients with AL amyloidosis (67-69). As mentioned above, when investigating patients with unexplained cardiac hypertrophy, serum and urine immunofixation together with serum FLC is an effective screening method for AL amyloidosis. If a monoclonal protein is found, abdominal fat pad and bone marrow aspirate/biopsy is then the next investigational step. If no monoclonal protein is detected, AL amyloidosis is unlikely and ATTR should be suspected. Gillmore et al (70) have shown that a positive bone scintigraphy together with the absence of a monoclonal protein can safely be used to diagnose ATTR cardiac amyloidosis without the need of endomyocardial biopsy.
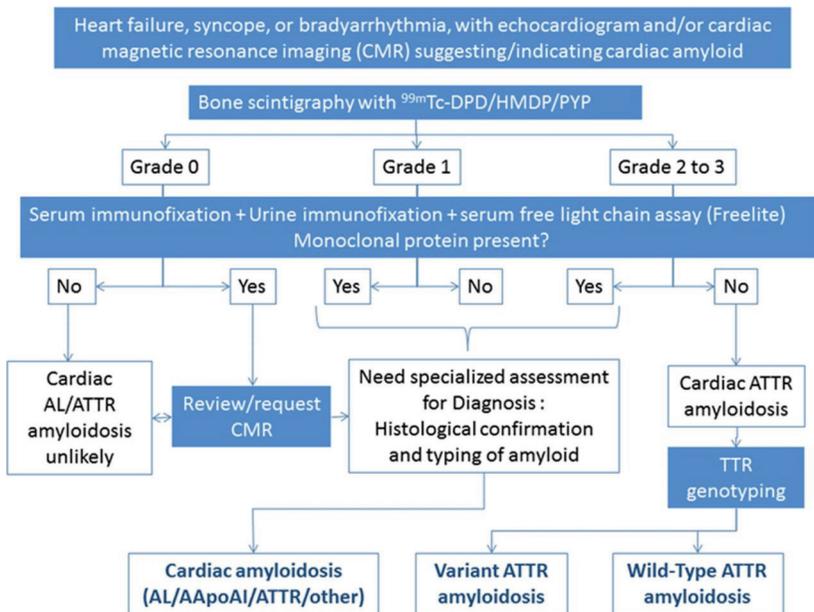
Figure 2. Algorithm for the diagnosis of cardiac amyloidosis, from Gillmore et al (70). Reproduced with permission from the publisher.

Effects on cardiac function

Cardiac dysfunction in AL amyloidosis is caused both by the disruption of tissue architecture by amyloid deposition and by the toxic effects of mainly the prefibrillar forms of the amyloidogenic light chains (oligomers). Amyloid deposition leads to expansion of the extracellular space, increased cardiac wall thickness and stiffening of the heart without compensatory dilatation. This results in a restrictive and diastolic dysfunction with elevated filling pressures and reduced stroke volume which involves both ventricles. In severe stages, the systolic function is also decreased (50). Amyloid deposition also occurs in the atria causing reduced contractility (71), as well as in the valves and perivascularly.

It has been shown that with the same degree of increased cardiac wall thickness, AL patients have more pronounced cardiac dysfunction and shorter survival than ATTR patients (72). Animal studies have shown that infused amyloidogenic light chains cause direct toxicity to zebra fish cardiomyocytes (73), and reduces the motility of the nematode C. elegans, which can be used as a model of the vertebrate heart (74). There is evidence that the amyloidogenic light chains cause impairment of cardiomyocyte metabolism, activation of apoptosis and increased oxidant stress. This causes decrease in cardiomyocyte contractility and relaxation (75-77), effects that are mediated through p38 MAPK (mitogen-activated protein kinase) signalling (78, 79). The p38 MAPK activation leads to Nt-proBNP release, which in AL amyloidosis is caused both by the cardiotoxic effect of the light chains and by the general mechanisms leading till Nt-proBNP release in heart failure (80).

Another factor influencing cardiac function might be myocardial oedema, which by CMR T2 (transverse relaxation time) mapping and histology has been shown to be increased in cardiac amyloidosis, more so in AL than in ATTR. Increased myocardial oedema on T2 mapping has also been associated with inferior survival in AL patients (81).

Angina and "infarction-like" conditions are prevalent in cardiac amyloidosis, and in a study of specimens from AL patients, 74% were found to have histological signs of myocardial ischemia (82). There is evidence of microvascular dysfunction which can be caused by endothelial dysfunction due to toxic effects of the light chains or by small vessel involvement of amyloidosis (83).

Assessment of cardiac function by PET

Apart from detection of cardiac amyloidosis, PET can be used to assess different aspects of cardiac function, using for example the tracer ^{11}C-acetate. Acetate is used in the mitochondria to convert nutrients and oxygen into ATP (adenosine triphosphate). Carbon dioxide is the main metabolite of acetate

oxidation, and leaves the tissue in direct proportion to the carbon oxidation, and is then finally exhaled. That is why radiolabelled acetate can be used with PET to measure oxidative metabolism. The high extraction and uptake into myocytes also make it suitable for measuring myocardial blood flow (MBF). The influx phase of ^{11}C-acetate is used to measure MBF and the efflux phase to measure MVO_2 (myocardial oxygen consumption). Cardiac output (CO) can be quantified with high accuracy and forward stroke volume calculated (CO/heart rate). Furthermore, left ventricular (LV) volumes, ejection fraction, LV mass and septal wall thickness (IVS) can be measured. Efficiency of the myocardium (MEE) is defined as the ratio of energetic output and the energy consumed, and can be calculated from ^{11}C-acetate PET (84). In the diagnosis of ischemic heart disease, ^{11}C-acetate PET can be used to measure perfusion and metabolism simultaneously, but due to the relatively long half-life of ^{11}C-acetate the use is limited when both rest and stress measures must be performed in the same session.

In cardiac amyloidosis, PET-studies have shown reduced MBF (85, 86), which is assumed to be caused by the obstruction of blood flow from the amyloid infiltration, or possibly from amyloid involving the blood vessel walls. Elevated ratio of right and left ventricular (RV/LV) MBF has been shown to be associated with increased pulmonary pressure and to have prognostic implications in AL and ATTR cardiac amyloidosis (87). A value of RV/LV MBF > 0.56 was shown to predict inferior survival, and was mainly driven by increased RV MBF, which could be related to an increased demand due to elevated RV afterload.

In one previous ^{11}C-acetate PET study of MEE in cardiac amyloidosis, MVO_2 was relatively unchanged, whereas MEE was reduced, indicating a poor energetic efficiency but without any severe effect on the oxidative metabolism (85).

Treatment

Until recently, there were no treatment regimens approved by medical agencies for AL amyloidosis. The daratumumab-VCd (bortezomib, cyclophosphamide, dexamethasone) combination was the first treatment to be approved in 2021, based on results of the ANDROMEDA study (88). The study was a randomized trial were patients received six 4-week cycles of VCd with or without the addition of daratumumab. The daratumumab group also continued to receive daratumumab for up to 24 cycles in total. The study showed a significantly higher rate of hematologic complete response (CR) in the daratumumab group (53.5 vs 18.1%), and also a significantly higher rate of organ responses. The study did not include patients with advanced stage cardiac involvement.

Otherwise, therapies are mainly adopted from what is used in myeloma. Randomized studies are rare, and treatment recommendations are mostly based on small phase 2 studies. Another issue has been incoherence in the reporting of results from clinical trials in AL amyloidosis. Since early mortality is high, response numbers are largely affected by whether results are reported as "intention-to-treat" or not, a problem that has been addressed in a publication by Comenzo et al (89). Furthermore, patients with advanced stage cardiac involvement, the patient group most in need of improved therapeutic options, are often excluded from clinical trials.

All treatments used in clinical practice today are directed towards the plasma cell clone, with the aim of eliminating the amyloidogenic light chains from the circulation, thereby preventing further amyloid formation and enabling reduction of existing organ deposition. There is still no evidence that the disease is curable, and patients need to be followed lifelong.

High dose melphalan

High dose melphalan and autologous stem cell transplantation (HDM/ASCT) has been performed since the 1990s and is still considered standard of care for eligible patients at many centres. There is only one randomized study comparing high dose melphalan to conventional chemotherapy (90). In this study, 100 patients were randomized to receive either HDM/ASCT or standard dose melphalan together with high dose dexamethasone. However, in the group assigned to receive HDM/ASCT, only 37 patients came to treatment (10 patients died, 1 declined treatment and 2 did not have enough stem cells). Out of these 37 patients, 9 died within 100 days from treatment, that is, treatment related mortality (TRM) was 24%. In an "intention-to-treat" analysis, the median overall survival (OS) was significantly longer in the group assigned to receive standard dose melphalan compared to high dose (56.9 vs 22.2 months), and no significant difference in response rates was seen between the groups either. However, no patient selection was made in the study, and high-risk patients were present in both arms (21 of the patients in the high dose melphalan group). When divided into high-risk and low-risk disease, there was no significant difference in OS between the standard dose and high dose melphalan groups.

Since then, it has been shown that patient selection can improve the results of HDM/ASCT, and a risk-adapted approach is now being used when assigning treatment for patients. In the later years, large amyloidosis centres have reported good long-term results with HDM/ASCT, with median OS between 7.3 and 10.4 years and hematologic CR rates of around 40-50% (91-93). The reported TRM is reduced from 7.4-8.7% to 1.1-3.3% in the later years. In the largest studied patient population including 1536 patients from the Centre for International Blood and Marrow Transplant Research registry, TRM was 20% in 1995-2000, 11% in 2001-2006 and 5% in 2007-2012 (94). 5-year survival

rate increased from 55% to 61% and 77% in the latest time period. They also reported superior survival in centres that performed more than four transplantations per year for AL amyloidosis.

Table 3. Previous studies on high dose chemotherapy in AL amyloidosis.

Author, year	Centre	Time-period	No of pts	TRM	CR rate	OS
Sharpley, 2019 (91)	UK	1994-2018	264	8.7% (18.8→1.1%)	52%	7.3 y
Sidiqi, 2018 (92)	Mayo Clinic	1996-2016	672	7.4% (14.5→2.4%)	40%	10.2 y
Gustine, 2022 (93)	Boston	1994-2021	648	8% (11.8→3.3%)	39%	7.6 y
D´Souza, 2015 (94)	International	1995-2012	1536	20→5%	30-37% (2001-2012)	55-77% 5y OS

TRM, treatment related mortality. CR, complete response. OS, overall survival.

Selection criteria for HDM/ASCT

The proportion of patients treated with high dose therapy has varied widely between centres, with around 30% reported from the United States (3) compared to 5-6% from European centres (2, 91), suggesting that varying selection criteria have been used. During the later years, a decreasing trend has been observed in the frequency of HDM/ASCT, probably due to stricter selection of patients for treatment (92).

Cardiac involvement is the main risk factor for treatment related mortality. Nt-proBNP > 5000 pg/mL and Troponin T > 0.06 ng/mL have been identified as risk factors for early mortality after HDM/ASCT. It has been suggested that patients with these risk factors should be excluded from high dose treatment (95). In 2022, guidelines for HDM/ASCT in AL amyloidosis were published by an International Society of Amyloidosis/European Haematology Association working group, including the mentioned cardiac biomarker levels as well as other parameters (96). The proposed eligibility criteria for high dose therapy were as follows:

- Age <70 years (>70 years may be discussed)
- At least one major organ involvement
- LV ejection fraction ≥40%, NYHA class <III
- Oxygen saturation ≥95%, diffusing capacity >50%
- Supine systolic blood pressure ≥90 mmHg
- ECOG performance status ≤2 unless limited by peripheral neuropathy

- Direct bilirubin <20 mg/L
- Nt-proBNP <5000 pg/mL
- Troponin T <0.06 ng/mL, troponin I <0.1 ng/mL or highly sensitive troponin T <75 ng/L
- eGFR >30 mL/min/1.73 m^2 if not on dialysis (otherwise the dose of melphalan should be reduced)
- No symptomatic arrhythmias or pleural effusions
- No uncompensated heart failure
- No factor X deficiency (<25% or active bleeding)
- No extensive gastrointestinal involvement with risk of bleeding

The role of induction therapy

The use of induction therapy before HDM/ASCT has increased over time (3, 91, 92), and in retrospective studies induction therapy has been associated with higher CR rate and longer OS after HDM/ASCT (93, 97, 98). One of the studies could not show an advantage with induction therapy in patients with bone marrow plasma cells (BMPC) less than 10%, however another retrospective analysis showed benefit with induction therapy independent of the amount of BMPC (93). There is one randomized study including 56 patients comparing bortezomib induction to no induction (99). With all subjects remaining on study at 12 months, the CR rate was significantly higher with bortezomib induction (67.9% vs 35.7%). Bortezomib based induction therapy can also make around 30% of patients initially considered ineligible for HDM/ASCT possible to treat (86).

Induction therapy might serve as a "test" of how well the patient tolerate treatment, and if toxicity or disease progression occur during induction therapy the patient may never proceed to high dose therapy. This might influence study results in advantage of induction therapy if "intention-to-treat" results are not reported.

Deferred HDM/ASCT to progression

Results are diverse regarding deferred HDM/ASCT to progression. A retrospective study by Manwani et al (100) showed significantly shorter OS from the time of ASCT when HDM/ASCT was deferred to progression compared to as consolidation. However, these results may be biased by the fact that having disease progression itself infers worse prognosis. On the other hand, Venner et al showed no significant difference in OS (not if measured from the time of ASCT or from diagnosis) with HDM/ASCT performed at progression compared to upfront (101).

HDM/ASCT in comparison to modern conventional treatment

There are no randomized studies comparing high dose to conventional chemotherapy in the era of modern treatment. With the recently approved

combination daratumumab-VCd in newly diagnosed AL amyloidosis, response rates are similar to what has been reported after HDM/ASCT (88). After 6 cycles of daratumumab-VCd, the hematologic CR rate was 53.3% compared to around 40-50% after HDM/ASCT. Organ response rates were 41.5% for cardiac and 53.0% for renal response with daratumumab-VCd. The reported rate of renal response with HDM/ASCT is between 31-32% (94) to 76% and 61% for cardiac response (93). A median duration of response of 12.3 years has been reported in patients achieving CR after HDM/ASCT (93), whereas the duration of response with daratumumab-VCd therapy is not yet evaluated. There are of course issues with comparing results between studies. The study populations differ and patients receiving high dose therapy is generally a highly selected group. The reporting of hematologic and organ responses from the retrospective studies depends on the methods and criteria used during that time-period. Results are also affected by whether results are reported for the "intention-to-treat" population or not.

The long overall and progression free survival reported after HDM/ASCT is the main advantage with HDM/ASCT, and it is not yet known whether the same long-term results can be obtained with daratumumab-VCd treatment. Treatment related mortality and morbidity is still an issue with HDM/ASCT, even though the TRM is markedly reduced over time it is still a few percent and higher than the around 0.5% (102, 103) observed in multiple myeloma. Based on that, the current recommendation is to refrain from high dose therapy in first-line treatment in patients achieving CR with induction therapy (96).

Treatment in advanced cardiac stage

Around 17% of AL amyloidosis patients have advanced (stage IIIb) cardiac involvement at diagnosis (2). For this group, median survival is short (4-6 months) (104-106) with only a small increase to 9 months in a recent report where 74% of patients received bortezomib or daratumumab based first-line therapy (107). Early mortality is high at around 40% within 3 months from diagnosis and has not decreased over time according to a recent European report (2).

Rapid and deep hematologic response is particularly important in this group, and can increase survival (104, 105, 107). Hematologic response of at least VGPR (very good partial response) at 1 month and an early cardiac response within 3 months is associated with prolonged survival (104, 107). However, the tolerability of treatments is of course an issue. For example, there are reports of cardiac toxicity from dexamethasone as well as bortezomib, and it is suggested that the timing and dosing of these drugs should be adapted (108, 109).

Patients with advanced cardiac stage are excluded from treatment with HDM/ASCT according to the guidelines described above, due to the high risk of treatment related mortality. In the ANDROMEDA study comparing

daratumumab-VCd to VCd, patients with advanced cardiac involvement (Nt-proBNP > 8500 ng/L or NYHA class IIIb/IV) were not included (88). 36% of patients in each arm were in stage III (Nt-proBNP ≥ 1800 ng/L and troponin T ≥ 0.025 ng/mL), these patients also benefitted from daratumumab-VCd. However, early mortality (within 60 days of starting treatment) was equal in the two study groups, indicating that the addition of daratumumab could not overcome the risk of early death.

The daratumumab-VCd combination has been evaluated retrospectively in 19 patients with stage IIIb cardiac disease (110). The starting dose of bortezomib was reduced in 50% of patients and omitted in one, dexamethasone was reduced to 10-20 mg/week while full dose cyclophosphamide and daratumumab was given from start. There were 3 deaths within 3 months (15.8%) which is better than the around 40% previously reported in stage IIIb patients. The authors stated that there were no treatment related deaths. However, it might be difficult to specify whether cardiac deaths are due to toxicity or not. Another retrospective study in stage IIIb patients has showed significantly higher hematologic and cardiac response rates, as well as prolonged survival, when daratumumab was included in first-line therapy (111).

Antibodies directed against the amyloid itself, and not the plasma cell clone, is an attractive approach in advanced cardiac involvement, with the hope of achieving a faster organ response. CAEL-101 is a monoclonal antibody directed towards amyloid proteins present in both kappa and lambda amyloid fibrils (112). A phase 1A/B study in 27 relapsed AL patients showed 67% organ responses, which were seen at in median 3 weeks from start of therapy. Phase 3 studies are now ongoing in stage IIIa and b patients in combination with standard of care plasma cell directed therapy. Another amyloid directed antibody under investigation is birtamimab that binds to an epitope of misfolded kappa and lambda light chains. The phase 3 VITAL study was closed early due to failure to meet endpoints in an interim analysis. However, in a post-hoc analysis of patients with Mayo stage IV disease, there was a significant improvement in survival at 9 months (113). A randomized study in stage IV patients is currently ongoing.

Prognosis

Cardiac biomarkers and staging systems

Cardiac involvement is the most important prognostic factor, and the cardiac biomarkers Nt-proBNP and troponins are the basis of prognostic systems used in AL amyloidosis. The Mayo Clinic staging system from 2004 used the thresholds Nt-proBNP 332 pg/mL, troponin T 0.035 ng/mL or troponin I 0.1 ng/mL (114). Elevation of both Nt-proBNP and troponin gave Mayo stage III,

one elevated stage II and none elevated stage I. Median survival for the groups was 4.1, 11.1 and 27.2 months respectively.

A revised Mayo staging system was published in 2012 with adjusted thresholds for Nt-proBNP (1800 pg/mL) and troponin T (0.025 ng/mL), and the inclusion of dFLC (difference between involved and uninvolved free light chain) with the threshold 180 mg/L (115). The new model yielded four different stages with scores 0-3 on these parameters, with median survival from diagnosis of 94.1, 40.3, 14 and 5.8 months respectively. This staging system was also validated using high sensitive troponin T measurement (116).

In a European collaboration study of 346 patients with 2004 Mayo stage III disease, Nt-proBNP > 8500 pg/mL and systolic blood pressure < 100 mmHg were identified as independent risk factors associated with inferior survival, suggesting that these parameters could be used to further risk stratify patients in Mayo stage III (117). In patients with both of these risk factors present, median OS was only 3 months, and with one of the risk factors present median OS was 6 months. In patients with either Nt-proBNP > 8500 pg/mL or systolic blood pressure < 100 mmHg, achieving hematologic CR was associated with improved survival, whereas achieving partial response (PR) did not lead to better survival.

In a retrospective European study of 230 patients treated upfront with VCd published in 2015 (106), Mayo stage III patients were sub-grouped into stage IIIa with Nt-proBNP < 8500 pg/mL and stage IIIb with Nt-proBNP ≥ 8500 pg/mL. Stage IIIb was the only independent risk factor for inferior survival.

In a comparison of the different staging systems Mayo 2004, Mayo 2012 and European 2015, the European system best predicted early mortality (at 1 year) (118). Mayo 2012, in which assessment of the plasma cell clone is included, outperformed the other systems regarding more long-term survival (at 3 years).

Table 4. Staging systems in AL amyloidosis.

Prognostic model	Measure	Stage	
Mayo 2004 with European modification	**1) Troponin** TnT≥0.035 ng/mL or TnI≥0.1 ng/mL or Hs TnT≥50 ng/L **2) Nt-proBNP** Nt-proBNP≥332 pg/mL or BNP≥81 ng/L	Stage I Stage II Stage IIIa Stage IIIb	No risk factors 1 risk factor 2 risk factors and Nt-proBNP<8500 pg/mL or BNP<700 pg/mL 2 risk factors and Nt-proBNP≥8500 pg/mL or BNP≥700 pg/mL
Mayo 2012	**1) Troponin** TnT≥0.025 ng/mL or Hs TnT≥40 ng/L **2) Nt-proBNP** Nt-proBNP≥1800 pg/mL or BNP≥400 pg/mL **3) dFLC**≥180 mg/L	Stage I Stage II Stage III Stage IV	No risk factors 1 risk factor 2 risk factors 3 risk factors

Plasma cell clone, FLC levels and cytogenetic abnormalities

Higher amount of bone marrow plasma cells and higher dFLC have been iden-tified as negative prognostic factors in AL amyloidosis (36, 119). In 730 pa-tients followed at the Mayo Clinic, the type of involved light chain, lambda or kappa, did not affect survival, but the presence of a heavy chain paraprotein was associated with a slightly better prognosis (119). Another study however reported shorter progression free and overall survival in lambda light chain amyloidosis compared to kappa, in patients treated with autologous stem cell transplantation (120).

Patients with low dFLC (< 50 mg/L), constituting around 15% of AL pa-tients, have less often and less advanced cardiac involvement, whereas renal involvement is more common and more severe in this group (120-122). Over-all survival is better in patients with low dFLC, who also have a survival ben-efit among patients within the same Mayo stage group. Patients with low dFLC (at least 20 mg/L) at diagnosis who achieve dFLC <10 mg/L after

treatment have longer overall and renal survival, suggesting that this should be the treatment goal in this patient group.

The most prevalent cytogenetic aberration in AL amyloidosis is translocation (11;14), present in around 50% of AL patients (123, 124). In patients treated with bortezomib first-line, t(11;14) has been associated with inferior prognosis (124-126). However, when treated with high dose melphalan, outcome is similar or better in patients with t(11;14) (127, 128). In a recent retrospective study including patients treated between 2016-2021, presence of t(11;14) was associated with higher frequency of swich to second-line therapy within one year (129). However, no effect on overall survival was seen, probably due to the possibility of receiving effective second-line treatment. The presence of t(11;14) is associated with sensitivity to the BCL2-inhibitor venetoclax, which has shown high efficacy in previously treated AL patients with t(11;14) (130). With daratumumab-VCd treatment, patients with t(11;14) had the same benefit of the addition of daratumumab as the whole daratumumab group (131).

Gain 1q21 is present in 20-30% of AL patients, and is overrepresented in patients with co-existing myeloma (123). Gain 1q21 has been associated with worse survival in patients treated with low dose melphalan first-line (132), but had no significant prognostic impact in a cohort treated with high dose melphalan (127).

The myeloma high-risk aberrations t(4;14), t(14;16) and del(17p) are uncommon in AL amyloidosis, around 2-3% each. These aberrations have been associated with inferior prognosis in patients treated with high dose melphalan, but not in patients treated with bortezomib (126, 127).

Cardiac function parameters

The degree of cardiac involvement at diagnosis is the major determinant of survival in AL amyloidosis. Even elevated Nt-proBNP or cardiac involvement detected by CMR, in patients with normal echocardiogram, are shown to be prognostic for survival (64).

Except from clinical (NYHA-class) and biochemical (Nt-proBNP, troponins) parameters, there are several echocardiographic and CMR measures that have been associated with inferior survival. Impaired longitudinal function measured with longitudinal strain (LS) is an early echocardiographic sign of cardiac amyloidosis. Several studies have shown independently prognostic value of LS in multivariable analysis involving cardiac biomarkers (133-137). Also, right ventricular dysfunction, typically assessed by TAPSE (tricuspid annular plane systolic excursion) is associated with poor prognosis in cardiac amyloidosis (138, 139). Other prognostic parameters are indexed stroke volume (SVI) and myocardial contraction fraction (MCF) calculated as LV stroke volume divided by LV myocardial volume. Both SVI and MCF are shown to be prognostic independent of cardiac biomarkers and MCF is highly correlated

to LS (136, 139, 140). According to a study by Milani et al, SVI, MCF and LS had similar prognostic value, and the authors suggested that SVI is used in clinical practice because it is routinely and easily assessed (136).

Treatment response

Hematologic response criteria and goal of treatment

The original response criteria (46) were updated in 2012 and four levels of hematologic response were defined, CR (negative serum and urine immuno-fixation and normal FLC ratio), VGPR (dFLC < 40 mg/L), PR (dFLC decrease > 50%) and no response (141). The level of FLC response is directly associated with survival, and correlate better with survival than monoclonal protein response in patients having intact immunoglobulins (142, 143). The absolute level of dFLC was a stronger predictor of survival than the percentage of FLC reduction, which is why the new response category VGPR with dFLC < 40 mg/L was established (141).

Studies have suggested a more stringent definition of CR, since both involved FLC (iFLC) ≤ 20 mg/L or below upper limit of normal (144, 145) and dFLC < 10 mg/L (146, 147) have performed better at predicting survival than the FLC ratio. However, not all studies have confirmed this finding (148). Recently published guidelines recommend CR with iFLC ≤ 20 mg/L or dFLC < 10 mg/L to be the goal of treatment (149). In patients with advanced cardiac stage (IIIb), achieving a rapid both hematologic and cardiac response is of special importance. Achieving VGPR within 1 month and cardiac response within 3 months is associated with superior survival and could be proposed as a goal of treatment (104, 107).

Assessment of MRD (measurable residual disease) by flow cytometry is established in myeloma and has been studied in AL amyloidosis. Retrospective studies have shown that undetectable bone marrow clonal plasma cells by flow cytometry (different techniques with varying sensitivity have been used) is associated with longer PFS, but without significant difference regarding OS (150-152). MRD by bone marrow flow cytometry could underappreciate disease because of varying presence of clonal plasma cells depending on the sample site. A recent study reported that, among 33 patients who were in CR and MRD negative using six-color flow cytometry (not next generation flow cytometry), four patients (12%) were positive by blood mass spectrometry (153). Further studies are needed to determine the value of this technique.

Evaluation of cardiac response

In the original organ response criteria published in 2005, cardiac response was defined as IVS decrease by 2 mm, 20% improvement in ejection fraction or

improvement by two NYHA classes (without increase in diuretic use), measures that are insensitive and observer dependent. Significant changes in Nt-proBNP are strongly associated with survival, and a new definition of cardiac response was established in 2012, with Nt-proBNP response defined as > 30% and > 300 pg/mL decrease if baseline Nt-proBNP was ≥ 650 pg/mL (141). Nt-proBNP can be used early (3 months after treatment initiation) to assess cardiac response. However, its use is limited in patients with renal failure since the level of Nt-proBNP is affected by the glomerular filtration rate, and it is also sensitive to temporary changes in fluid balance. In patients receiving IMiD (immunomodulatory drug) treatment, rises in Nt-proBNP is observed despite hematologic response, however, in this situation Nt-proBNP increase is still associated with worse outcome.

Further grading of cardiac response has been suggested by Muchtar et al, who proposed four levels of response with significantly differentiated survival rates: 1) cardiac CR, nadir Nt-proBNP ≤ 350 pg/mL, 2) cardiac VGPR, > 60% reduction in Nt-proBNP but not meeting cardiac CR definition, 3) cardiac PR, 31-60% reduction in Nt-proBNP and 4) non-responder, ≤ 30% reduction in Nt-proBNP (145, 154). The graded cardiac response performed better at predicting survival than the two-level response from one year after start of treatment. Median time to at least cardiac PR was 9.4 months (IQR 4.7–15.9).

In general, a complete hematologic response leads to the best outcome. However, the level of hematologic response at which organ response occur can vary between patients. The use of a combined hematologic and organ response score has been suggested, in order to better assess the overall response for the individual patient (155).

When it comes to imaging parameters, improvement in LS after treatment is associated with longer overall survival, and studies indicate superior prognostic performance compared to Nt-proBNP. It has been suggested that LS is incorporated in the staging system and also in the response assessment criteria in AL amyloidosis (134, 156). Reduction in ECV measured with CMR has also been associated with improved outcome in AL amyloidosis (157, 158).

Aims of the thesis

The overall aims of the thesis were to increase the knowledge about the epidemiology of AL amyloidosis, to study imaging methods for early diagnosis and prognosis assessment in cardiac AL amyloidosis, and to evaluate the long-term results of high dose chemotherapy in AL amyloidosis with focus on patients with cardiac involvement.

The use of high dose chemotherapy in AL amyloidosis is debated mainly because of the high mortality and morbidity that has been associated with the treatment. Studies from specialized amyloidosis centres have shown a reduction in the treatment related mortality over time, and superior results in centres performing > 4 high dose treatments per year. In **paper I** we aimed to evaluate the long-term results of all patients treated with high dose therapy for AL amyloidosis in Sweden during the first 15 years after the treatment was introduced. We sought to compare results from our Swedish conditions, where this treatment is performed in a decentralized setting, to the outcomes reported from the large amyloidosis centres regarding response, survival and treatment related mortality. We specifically wanted to evaluate results in patients with cardiac involvement, who have the highest risk of treatment related mortality, and analyse whether mortality has changed over time.

Cardiac involvement is the major determinant of prognosis in AL amyloidosis, and when diagnosed in advanced stages, prognosis is dismal. Echocardiography is the most used imaging method for diagnosis of cardiac amyloidosis, but many of the findings on echocardiography are unspecific for cardiac amyloidosis and occur late in the disease process. Previous pilot-studies have shown that PET with the amyloid binding tracer ^{11}C-PIB (initially developed for the diagnosis of Alzheimer's disease) can detect cardiac amyloidosis. **Paper II** aimed at determining the sensitivity and specificity of ^{11}C-PIB PET in detecting cardiac amyloidosis and evaluate differences in uptake in AL and ATTR amyloidosis. We also wanted to examine whether ^{11}C-PIB PET could detect cardiac amyloidosis in a group of patients without demonstrated heart involvement according to standard criteria, that is, if PET was able to detect cardiac amyloidosis earlier than echocardiography.

Since the degree of cardiac involvement is the most important prognostic factor, staging systems in AL amyloidosis are based on the level of cardiac biomarkers, mainly Nt-proBNP. However, the level of Nt-proBNP is not specific for the degree of cardiac involvement, it is also influenced by the

glomerular filtration and fluid balance, and often increases with IMiD treatment. Several cardiac function parameters have been investigated as prognostic markers in AL amyloidosis, such as longitudinal strain, stroke volume and myocardial contraction fraction. In **paper III** we wanted to examine the prognostic value of myocardial external efficiency from ^{11}C-acetate PET, a measure that incorporates oxygen consumption as well as mechanical parameters. Since oxygen metabolism could possibly be altered in cardiac amyloidosis, we hypothesized that the prognostic power would be increased compared to parameters based only on mechanical work.

There are few population-based epidemiological studies in AL amyloidosis. Many of the reports on the incidence and prevalence of AL amyloidosis are based on estimations, and the studies with verified cases include only a small number of patients. Survival data mainly come from large amyloidosis centres, where an improvement is seen over time. In **paper IV** we sought to determine the incidence, prevalence and survival of AL amyloidosis in Sweden, and evaluate whether the improvements seen regarding survival also applies to our conditions. The aim was also to evaluate whether the incidence and prevalence has changed during the last two decades, considering the increased awareness and survival of the disease. We also wanted to evaluate factors influencing overall and early mortality, such as cardiac involvement, and whether early mortality has changed over time.

Patients and methods

Paper I

We retrospectively collected data on all patients treated with HDM/ASCT in Sweden from 1994 when treatment was introduced until 2009. Patients were identified from registries at the eight different centres in Sweden were HDM/ASCT was performed. Patients with symptomatic myeloma (defined as myeloma diagnosis with CRAB criteria) or lymphoma were excluded. Data was retrieved from the time of diagnosis, at admission for HDM/ASCT, at 3 months, 6 months, 1 year and 2 years after HDM/ASCT, at best response and at progression. Follow-up was ended in April 2014. Response, treatment related mortality and overall survival were analysed, as well as factors influencing survival. The original consensus criteria from 2005 (46) were used to define organ involvement, response and progression. Changes in treatment related mortality between the earlier part of the studied period and the later were examined. Survival was analysed using Kaplan-Meier method, and differences between survival curves were measured with the log-rank test. The chi square test was used to evaluate differences in categorical values.

Paper II

36 patients with known cardiac amyloidosis (CA) were enrolled in the first part of the study, 15 patients with AL and 21 with ATTR amyloidosis, as well as two control groups consisting of healthy volunteers (n = 8) and patients with non-amyloid cardiac hypertrophy (n = 7). Subjects were examined with ^{11}C-PIB PET and echocardiography to establish the sensitivity and specificity of ^{11}C-PIB PET for the diagnosis of CA, and the optimal cut-off values for the two semi-quantitative measures SUVR (standardized uptake value ratio) and RI (retention index). We then applied these cut-off values prospectively to a group of patients with systemic amyloidosis (5 AL and 6 hereditary ATTR) without increased cardiac wall thickness.

The CA patients were diagnosed either by endomyocardial biopsy (n = 24) or by echocardiography showing hypertrophy together with amyloid detected on abdominal fat pad biopsy (n = 11), one of the AL patients had a positive fat pad biopsy and typical CMR findings for CA, even though echocardiography did not show increased wall thickness.

The majority of AL patients (12 out of 15) had received plasma cell directed therapy before study inclusion. Among the ATTR patients, 16 had wild-type and 5 hereditary ATTR, out of these 3 had the Danish Leu111Met mutation and 2 had the Swedish His88Arg mutation. The hypertrophic control group consisted of 4 patients with idiopathic hypertrophic cardiomyopathy and 3 with hypertrophy caused by hypertension.

All subjects were examined with ^{11}C-PIB PET and echocardiography, which were performed at two sites (Uppsala, Sweden and Aarhus, Denmark). PET/CT was performed according to identical protocols at the two sites, after ^{11}C-PIB injection a dynamic scan was performed during 35 minutes. All PET examinations were evaluated by two different observers blinded to the diagnosis of the subject, both by visual inspection and by the two semi-quantitative measures SUVR and RI, with good agreement between the readers. Regions of interest were drawn in the left atrial blood pool, and in the left ventricular wall, from which time-activity curves were created. Summed images from 10-20 minutes after ^{11}C-PIB injection were used to calculate the myocardium/blood SUVR, and for RI the mean tissue concentration between 10-20 minutes was divided by the integral of the blood time-activity curve from 0-15 minutes after ^{11}C-PIB injection.

ROC (receiver operating characteristic) curves were used to determine the cut-off values of SUVR and RI that discriminated CA from controls with the highest sensitivity and specificity. Spearman rank correlation or ordinal logistic regression was used to evaluate correlations between PET measures and clinical/echocardiography parameters.

Paper III

The study included 48 subjects with both AL (n=23) and ATTR (n = 25) cardiac amyloidosis as well as 20 controls (14 healthy volunteers and 6 with non-amyloid hypertrophic cardiomyopathy). ATTR patients comprised 18 wild-type and 7 hereditary ATTR, 4 with the Leu111Met mutation, 2 with the His88Arg mutation and one patient with unknown type of mutation.

The CA patients were diagnosed with endomyocardial biopsy (33 patients) or by abdominal fat pad biopsy together with echocardiography or CMR (15 patients). Hypertrophic controls consisted of 3 patients with hypertensive heart disease and 3 with idiopathic hypertrophic cardiomyopathy.

All subjects were examined at the two sites, Uppsala and Aarhus, with ^{11}C-acetate PET and echocardiography. Subjects were also examined with ^{11}C-PIB PET as part of a previous study (55) and the study in paper II. Subjects from Uppsala also went through ^{15}O-water PET, which is not included in the current paper.

[11]C-acetate PET was performed according to the same protocol at the two sites. After ^{11}C-acetate injection, images were collected during 27 minutes.

Previously described methods were used to calculate myocardial oxygen consumption (MVO_2) (159). Myocardial external efficiency (MEE) was calculated through the following formula as previously described (160):

$$MEE = \frac{EW \times 1.33 \times 10^{-4}}{LV\ mass \times MVO_2 \times 20} \times 100$$

EW (external work) was calculated as the product of forward stroke volume (FSV), heart rate and mean arterial blood pressure.

Survival of CA patients was obtained and clinical, echocardiographic and ^{11}C-acetate PET parameters were examined in univariable and multivariable cox regression analysis. ROC curves were used to determine cut-off values for the ^{11}C-acetate PET parameters that best discriminated deceased from surviving CA patients. Survival analysis was performed using the established cut-off values for the ^{11}C-acetate PET parameters in all CA patients and in AL and ATTR respectively.

Paper IV

Through the pathology departments at Uppsala University Hospital and Karolinska University Hospital, we identified all samples that were assigned the code for amyloidosis (Snomed code M55100) during the time period 2000-2020. Patients not registered as inhabitants of the Uppsala and Stockholm County according to the Swedish population registry were excluded. The pathology reports were reviewed and cases with a new diagnosis of systemic AL amyloidosis during the time period were selected. If the diagnosis was uncertain from the pathology report, a review of the patient records was performed to ensure the correct diagnosis. Localized AL amyloidosis (including nodular amyloidosis of the lungs) were not included and neither were a few patients in which AL amyloidosis had been detected but who did not have any organ involvement.

Amyloid was detected through positive Congo red staining and typing was performed with immunohistochemistry or western blot. Cases with an uncertain amyloid typing were included as AL amyloidosis if they had a diagnosis of myeloma or a monoclonal protein present.

For residents in Uppsala County, all amyloid diagnostics is performed either at the specialized amyloid laboratory in Uppsala or sent to Karolinska University Hospital (renal or cardiac biopsies), and are thereby included in this study. In the Stockholm County on the other hand, smaller pathology departments might have performed amyloid diagnostics in some cases, and these may be missed in this report. That is why the cases from Uppsala County were used for incidence and prevalence calculations, and patients from Stockholm County were included in the treatment and survival analyses.

For all identified cases of systemic AL amyloidosis from Uppsala and Stockholm County a review of the patient records was performed, clinical and treatment characteristics were retrieved as well as survival time.

The incidence rate was calculated as the number of patients diagnosed each year by the population count for Uppsala County for the corresponding year. Age- and sex-adjusted incidence rate was calculated through direct standardization to the 2010 Swedish population. The 5-year limited duration prevalence was calculated for each year from 2004-2020 as the number of patients diagnosed during 5 years back in time and still alive at the end of the year of interest, divided by the population count for that year. 20-year prevalence was calculated for year 2020 using the same method. Poisson regression was used to analyse trends in incidence rate and prevalence over time. Survival analysis was performed using the Kaplan-Meier estimate and univariable and multivariable cox regression analyses were performed to examine factors influencing survival.

Ethical considerations

The studies included in this thesis were all approved by the Swedish Ethical Review Authority. The PET-studies that included patients from Aarhus, Denmark, were also approved by the Central Denmark Region Committees on Health Research Ethics. The studies followed the principles of the Declaration of Helsinki. Subjects in the PET studies (paper II and III) were given oral and written information, and signed informed consent before entering the study. These studies were also approved by the Radiation Protection Committee.

The subjects from Uppsala (healthy volunteers, hypertrophic controls and amyloidosis patients) included in paper II and III performed three PET examinations in the same session, with the tracers ^{11}C-PIB, ^{11}C-acetate and ^{15}O-water. For patients enrolled in Aarhus, ^{11}C-PIB and ^{11}C-acetate PET were performed. Together with the PET examinations, a low-dose CT scan of the chest was performed without the use of contrast agent. All three PET-tracers used were approved substances used in clinical practice (^{11}C-PIB is used in Alzheimer's disease), and except from the radiation exposure there are no known side-effects.

The radiation dose from one ^{11}C-PIB PET examination including low dose CT scan is 2.6 mSv, from ^{11}C-acetate 2.2 mSv and from ^{15}O-water 0.9 mSv. The total radiation dose was 5.7 mSv for Uppsala subjects and 4.8 mSv for Aarhus subjects, which can be put in relation to the 1-2 mSv yearly background radiation in Sweden. There were no fertile women examined in the study, and we therefore did not perform any pregnancy tests before examinations.

The subjects needed to be fasting for 4 hours before examination. Two peripheral venous catheters were inserted for tracer injection. The total time for all three PET exams was about four hours inclusive of preparations. 5 of the CA subjects also had an arterial catheter inserted for metabolite analysis, which could have caused some discomfort for the patients, and a risk of bleeding that was reduced by the procedure being performed by an experienced professional and the insertion site carefully observed.

Echocardiography was also performed in association with the PET exams, also in the healthy volunteers, this exam caused no discomfort to the subjects. If any abnormalities were found in the healthy volunteers, they were referred for further evaluation as in clinical routine.

Paper I and IV were retrospective analyses, and a substantial proportion of patients were deceased at the time of data collection. Informed consent was therefore not obtained, and was not required according to the Ethical Review Authority. The review of patient records was approved by the responsible clinic managers at the local hospitals.

Data obtained from the studies was stored in protected files, locked in at the hospital departments. Results used for presentation or publication was deidentified. For the CA and hypertrophic patients included in the PET studies, results of examinations were made available in the patient´s medical records.

In a broader perspective, PET is a relatively expensive imaging method not readily available, and if shown to be valuable in the clinic, the varying accessibility could be an ethical consideration.

Results and discussion

Paper I

During the studied time period 72 patients (39 men and 33 women) with a median age of 59 years (range 41-69) at HDM/ASCT were treated. Organ involvement was renal in 79%, cardiac in 40% and hepatic in 24%. The number of these organs involved were 1 in 57%, 2 in 31% and 3 in 8%, 4% had only gastrointestinal involvement. The patients with renal involvement had a median 24 h urine albumin of 5125 mg (range 505-27300 mg), and in patients with cardiac involvement median cardiac wall thickness was 16.5 mm (range 13-20 mm).

36% received induction therapy before HDM/ASCT, the most common regimens being vincristine/doxorubicin/dexamethasone and cyclophosphamide/dexamethasone. Bortezomib containing induction was not used in any of the patients. Median time from diagnosis to HDM/ASCT was 5.5 months in patients who received induction therapy and 3 months in patients who received HDM/ASCT directly. 97% of patients were treated with HDM/ASCT as part of first-line therapy, and the remaining two patients received HDM/ASCT due to insufficient response to primary treatment. 57% of patients received full dose (200 mg/m^2) melphalan and in the remaining patients, varying degree of dose reductions were made.

Overall response rate, at least partial organ or hematologic response, at any time point, was 64% out of all patients treated, 57% achieved organ response and 7% only hematologic response. Time to response was in median 4 months from HDM/ASCT. In responding patients, 61% had progression during the study period at a median of 3.5 years after HDM/ASCT.

Overall survival from the time of ASCT was in median 98 months (95% CI 61-135) or 8.2 years, with 5-year survival 63.9% and 10-year survival 43.4%. Median survival was significantly longer in patients without cardiac involvement, 135 months (11.3 years) versus 49 months with cardiac involvement, p = 0.001. Significantly longer survival was also seen in patients with one organ involved (median 135 months) compared to two or more organs (median 56 months), p = 0.009. There was no significant difference in survival between the patients who received induction treatment and those who did not, and neither between patients who received a higher dose of melphalan (160-200 mg/m^2) and those who received a lower dose (100-140 mg/m^2).

TRM defined as all mortality within 100 days from ASCT was 12.5%. In patients with cardiac involvement TRM was 17.2%, compared to 9.3% in patients without cardiac involvement. When comparing the earlier time period (1994-2001) to the later (2002-2009), early mortality decreased from 23.8% to 7.8%.

The study showed that long overall survival was achieved with HDM/ASCT. The median overall survival of 8.2 years is similar to the 7-10 years that has been reported from specialized amyloidosis centres (91-93). 22% of patients were alive and without disease progression or further treatment at a median follow-up time of 8.3 years (up to 14.5 years), which shows that a proportion of patients can achieve very long-term remissions. The majority of these patients (63%) had only renal involvement. Median survival in patients with only renal involvement was 13.9 years.

As expected, cardiac involvement was associated with inferior survival. So was involvement of 2 or more organs, however, in the patients with ≥ 2 organs involved, 79% had heart involvement compared to 16% in the group with one organ involved.

Unlike what has been shown in other studies (93, 97, 98), induction therapy did not improve outcome. However, no patients received bortezomib-containing induction, which has shown the greatest benefit (99). Induction therapy can serve as a test of whether the patient is fit for HDM/ASCT, and patients might have died or progressed during induction therapy. However, we did not see an increase in early deaths among patients not receiving induction therapy.

TRM decreased to 7.8% in the later part of the time period (2002-2009). This is in line with results from large amyloidosis centres; TRM of 4.4% is reported from Boston during the period 2003-2011, 6.2% from UK in 2007-2012 and 8.6% from the Mayo Clinic in 2003-2009. All centres have reported a decreasing trend in TRM which has been attributed mainly to a stricter selection of patients for HDM/ASCT. During the period of this study, cardiac biomarkers were not widely used to assess the degree of cardiac involvement but according to the grade of hypertrophy, patients with advanced cardiac involvement were probably treated.

Limitations of the study include the retrospective design which confers inconsistencies in how patients are followed, and a selection bias that leads to patients with less advanced disease being chosen for HDM/ASCT. An "intention-to-treat" analysis was not possible because only patients who went through treatment with HDM/ASCT were identified. Furthermore, the study was performed during a time-period when analysis of FLC and cardiac biomarkers were not widely used, which confers that information on the Mayo stage of patients and the rate of complete remissions are lacking. Furthermore, without the use of cardiac biomarkers, the methods for evaluating cardiac response are insensitive, probably leading to an underestimation of cardiac responses.

In conclusion, long overall survival was reached with HDM/ASCT, even in a decentralized setting. TRM was comparable to that reported from larger centres from this time period and was decreasing over time.

Paper II

At visual inspection, all of the CA patients, both AL and ATTR, were assessed as positive at ^{11}C-PIB PET, and none of the hypertrophic or healthy controls. A higher myocardium/blood SUVR was seen in CA patients compared to controls from 3.5 minutes after ^{11}C-PIB injection. At 10-20 minutes after ^{11}C-PIB injection the largest difference was seen between CA and controls, with median SUVR for AL 2.61 (IQR 2.61), ATTR 1.64 (IQR 0.62), hypertrophic controls 0.88 (IQR 0.26) and healthy controls 0.87 (IQR 0.26), $p < 0.001$ comparing CA patients to controls. RI was also significantly higher in CA than in controls ($p < 0.001$) with median RI at 10-20 minutes for AL 0.086 min^{-1} (IQR 0.075), ATTR 0.045 min^{-1} (IQR 0.014), hypertrophic controls 0.029 min^{-1} (IQR 0.005) and healthy controls 0.033 min^{-1} (IQR 0.005). There was no significant difference between the hypertrophic and healthy controls regarding SUVR ($p = 0.694$) or RI ($p = 0.054$).

ROC curves were used to determine the cut-off values of SUVR and RI that best discriminated CA from controls. The SUVR cut-off 1.09 best separated CA from controls, with AUC (area under the curve) of the ROC curve 0.98 (95% CI 0.94-1.00), sensitivity 94% (95% CI 80-99%) and specificity 93% (95% CI 66-100%). The highest sensitivity and specificity for the method was seen in AL patients where the SUVR cut-off 1.4 separated AL from controls with 100% (95% CI 88-100%) accuracy. With the RI cut-off value 0.037 min^{-1} CA could be separated from controls with a sensitivity of 94% (95% CI 80-99%) and specificity 100% (95% CI 75-100%). The RI cut-off 0.040 could completely discriminate AL from controls.

In the group of patients with systemic amyloidosis but without evidence of cardiac involvement according to echocardiography, 5 out of 11 were PET positive according to both readers, and another 3 according to one of the readers. RI was significantly higher in the predisposed group compared to controls, SUVR was numerically higher but did not reach statistical significance. In 2 of the patients (1 AL and 1 ATTR) endomyocardial biopsies were performed at follow-up, and both were positive for amyloid.

There were some correlations between clinical/echocardiography parameters and SUVR/RI shown in detail in the article.

The study confirmed that ^{11}C-PIB PET can detect cardiac amyloidosis of both AL and ATTR subtype with high accuracy. The method can distinguish cardiac amyloidosis from non-amyloid hypertrophic hearts, as well as healthy,

with high sensitivity and specificity, and may therefore be a valuable tool in the diagnosis of cardiac amyloidosis.

In the group of amyloidosis patients without increased wall thickness, there was a high prevalence of ^{11}C-PIB positivity, indicating that the method is more sensitive than echocardiography. The same finding is reported from a previous study with ^{11}C-PIB PET in ATTR mutation carriers (161).

In comparison to LGE measurement from CMR, that in a meta-analysis showed a sensitivity of 85% and specificity of 92% (162), the accuracy of ^{11}C-PIB PET seem to be at least comparable. ^{11}C-PIB binds directly to amyloid, as opposed to LGE measurement and echocardiography that mainly show indirect signs of amyloidosis, which might make this method more accurate.

Bone scintigraphy has shown high sensitivity and specificity for ATTR CA, but is often negative in AL. In contrast to bone scintigraphy, ^{11}C-PIB PET shows a higher uptake in AL patients compared to ATTR and may therefore be more useful in the diagnosis of AL CA.

There seems to be some correlations between ^{11}C-PIB uptake and clinical/echocardiographic parameters of cardiac involvement, which could indicate that the level of ^{11}C-PIB uptake is partly related to the degree of cardiac involvement. However, ^{11}C-PIB uptake also varies between amyloid fibril types. Pilebro et al showed that ^{11}C-PIB binds with varying affinity for different types (full-length and fragmented) of ATTR fibrils (161). The level of ^{11}C-PIB uptake in AL cases are even more varying than in ATTR, which could indicate differences in ^{11}C-PIB binding depending on the individual fibril structure in AL patients as well.

The degree of ^{11}C-PIB uptake could also be influenced by myocardial blood flow, which could cause less ^{11}C-PIB to be delivered to the tissue. This could contribute to the lower ^{11}C-PIB uptake seen in ATTR since these patients have thicker cardiac walls and thereby possibly lower perfusion. However, a previous pilot-study could not show any association between ^{11}C-PIB uptake and myocardial perfusion measured with ^{11}C-acetate PET (55).

In this study, visual inspection was more accurate than the semi-quantitative measures SUVR and RI. This can be due to heterogenous amyloid deposition that might be missed in SUVR and RI measurement. It may also be easier visually to distinguish uptake in myocardium from spill-in from surrounding tissues. If visual assessment is inconclusive, we recommend the use of the simple measure SUVR at 10-20 minutes after ^{11}C-PIB injection at first hand, and using the stated cut-off value 1.09.

Limitations of the study include the relatively small number of subjects when divided into amyloid subtypes, especially in the prospective group, and also the varying disease stages of patients with the majority of AL patients having received treatment that could possibly affect ^{11}C-PIB uptake.

In conclusion, ^{11}C-PIB PET was highly accurate in detecting CA, especially AL, and could be a useful method to rule in or out amyloidosis in patients with unexplained diastolic heart failure. Findings also indicated

that ^{11}C-PIB PET can detect CA at an earlier stage compared to echocardiography and might be a useful tool for early diagnosis of CA.

Paper III

MVO_2 was significantly reduced in CA patients compared to healthy controls (0.08 vs 0.10 mL/min/g, p = 0.003), but not significantly different in CA compared to hypertrophic controls. MVO_2 did not differ significantly between AL and ATTR (0.08 vs 0.07 mL/min/g, p = 0.252), and was not significantly different in surviving compared to deceased CA patients, neither in AL nor in ATTR.

MEE was significantly lowered in CA compared to both hypertrophic and healthy controls (14 vs 28 and 27% respectively, p <0.001), but without significant difference between deceased and surviving CA patients. There was no significant difference between AL and ATTR patients.

The ratio of FSV and LVM from ^{11}C-acetate PET was also significantly reduced in CA, compared to both hypertrophic and healthy controls (0.29 vs 0.58 and 0.82 mL/g, p = <0.001) and also significantly lowered in deceased patients compared to survivors. FSV/LVM was significantly lower in ATTR compared to AL.

In ROC analysis the best cut-off value of MEE in discriminating deceased from surviving CA patients was 15.7% with AUC 0.63 (95% CI 0.47-0.79). FSV/LVM was the ^{11}C-acetate PET parameter that best discriminated deceased from surviving, both AL and ATTR patients. The optimal prognostic cut-off value was 0.27 mL/g with AUC 0.72 (95% CI 0.57-0.87), in AL patients the best cut-off was 0.39 mL/g.

When using the cut-off values above, survival was significantly longer with high MEE and FSV/LVM compared to low (p = 0.032 and p < 0.001 respectively). When analysing AL and ATTR separately there was a significant survival difference with high compared to low FSV/LVM, but not significant for MEE.

In univariable analysis, NYHA-class, Nt-proBNP and the ^{11}C-acetate PET parameters FSV/LVM and MEE were the strongest prognostic factors. Out of the ^{11}C-acetate PET parameters, FSV/LVM was the strongest survival predictor with hazard ratio 0.56 per 0.1 mL/g (95% CI 0.39-0.81, p = 0.002), and independently prognostic in a multivariable model including the strongest prognostic ^{11}C-acetate PET parameters MEE and FSVI. FSV/LVM remained significantly prognostic in bivariable analysis with all the other univariable predictors.

The study showed that MEE and FSV/LVM are reduced in cardiac amyloidosis and that lower levels of both MEE and FSV/LVM are associated with inferior survival. The ^{11}C-acetate PET parameter that best predicted survival was

FSV/LVM, this measure is similar to MCF that was previously shown to be prognostic in CA (139). MCF is calculated as LV stroke volume by LV myocardial volume, and usually the geometric total SV is used, and not the forward SV. This might be a disadvantage with MCF, since the presence of valvular insufficiencies often seen in CA is not taken into account.

With increasing amyloid burden, the stroke volume decreases and mass increases, which should make FSV/LVM a sensitive marker of disease progression. FSV/LVM was lowered in the surviving CA patients as well, which suggests that it is not only a marker of advanced disease. In a previous study, SV had a high probability of being abnormal from early disease stages and MCF had a progressively increasing likelihood of being abnormal with higher amyloid burden (139).

In this study, FSV/LVM was a better prognostic marker than all the echocardiographic parameters. However, due to the retrospective nature of the study, LS was missing in a substantial proportion of CA patients (11 out of 48), and was therefore not included in the survival analyses. LS has previously been shown to be highly prognostic in CA (134).

MEE was significantly reduced in CA, which reflects poor energetic efficiency. The measure incorporates FSV/LVM and also MVO_2. The inclusion of oxidative metabolism did not seem to add prognostic value to MEE in either subtype of CA. However, MVO_2 is measured in relation to myocardial mass, which in amyloidosis consists not only by viable myocytes but also extracellular amyloid deposition that is not contributing to the oxygen metabolism. If correction for extracellular mass was performed, MVO_2 would probably be higher.

FSV/LVM or MCF can be measured with other PET perfusion tracers, as well as with CMR or echocardiography, and in patients that are evaluated for cardiac symptoms lowered values might be indicative of cardiac amyloidosis.

Limitations of the study include the low patient numbers, especially when separated in AL and ATTR, the groups are too small to draw conclusions about differences between the subtypes. Different mechanisms are involved in AL compared to ATTR CA, including more blood vessel involvement as well as toxic effects on the myocytes in AL amyloidosis, making it reasonable to analyse the two groups separately. Furthermore, including measurement of ECV by for example using combined PET/CMR would be valuable for correction of MVO_2 for different levels of ECV.

In conclusion, reduced MEE was associated with shorter survival in CA patients, but FSV/LVM was the strongest survival predictor and the only independently prognostic ^{11}C-acetate PET parameter in multivariable analysis.

Paper IV

During 2000-2020 a total of 302 new cases of systemic AL amyloidosis were identified, 76 from Uppsala and 226 from Stockholm County. The crude incidence rate for Uppsala County was 10.8 (95% CI 8.6-13.5) per million person-years, and the age- and sex-adjusted incidence rate was 12.0 per million person-years (95% CI 9.3-14.7). There was no significant change in the incidence rate during the studied time period, p = 0.153. The 5-year limited duration prevalence increased numerically during the period from 9.9 cases per million inhabitants in 2004 to 38.6 in 2020, but the trend was not statistically significant (p = 0.434). The 20-year prevalence for 2020 was 48.9 (95% CI 31.3-76.4) per million inhabitants for Uppsala County.

Baseline and treatment characteristics are presented in detail in the manuscript. Comparing the earlier part of the studied time period (2000-mid 2010) to the later (mid 2010-2020), significantly fewer patients were diagnosed at autopsy in the later period, and the proportion of patients receiving palliative treatment was significantly lower. First-line therapy differed markedly between the time periods, with a significant increase in the use of proteasome inhibitor, IMiD and CD38-anibody based treatments in the later time period, and a decrease in the use of chemotherapy based regimens (except for high dose chemotherapy). The use of high dose therapy was not significantly different between time periods, but induction therapy before HDM/ASCT was used more in the later period.

Median overall survival from the time of diagnosis was 21 months (95% CI 14.5-27.5). In the later time period survival was significantly longer than in the early period, 28 months (95% CI 18.3-37.7) compared to 13 months (95% CI 7.6-18.4), p < 0.001. Significantly shorter survival was seen in patients with cardiac involvement (HR 2.04, 95% CI 1.41-2.93, p < 0.001) and in patients aged > 70 years at diagnosis (HR 1.54, 95% CI 1.16-2.06, p = 0.003). Patients who underwent high dose chemotherapy had superior survival (HR 0.30, 95% CI 0.17-0.53, p < 0.001), median survival in patients receiving high dose therapy was 266 months (95% CI 77.5-454.5) or 22.2 years.

Mortality within 6 months from diagnosis was less frequent in the later time period compared to the earlier (25.6% vs 38.2%, p = 0.022). Heart involvement and not receiving high dose therapy was associated with increased risk of early death, and causes of early death were predominantly heart related. TRM defined as all death within 100 days of ASCT was reduced from 15.8% in the early time period to 5.3% in the late period.

In this study that included only confirmed cases of systemic AL amyloidosis, we showed that the standardized incidence was 12.0 (95% CI 9.3-14.7) per million person-years for Uppsala County of Sweden, which is consistent with the incidence of 12 (95% CI 8-16) per million person-years previously

reported from Olmsted County, Minnesota using similar methodology (26). The incidence did not change significantly during the studied time period.

We believe that this study as near as possible captures all cases diagnosed with AL amyloidosis in this region, and since only confirmed cases are included, accuracy should be higher than for incidence reports based on estimations from registries. Since the incidence is consistent with that reported from Olmsted County, Minnesota it should be generalizable to populations of the Western world. However, there are indications of varying incidence of plasma-cell disorders including AL amyloidosis between ethnicities, and the incidence could probably not be applicable to all populations globally.

With regards to the prolonged survival that is reported in our and other studies, an increase in prevalence would be expected over time. In our study we could not show any statistically significant increase in the 5-year prevalence during the studied time period. Other studies have reported an increasing prevalence of AL amyloidosis over time (28, 30, 34).

This study includes a truly population-based cohort of patients, which is reflected by the higher median age, larger proportion of patients receiving palliative treatment, and inferior survival compared to what is reported from a European observational study (2). In our study there was a significant reduction in patients diagnosed at autopsy, and in patients receiving palliative treatment between the early and late part of the time period, which can indicate that patients are being diagnosed at an earlier disease stage. There has also been a shift in treatment pattern between the early and late time period, that has probably contributed to the significant improvement in survival that is observed.

There was also a significant reduction in early mortality (< 6 months from diagnosis) in the later time period, which is encouraging since the European study mentioned above reported consistently high early mortality over time (2). The reduction in early deaths is probably mainly due to earlier diagnosis. In the randomized ANDROMEDA study, more effective treatment with the addition of daratumumab did not affect early mortality (< 60 days from starting treatment), so the change in treatment patterns has probably impacted less on the reduced early mortality. As expected, cardiac involvement was the most important risk factor for both overall and early mortality.

Treatment with HDM/ASCT was associated with better prognosis, both long-term and within 6 months from diagnosis. This is likely at least partly due to selection of patients with less advanced organ involvement for HDM/ASCT. There was a significant reduction in TRM between the early and late time period, which can indicate more adequate patient selection. The frequency of HDM/ASCT did not differ significantly between time periods, but if patients generally were diagnosed earlier, there could have been a selection of patients with less advanced disease for HDM/ASCT. The use of induction therapy, and mainly bortezomib based induction was also higher in the later

time period, which has previously been associated with improved outcome after HDM/ASCT (93).

Limitations of the study include the homogenous population with the vast majority being of Caucasian origin, reducing the generalizability of the incidence to other populations. Although this study was based on around twice as many cases as the previous comparable incidence study from Olmstead County (26), the number is still quite small. We also lack information on important prognostic factors such as cardiac stage and treatment response.

In conclusion, we could determine the standardized incidence for systemic AL amyloidosis to 12.0 (95% CI 9.3-14.7) per million person years for Uppsala County, which is consistent with previous similar reports. There was no significant change in the incidence rate over time. A numerical increase in the 5-year prevalence was observed, but the trend was not statistically significant. Prolonged overall survival and decreased early mortality was seen over time.

Concluding remarks and further perspectives

In **paper I** we could confirm that long overall survival and long-term remissions can be obtained with HDM/ASCT in AL amyloidosis, also in decentralized settings. The rate of TRM is decreasing over time and, as shown in paper IV and other studies, has continued to decrease. TRM is getting close to what is seen with HDM/ASCT in myeloma, indicating that the current selection criteria are accurate. In paper IV, that included a later time period, we could also show that median survival in high dose treated patients was as long as 22 years.

However, with the recently introduced more effective first-line therapy daratumumab-VCd, 53% of patients reached CR. The current recommendation is not to proceed to HDM/ASCT if CR is already reached, even though there is lack of data on PFS with daratumumab-VCd only, compared to HDM/ASCT. With regards to the effectiveness of the current standard first-line treatment, results regarding PFS from a randomized study would take time, and validated surrogate endpoints are currently lacking. MRD with bone marrow flow cytometry is used in myeloma, but studies indicate that this is not a highly sensitive measure of deep response in AL amyloidosis, and more studies are needed on blood mass spectrometry in this situation. According to paper IV the frequency of high dose treatment was not significantly different between time periods. However, the most recent time period, after introduction of the daratumumab-VCd treatment and updated treatment guidelines, was not included and likely the rate of high dose therapy will decrease.

In **paper IV** we could show that the population-based incidence of AL amyloidosis in Sweden is around 12 per million person-years and has not changed significantly over the last two decades. Our study indicates that there is still an underdiagnosis since a proportion of patients are not diagnosed until autopsy, and that patients are still diagnosed in late stages of cardiac involvement when prognosis is poor. This again highlights the need for increased awareness of this disease, especially among cardiologists. The increasing treatment possibilities in both AL and ATTR will hopefully lead to more patients being evaluated for cardiac amyloidosis in general. When investigating patients with possible CA, looking for a monoclonal protein should be done early as a screening method for AL, as well as bone scintigraphy for ATTR. In unclear cases, our study (**paper II**) shows that ^{11}C PIB PET can be used to rule in or out CA, especially of AL subtype, with high accuracy. Our and other studies

have shown that amyloid PET as well as CMR can detect cardiac AL amyloidosis earlier than echocardiography, and even earlier than Nt-proBNP according to a study by Cohen et al (63). Recently, consensus guidelines (47) have included typical findings on amyloid PET or CMR as criteria for cardiac involvement of AL amyloidosis.

However, the exact role for PET and CMR in CA diagnostics is not yet established, and studies are lacking directly comparing the accuracy of the two methods. Availability and cost may also be issues with both of these modalities compared to echocardiography. Regarding CMR, ECV measurement has shown higher accuracy compared to LGE in detecting CA, with a sensitivity of 89% and specificity of 98.6% reported from a meta-analysis including both AL and ATTR CA (163). Furthermore, T1 and T2 mapping has shown high diagnostic performance (164), with the advantage of not requiring contrast. Amyloid PET might have the advantage of possibly being able to distinguish between amyloid subtypes. A study with ^{18}F-florbetaben showed delayed tracer uptake in AL patients compared to ATTR, that could differentiate the two groups (56). A potential role of ECV measurement in follow-up after treatment has been shown (157, 158), whereas the possible role of amyloid PET in follow-up after treatment has not yet been evaluated.

When it comes to prognostication of cardiac AL amyloidosis, we could show in **paper III** that the ^{11}C-acetate PET parameter FSV/LVM is highly prognostic in CA. This measure can be obtained from other imaging modalities as well, and except from being a prognostic marker it might serve as an indication of possible CA diagnosis. Both PET and CMR can be used to non-invasively assess the coronary flow reserve, in the investigation of patients with suspected coronary syndromes (165). In this situation, the finding of a lowered FSV/LVM can give an indication of cardiac amyloidosis.

In our study we were not able to compare FSV/LVM to the other proposed prognostic imaging parameters LS and ECV, and we did not have enough AL patients to draw robust conclusions in this group separately. The pathophysiology of cardiac amyloidosis is complex, especially in the AL subtype, several mechanisms leading to cardiac dysfunction are involved. Compared to ATTR, AL patients have more vascular amyloid deposition, higher grade of myocardial oedema and more toxic effects on the myocytes involved. The degree of influence of these various components may vary between AL patients, and also at different stages of the disease. To evaluate the effects on for example oxidative metabolism, larger study groups are needed, and also combined PET/CMR studies to be able to correct for ECV.

Populärvetenskaplig sammanfattning på svenska

Amyloidos är en grupp sjukdomar som kännetecknas av inlagring av amyloid (aggregat av olika typer av proteiner) i olika organ i kroppen. Detta leder till att organen med tiden fungerar allt sämre. Vilken slags amyloidos det rör sig om definieras utifrån den typ av protein som lagrats in. Den allmänt mest kända amyloidossjukdomen är Alzheimers sjukdom där proteinet beta-amyloid lagras in i hjärnan. När det gäller systemisk amyloidos som drabbar flera organ i kroppen är lättkedje (AL) och transthyretin (ATTR) amyloidos de två vanligaste typerna.

Vid AL-amyloidos är den grundläggande orsaken en patologisk klon av plasmaceller (antikroppsbildande vita blodkroppar) i benmärgen som producerar avvikande lätta immunglobulinkedjor, det vill säga delar av antikroppar. Dessa har förmågan att bilda amyloidstruktur och lagras in i olika organ. Orsaken till att man drabbas av sjukdomen är okänd. Det är en ovanlig sjukdom som är något vanligare hos män än hos kvinnor, och oftast drabbar personer i 60–70 års åldern.

Hjärtat är ett av de vanligaste organen som kan drabbas vid både AL- och TTR-amyloidos vilket är särskilt allvarligt på grund av hög dödlighet i hjärtsvikt och rytmrubbningar. Vid AL-amyloidos ses hjärtengagemeng hos cirka 80% och graden av hjärtpåverkan är den viktigaste faktorn för överlevnad vid sjukdomen. Sjukdomen kan indelas i stadier utifrån framför allt nivån av hjärtbiomarkörer, vilket ger information om prognosen. Vid det svåraste stadiet som betecknas IIIb är överlevnaden endast cirka 6 månader trots behandling.

AL-amyloidos kan yttra sig på en mängd olika sätt beroende på vilka organ som drabbas, och fynden vid undersökningar är inte specifika för sjukdomen. Därför är det vanligt att diagnosen fördröjs och att patienterna har utvecklat en allvarlig organpåverkan när diagnosen ställs. Detta medför sämre möjlighet för patienten att tolerera behandling, och mindre chans att organfunktionen ska kunna återhämta sig. Det är alltså viktigt att diagnosen ställs tidigt i förloppet för prognosen vid sjukdomen.

Dagens behandling riktar sig mot de avvikande plasmacellerna i benmärgen, som är grundorsaken till sjukdomen. Cellgifter av olika typ används liksom modernare mer riktade typer av behandlingar.

Arbete I syftade till att undersöka långtidsresultaten av högdos cellgiftsbehandling (melfalan), med stöd av egna stamceller, vid AL-amyloidos.

Behandlingen är omdiskuterad på grund av den höga dödligheten i samband med behandling som tidigare har rapporterats. Studier från specialiserade amyloidoscentra har visat minskad dödlighet vid behandling på senare tid, och bättre resultat om behandling utförs vid centra med hög erfarenhet. Någon tidigare studie av behandlingsresultat från Sverige, där denna vård är decentraliserad, har inte gjorts. Studien syftade till att utvärdera effekt, överlevnad och behandlingsrelaterad mortalitet efter högdos cellgiftsbehandling vid AL-amyloidos, och om någon förändring avseende dödlighet i samband med behandling har skett över tid i Sverige.

Vi fann att 64% av patienterna hade effekt av behandlingen. Överlevnaden från behandling var i median 8,2 år, med 5-årsöverlevnad 63,9% och 10-årsöverlevnad 43,4%. Längre överlevnad sågs hos de patienter som inte hade hjärtengagemang, och hos de där endast ett organ var påverkat av amyloidos. Vi fann ingen säker skillnad beroende av om förbehandling med cellgifter gavs innan högdosbehandlingen eller inte, och inte heller beroende av om full dos melfalan eller reducerad dos gavs. Dödligheten inom 100 dagar från stamcellsinfusion var 12,5%, och högre hos patienter med hjärtengagemang. Sett över tid minskade dödligheten från 23,8% under den tidigare delen av den studerade perioden jämfört med 7,8% under den senare.

I **arbete II** undersökte vi en ny metod för att diagnostisera hjärtamyloidos i ett tidigt skede. Avbildningsmetoden positronemissionstomografi (PET) med spårämnet ^{11}C-PIB har använts för att påvisa amyloidos vid Alzheimers sjukdom. ^{11}C-PIB binder till amyloid av olika typer, och har i pilotstudier visats kunna påvisa amyloidos i hjärtat. I studien undersökte vi 36 patienter med hjärtamyloidos, både AL och ATTR, samt 8 friska kontroller och 7 kontroller med förtjockat hjärta av annan orsak än amyloidos. Samtliga forskningspersoner genomgick PET av hjärtat med användning av spårämnet ^{11}C-PIB, samt även hjärtultraljud. Upptaget av ^{11}C-PIB i hjärtat värderades både visuellt som positivt/negativt och genom beräkning av måtten SUVR och RI vilka ger en kvantifiering av ^{11}C-PIB-upptaget. De gränsvärden för SUVR och RI som bäst skiljde ut patienterna med hjärtamyloidos från kontrollerna applicerades sedan på en grupp amyloidospatienter utan diagnostiserat hjärtengagemang enligt gängse kriterier. Alla undersökningar utvärderades blindat av två oberoende observatörer med expertis inom hjärt-PET.

Vi fann att alla patienter med hjärtamyloidos var positiva vid PIB-PET utifrån den visuella bedömningen, men ingen av kontrollerna, varken de friska eller de med hjärtförtjockning av annan orsak. Både SUVR och RI var högre hos hjärtamyloidos patienterna jämfört med kontrollerna. SUVR-gränsvärdet 1,09 kunde skilja hjärtamyloidos från kontroller med högst sensitivitet (94%) och specificitet (93%), och för RI gav tröskelvärdet 0,037 min^{-1} högst sensitivitet (94%) och specificitet (100%). Högre sensitivitet och specificitet för båda måtten sågs hos AL patienter jämfört med ATTR. I gruppen utan diagnostiserat hjärtengagemang var 5 av 11 positiva vid visuell bedömning av

båda observatörerna. Hos två av de PET-positiva patienterna utfördes hjärtbiopsi vid uppföljning som utföll positivt för amyloidos.

Arbete III syftade till att utvärdera om mått på hjärtats verkningsgrad (MEE, myocardial external efficiency) kan användas för prognostisk information vid hjärtamyloidos. PET av hjärtat med spårämnet ^{11}C-acetat kan användas för att mäta hjärtats syrgasförbrukning och ger även mått på mekaniskt arbete såsom slagvolym. Därmed kan hjärtats verkningsgrad beräknas. Kvoten mellan slagvolym och vänsterkammarmassa har i tidigare studier visats vara prognostiskt vid hjärtamyloidos. Vi ville undersöka om hjärtats syrgasförbrukning och verkningsgrad bättre kunde förutsäga överlevnad. 48 patienter med hjärtamyloidos (både AL och ATTR), samt 20 kontroller undersöktes med ^{11}C-acetat PET och hjärtultraljud. Patienterna följdes upp med avseende på överlevnad. Parametrar från undersökningarna utvärderades statistiskt vad gäller deras inverkan på överlevnaden.

Studien visade att MEE var sänkt hos patienterna med hjärtamyloidos jämfört med kontrollerna, men utan statistiskt säkerställd skillnad mellan de som avlidit och de överlevande. Det tröskelvärde avseende MEE som bäst kunde skilja de avlidna från de överlevande var 15,7%. Överlevnaden var sämre för patienterna med MEE <15,7% jämfört med för de med MEE>15,7%, dock var skillnaden inte statistiskt säkerställd när AL- och ATTR-amyloidos analyserades var för sig. Bäst separation mellan avlidna och överlevande erhölls med kvoten mellan slagvolym och massa (FSV/LVM) där patienter med nivå under tröskelvärdet 0,27 ml/g hade tydligt sämre överlevnad, en skillnad som var statistiskt säkerställd även för AL- och ATTR-amyloidos för sig. När effekten på överlevnad av flera ^{11}C-acetat PET parametrar undersöktes tillsammans, fann vi att FSV/LVM var det mått som bäst kunde förutsäga överlevnaden.

I **arbete IV** ville vi undersöka förekomsten och överlevnaden av AL-amyloidos i Sverige, detta då det råder brist på sådana populationsbaserade epidemiologiska studier inom AL-amyloidos. Eftersom sjukdomen alltid diagnostiseras med vävnadsbiopsi sökte vi efter alla patienter som under perioden år 2000–2020 erhållit diagnosen amyloidos i databaserna på patologlaboratorierna vid Akademiska sjukhuset i Uppsala och Karolinska sjukhuset i Stockholm. Alla utlåtanden från vävnadsbiopsierna granskades, och för alla oklara fall utifrån patologutlåtandet liksom alla identifierade fall av AL-amyloidos utfördes journalgranskning. Endast patienter folkbokförda i Uppsala respektive Stockholms län med verifierad nydiagnostiserad AL-amyloidos under den aktuella tidsperioden inkluderades. Incidensen (antal nya fall per invånare och år) och prevalensen (antal som lever med diagnosen efter en viss tidsperiod) samt överlevnaden undersöktes, liksom eventuell förändring över tid.

Vi fann en incidens av systemisk AL-amyloidos, standardiserad utifrån befolkningens ålder och kön, på 12 nya fall per miljon invånare och år. Incidensen ökade inte signifikant under perioden. 5-års prevalensen ökade under perioden, men trenden var inte statistiskt säkerställd. Medianöverlevnaden från diagnos var 21 månader, och ökade från 13 månader för patienter

diagnostiserade under den tidigare delen av tidsperioden till 28 månader under den senare delen. Sämre överlevnad sågs hos patienter med hjärtengagemang, ålder> 70 år vid diagnos och hos patienter som inte genomgick högdos cellgiftsbehandling. Tidig dödlighet inom 6 månader från diagnos minskade från 38,2% under den tidigare delen av tidsperioden, till 25,6% under den senare delen.

Sammanfattningsvis har denna avhandling bidragit med kunskap inom epidemiologi, diagnostik, behandling och prognostisering vid AL-amyloidos, särskilt när det gäller patienter med hjärtengagemang. Vi har visat att goda resultat kan uppnås med högdos cellgiftsbehandling vid AL-amyloidos även vid decentraliserad vård. Dock är det i dagsläget oklart hur denna behandling står sig i relation till modern konventionell behandling, där jämförande studier hittills saknas. Vi har även visat att PET med spårsubstansen ^{11}C-PIB är en metod som kan diagnostisera hjärtamyloidos med mycket hög träffsäkerhet. Metodens plats i relation till andra diagnostiska metoder vid hjärtamyloidos behöver dock studeras ytterligare. När det gäller faktorer som kan förutsäga överlevnad vid hjärtamyloidos visar vår och andras studier att kvoten mellan slagvolym och vänsterkammarmassa är starkt prognostisk för överlevnad, och kan addera information till kända prognostiska markörer. Vi har även kunnat beräkna incidensen av AL-amyloidos i Sverige, och visa att den totala liksom den tidiga överlevnaden har förbättrats över tid vilket indikerar att tidigare diagnostik och förbättrad behandling av patienterna har givit resultat.

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Paper I

ORIGINAL ARTICLE

Outcome of AL amyloidosis after high-dose melphalan and autologous stem cell transplantation in Sweden, long-term results from all patients treated in 1994–2009

S Rosengren[1], U-H Mellqvist[2], H Nahi[3], K Forsberg[4], S Lenhoff[5], O Strömberg[3], L Ahlberg[6], O Linder[7] and K Carlson[1]

High-dose melphalan and autologous stem cell transplantation (HDM/ASCT) is widely used in immunoglobulin light chain (AL) amyloidosis, but the benefit is debated mainly because of the high treatment-related mortality (24% in a randomised study comparing HDM/ASCT with oral melphalan/dexamethasone). We report here on the long-term outcome of all patients treated with HDM/ASCT for AL amyloidosis in Sweden between 1994 and 2009. Seventy-two patients were treated at eight Swedish centres. Median follow-up was 67.5 months. At least partial response (organ or haematological) was seen in 64% of the patients. Median overall survival was 98 months or 8.2 years, with 5-year survival 63.9% and 10-year survival 43.4%. In patients with cardiac involvement or multiple organ involvement, survival was significantly shorter, median overall survival 49 and 56 months, respectively. All mortality within 100 days from ASCT was 12.5% for all patients and 17.2% in the patients with cardiac involvement. For patients treated in the earlier time period (1994–2001), 100-day mortality was 23.8% compared with 7.8% in the later period (2002–2009). In conclusion, long survival times can be achieved in patients with AL amyloidosis treated with HDM/ASCT, also in smaller centres. Early mortality is high, but with a decreasing trend over time.

INTRODUCTION

AL amyloidosis is a clonal plasma cell disorder in which excessive Ig light chains are produced and form amyloid fibrillar deposits in, for example, heart, kidney and liver that eventually lead to organ failure and death. Especially in patients with heart involvement, mortality is high owing to progressive heart failure and arrhythmia.[1] The prognosis is poor; untreated and also when treated with oral melphalan and prednisone, survival is about 1–2 years. In patients with symptomatic cardiac amyloidosis, median survival without treatment is only 6 months. Involvement of multiple organs is also a poor prognostic sign.[2] Because of the organ dysfunction, patients with AL amyloidosis are sensitive to the toxic effects of chemotherapy, which may limit the treatment possibilities.

Treatment with high-dose melphalan and autologous stem cell transplantation (HDM/ASCT) has resulted in prolonging survival to 4–6 years, but the benefit is debated mainly because of the high treatment-related mortality that has been around 15%.[3–7] The advantage with HDM/ASCT could also be overestimated because of selection bias, although a case–control study with 63 AL patients undergoing HDM/ASCT and 63 matched controls showed longer survival for the patients treated with HDM/ASCT, with 4-year overall survival rates of 71% compared with 41%.[8] In one randomised study, HDM/ASCT was not shown to be superior to standard-dose melphalan plus high-dose dexamethasone,[7] however, patient selection for HDM/ASCT was not optimal in that study and treatment-related mortality was high (24%). If prognostic markers such as cardiac troponins

and NT-proBNP are used to select patients for HDM/ASCT, outcome can be improved.[9]

In Sweden, patients with AL amyloidosis have been treated with HDM/ASCT since 1994, but no evaluation of the outcome has been made previously, and it is not known whether the increased knowledge about, for example, patient selection has improved the results in our country. The purpose of this retrospective study was to examine the long-term outcome of HDM/ASCT in Sweden for the first 16 years since the treatment was introduced, and whether the results have changed over time.

SUBJECTS AND METHODS

Data were collected retrospectively on all patients treated with HDM/ASCT from 1994 until 2009 through contacts at the eight centres in Sweden where the treatment was performed. Follow-up was ended in April 2014. The study was performed with approval of the local Ethics Committee. Only patients with amyloid classified as AL were included. Patients with AL amyloidosis associated with lymphoma or symptomatic myeloma, and those with only soft-tissue involvement were excluded. Symptomatic myeloma was defined as 10% or more plasma cells in the bone marrow and skeletal changes, or other CRAB criteria attributable to myeloma.

Criteria from the 10th International Symposium on Amyloid and Amyloidosis were used to establish organ involvement, response and progression.[10] The aim was to collect data from diagnosis, at admission for HDM/ASCT, 3 months, 6 months, 1 year and 2 years after HDM/ASCT, at best response and at progression. Although in many patients, data from all these occasions were not available.

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The majority of patients were treated in a time period when free light chain analysis was not in clinical use. Only in 16 of the 72 patients (22%), was free light chain analysis available for follow-up.

IBM SPSS version 21 (Armonk, NY, USA) was used for statistical analyses. Survival was estimated using Kaplan–Meier Analysis, and the log-rank test was used to evaluate differences between survival curves. The χ^2-test was used to compare categorical variables.

RESULTS

Seventy-two patients (39 men and 33 women) were treated with HDM/ASCT between 1994 and 2009. Median age at time of treatment was 58.5 years (range 41–69). Median follow-up was 67.5 months (range 0–232) from the time of HDM/ASCT.

Fifty-two per cent had elevated plasma cells (3% or more) in the bone marrow, with a median plasma cell count of 7.3%. Forty-one per cent had M-protein in the blood with a median level of 8 g/L and 48% had measurable Bence Jones proteinuria (median 110 mg/L).

Fifty-seven patients (79%) had kidney involvement, 29 (40%) had cardiac involvement and 17 (24%) had liver involvement. In six patients (8%), all three of these organs were involved, in 22 patients (31%), two of the organs were involved and in 41 patients (57%), one of the organs was involved. In three patients (4%), none of these three organs was involved, these patients had only gastrointestinal involvement.

In the patients with renal involvement, the median 24 h urine albumin was 5125 mg per day (range 505–27 300). The patients with heart involvement had a median cardiac wall thickness of 16.5 mm (range 13–20).

Twenty-four patients (36%) had received induction treatment before HDM/ASCT; VCR/adriamycin/dexamethasone ($n = 10$), cyclophosphamide/dexamethasone ($n = 6$), melphalan/prednisone ($n = 4$), VCR/adriamycin/cyclophosphamide/prednisone ($n = 3$), only steroids ($n = 2$) and cyclophosphamide/VCR/prednisone ($n = 1$). Stem cells were mobilised with G-CSF alone (69%, $n = 50$), cyclophosphamide and G-CSF (28%, $n = 20$) or other regimens (3%, $n = 2$). In all except two patients, HDM/ASCT was performed as part of primary therapy. The remaining two patients were initially treated with oral melphalan and prednisone with insufficient response.

The given dose of melphalan was 200 mg/m^2 in 41 patients (57%), 160 mg/m^2 in 1 patient (1%), 140 mg/m^2 in 18 patients (25%), 120 mg/m^2 in 1 patient (1%) and 100 mg/m^2 in 11 patients (15%). The reasons for dose reduction are not known.

The median time from diagnosis to HDM/ASCT was 5.5 months (range 2–126) for the patients that were treated before HDM/ASCT and 3 months (range 1–29) for the patients who did not receive induction treatment.

Response (at least partial response), either organ or haematological, was seen in 46 patients (64%); 41 patients (57%) had organ response and 5 patients (7%) had only haematological response. Median time to response was 4 months (range 1–16).

Median event-free survival, measured from the time of SCT until progression (organ or haematological) or death, was 41 months (range 0–175; Figure 1).

Treatment at progression was melphalan/prednisone or dexamethasone ($n = 10$), velcade/dexamethasone ($n = 8$), thalidomide ($n = 4$), VCR/adriamycin/dexamethasone ($n = 2$), only steroids ($n = 2$), cyclophosphamide/velcade/dexamethasone ($n = 1$), cyclophosphamide/thalidomide/dexamethasone ($n = 1$) and velcade/melphalan/dexamethasone ($n = 1$). Eight patients were not treated at progression.

Indication for treatment could be either organ or haematological progression.

Five patients underwent a second HDM/ASCT, in all cases at first progression after the initial HDM/ASCT. Median time between the first and second HDM/ASCT was 9.8 years (range 1.5–10.3). Four out of the five patients achieved at least partial response after the second HDM/ASCT. Three of these patients have received treatment again after the second HDM/ASCT, after a period of 6.5, 2.1 and 2.1 years, respectively, and in the fourth patient, disease progression has not occurred 1.5 years after the second HDM/ASCT.

Overall median survival, measured from the time of SCT until death or end of follow-up, was 98 months (range 0–232) or 8.2 years, with 1-year survival 77.8%, 5-year survival 63.9% and 10-year survival 43.4%. For patients without cardiac involvement, median survival was significantly longer, 135 months compared with 49 months ($P = 0.01$) in the patients with cardiac involvement (Figure 2). Patients with only one involved organ also had significantly longer survival with median survival 135 months
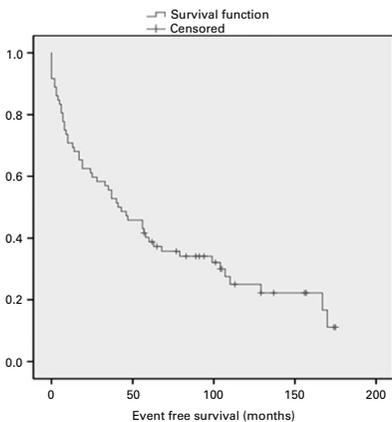
Figure 1. Event-free survival measured from SCT until progression or death. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.
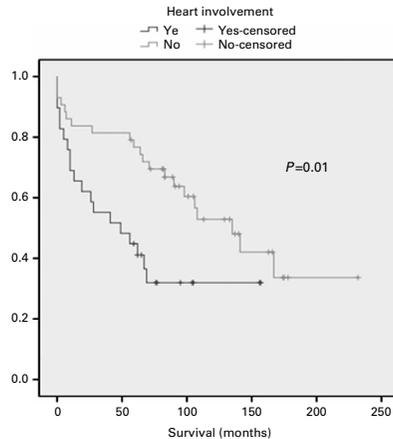
Figure 2. Survival according to heart involvement. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.
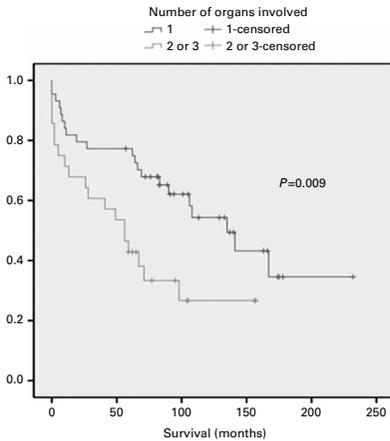
Figure 3. Survival according to the number of organs involved. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.

Table 1. Complications considered treatment-related

Complication	Number of patients
Febrile neutropenia	47 (64.4%)
Severe mucositis	17 (23.3%)
Renal failure (new or significantly worsened)	6 (8.2%)
Atrial arrhythmia	5 (6.8%)
Heart failure (new or significantly worsened)	3 (4.1%)
Sudden cardiac death	3 (4.1%)

Figure 4. The total number of patients treated each year. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.

compared with 56 months in patients with two or more involved organs, $P = 0.009$ (Figure 3). There was no significant difference in survival between the patients who had received treatment before HDM/ASCT and those who had not.

Median survival for patients who received a higher dose of melphalan (160–200 mg/m^2) was 124 months compared with 82 months with a lower dose (100–140 mg/m^2), but the difference was NS, $P = 0.175$.

Mortality within 100 days from ASCT was 12.5% for all patients. Causes of death were sudden cardiac death ($n = 3$), heart failure ($n = 3$) and infection ($n = 3$). Half of the patients that died from cardiac causes had received induction treatment. All complications that were considered treatment-related are listed in Table 1.

For patients with heart involvement, 100-day mortality was higher than for those without heart involvement, 17.2% compared with 9.3% (NS). Patients treated in the earlier period (1994–2001) also had higher mortality the first 100 days, 23.8% compared with 7.8% (NS) in the later period (2002–2009). The proportion of patients with cardiac amyloidosis or involvement of two or more organs did not differ significantly between the earlier and later time period.

Six patients have received a kidney transplant. Four of them received the transplant after HDM/ASCT and one of them before. In one of the patients, kidney transplantation was performed directly prior to a second HDM/ASCT. Five of the six patients receiving renal transplants are alive, and not on dialysis, with a median follow-up of 140 months (11.7 years). The sixth patient died in disease progression 4.5 years after HDM/ASCT.

Two patients have received a heart transplant, one before and one after HDM/ASCT, both these patients died (2.5 and 4.5 years after the heart transplant).

The number of patients treated with HDM/ASCT during the studied 16-year period varied between 5 and 11 per million inhabitants in the different regions of the country. During the studied period, between 0 and 11 patients were treated per year, with a tendency towards fewer patients in the later years (Figure 4).

DISCUSSION

This study shows that HDM/ASCT, to a substantial degree, leads to response and, in some patients, long-term remission is achieved. The response rate of 64% is comparable to results reported previously.[4,11] Overall median survival for the whole patient group was 98 months (8.2 years), with the longest surviving patient being alive 19 years after treatment. Five- and 10-year overall survival was 64% and 43%, respectively. These results are notably good considering that treatment was performed in relatively small centres not specialised in amyloidosis, and includes patients treated in a period when experience with HDM/ASCT in AL amyloidosis was limited. A small number of patients received a second HDM/ASCT, most of them achieved a long remission after the second treatment as well.

For patients with cardiac involvement, survival is worse, with a median survival of only 49 months. Mortality within 100 days from HDM/ASCT is also higher in this group (17.2%). The degree of cardiac dysfunction has been shown to be one of the most important prognostic factors for survival in AL amyloidosis and outcome after HDM/ASCT, and can be assessed by markers such as NT-proBNP and cardiac troponins.[12,13] These markers are now used in staging systems for AL amyloidosis and in the selection of patients for HDM/ASCT, to reduce the transplant-related mortality. This study includes patients treated in a time period when cardiac markers were not used, and therefore patients with substantial cardiac dysfunction were probably treated.

Patients with involvement of two or more organs also have worse survival (median 56 months). Previous studies have shown that the number of organs involved is an important prognostic factor, with higher number of organs involved being associated with shorter survival times and higher mortality after treatment with HDM/ASCT.[4,5] It has been suggested that the number of organs involved should be included in a staging system for risk assessment, but this has been questioned because of the diversity

in how the number of organs is counted and the belief that the degree of organ dysfunction is more important.[9]

It has earlier been shown that induction therapy result in a substantial number of deaths prior to HDM/ASCT, but reduced transplant-related mortality.[14] In this study however, treatment before HDM/ASCT was not associated with significantly better survival. And early cardiac death was not more common among the patients that had not received induction treatment.

Many patients (42%) in this study received a reduced dose of melphalan. It has been shown earlier that dose reduction of melphalan (to reduce transplant-related toxicity) is related to lower response rates.[15] In this study, dose reduction was not associated with shorter survival that was statistically significant.

The high 100-day mortality (12.5%) in this study is on a level with what is reported by others.[3–6] For patients treated during the later part of the period, the early mortality was lower, 7.8% compared with 23.8% in the earlier period, although the difference was not statistically significant possibly owing to small patient groups. Other studies have shown improved outcomes in the later years, with higher response rate, lower early mortality (to around 5%) and longer overall survival.[16,17] This is probably owing to better selection of patients for HDM/ASCT, and improvements in the transplant procedures and supportive care. Unfortunately, this does not seem to apply for patients with cardiac amyloidosis. A recent report on 1536 patients from the Center for International Blood and Marrow Transplant Research database could not show any statistically significant improvement in survival over time for patients with heart involvement.[17]

In this study, six patients underwent organ transplantation, which was performed either before or after HDM/ASCT. This reflects the fact that it is not known which sequence is superior. In one retrospective study with 19 AL patients who underwent kidney transplantation, no significant difference in graft or patient survival was seen when HDM/ASCT was performed before compared with after kidney transplantation. Recurrence of AL amyloidosis in the kidney graft did also not differ significantly between the groups.[18]

Considering the varying frequency of HDM/ASCT between the regions of the country, different criteria for treatment have probably been used. There is a need for more coherence when it comes to selecting patients for SCT and also in the follow-up routines.

In summary, this study shows that good results can be obtained treating AL amyloidosis with HDM/ASCT, also in small centres. Early mortality is high, but with a decreasing trend. In cardiac amyloidosis, prognosis is still poor, and there is a need for safer therapy and also earlier diagnosis in this group.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Paper II

MINI-FOCUS ISSUE: CARDIAC AMYLOIDOSIS

ORIGINAL RESEARCH

Diagnostic Accuracy of [11C]PIB Positron Emission Tomography for Detection of Cardiac Amyloidosis

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ABSTRACT

OBJECTIVES This dual-site study evaluated the diagnostic accuracy of the method.

BACKGROUND Pittsburgh compound ([11C]PIB) positron emission tomography (PIB-PET) has shown promise as a specific and noninvasive method for the diagnosis of cardiac amyloidosis (CA).

METHODS The study had 2 parts. In the initial study, 51 subjects were included, 36 patients with known CA and increased wall thickness (15 immunoglobulin light chain [AL] and 21 transthyretin [ATTR] amyloidosis) and 15 control patients (7 were non-amyloid hypertrophic and 8 healthy volunteers). Subjects underwent PIB-PET and echocardiography. Sensitivity and specificity of PIB-PET were established for 2 simple semiquantitative approaches, standardized uptake value ratio (SUVR) and retention index (RI). The second part of the study included 11 amyloidosis patients (5 AL and 6 hereditary ATTR) without increased wall thickness to which the optimal cutoff values of SUVR ($>$1.09) and RI ($>$0.037 min^{-1}) were applied prospectively.

RESULTS The diagnostic accuracy of visual inspection of [11C]PIB uptake was 100% in discriminating CA patients with increased wall thickness from controls. Semiquantitative [11C]PIB uptake discriminated CA from controls with a 94% (95% confidence interval [CI]: 80% to 99%) sensitivity for both SUVR and RI and specificity of 93% (95% CI: 66% to 100%) for SUVR and 100% (95% CI: 75% to 100%) for RI. [11C]PIB uptake was significantly higher in AL-CA than in ATTR-CA patients (p $<$ 0.001) and discriminated AL-CA from controls with 100% (95% CI: 88% to 100%) accuracy for both the semiquantitative measures. In the prospective group without increased wall thickness, RI was elevated compared to controls (p $=$ 0.001) and 5 of 11 subjects were evaluated as [11C]PIB PET positive.

CONCLUSIONS In a dual-center setting, [11C]PIB PET was highly accurate in detecting cardiac involvement in the main amyloid subtypes, with 100% accuracy in AL amyloidosis. A proportion of amyloidosis patients without known cardiac involvement were [11C]PIB PET positive, indicating that the method may detect early stages of CA. (J Am Coll Cardiol Img 2020;13:1337–47) © 2020 by the American College of Cardiology Foundation.

ABBREVIATIONS AND ACRONYMS

AL = immunoglobulin light chain amyloidosis

ATTR = transthyretin amyloidosis

AUC = area under the curve

CA = cardiac amyloidosis

CI = confidence interval

IQR = interquartile range

IVST = interventricular septum thickness

LVEF = left ventricular ejection fraction

MRI = magnetic resonance imaging

NYHA = New York Heart Association

PET = positron emission tomography

PIB = Pittsburgh compound B

RI = retention index

ROC = receiver operating curve

SUVR = standardized uptake value ratio

TAPSE = tricuspid annular plane systolic excursion

Amyloidosis is a group of diseases characterized by misfolded protein deposition in various organs, leading to progressive organ failure. The 2 most prevalent types are immunoglobulin light chain (AL) and transthyretin (ATTR) amyloidosis. In both these forms, the heart is commonly involved, causing increased myocardial wall thickness and eventually heart failure. Patients with advanced cardiac amyloidosis (CA) have poor outcome, especially in severe AL-CA, where survival is short despite treatment. Diagnosis is often delayed because of the unspecific symptoms and clinical findings, which causes patients to be diagnosed in advanced disease stages where treatment possibilities are limited (1). In the past few years, progress has been made in the treatment of both AL and ATTR amyloidosis, with development of new and promising therapeutic options (2-7). However, the poor prognosis in patients diagnosed with advanced CA has not yet been overcome, and earlier diagnosis is necessary to make these patients benefit from the therapeutic improvements.

Therefore, accurate diagnostic methods that promote early diagnosis of CA are crucial. Endomyocardial biopsy is considered the gold standard for diagnosis of CA, even though the method is invasive and amyloid deposits can be missed because of patchy distribution (1). In clinical practice, cardiac involvement is considered evident in cases with biopsy-proven amyloidosis in any localization (mainly fat pad biopsy) together with typical findings on echocardiography (8,9). However, the sensitivity of fat pad biopsy for CA diagnosis is only 15% and around 45% to 65% in wild-type and hereditary ATTR, respectively, and 85% in AL (10,11). Furthermore, findings on echocardiography are not specific for amyloidosis and the method is not sensitive enough to detect early stages of CA.

Magnetic resonance imaging (MRI) is often used to detect CA; however, the method is not suitable in patients with renal impairment (which is quite common in AL-amyloidosis) because of the risk of nephrotoxicity from the gadolinium contrast and may be contraindicated in patients with devices such as pacemaker. There is also a risk that "patchy" distribution of amyloidosis can be missed (1).

Scintigraphy with bone-seeking phosphate derivatives has a very high sensitivity in ATTR-CA, both wild-type and hereditary, and together with the absence of a monoclonal protein, a positive bone scintigraphy performs equally to endomyocardial biopsy for the diagnosis of ATTR-CA (12). However, the method is not useful in AL-CA, where most cases are negative and was only evaluated in subjects where echocardiography or cardiac MRI had already shown suspected amyloidosis. Thus, the ability of the method to detect early stages of CA is unclear.

The positron emission tomography (PET) tracer [^{18}F] florbetapir has shown promising diagnostic performance, although there are still no larger studies in cardiac amyloidosis (13,14).

Pittsburgh compound B, [^{11}C]PIB, is the original amyloid-binding tracer for PET and was constructed to detect brain amyloid deposits in vivo in Alzheimer disease (15). The diagnostic utility of PIB-PET in CA has been examined in 5 smaller, single-center studies indicating promising diagnostic performance (16-20). However, the method has not been evaluated in multicenter settings and has not been prospectively applied to clinical cases.

The aim of our dual-center study was to further examine PIB-PET in a larger cohort of amyloidosis patients and controls than what has previously been studied to establish the sensitivity and specificity of the method. We also included a prospective cohort of amyloidosis patients without increased wall thickness on echocardiography to examine the performance of PIB-PET in detection of early CA.

METHODS

SUBJECTS. Thirty-six patients with known CA were included in the study, 15 with AL and 21 with ATTR amyloidosis (16 wild-type and 5 hereditary). There were 2 control groups consisting of healthy volunteers (n = 8) and patients with non-amyloid cardiac hypertrophy (n = 7). CA and control subjects were used to establish cutoff values for the 2 previously proposed, semiquantitative approaches to measuring [^{11}C]PIB uptake in the myocardium.

The best cutoff values were then prospectively applied to 11 patients with evidence of amyloid systemically but without known cardiac involvement (5 patients with AL amyloidosis and 6 with transthyretin mutation). No subject had previously undergone PIB-PET.

In the CA groups, diagnosis was confirmed either by endomyocardial biopsy (n = 24) or by abdominal fat pad biopsy together with echocardiography (n = 12). According to the definition of organ involvement by Gertz et al. (8), cardiac amyloidosis was defined as left ventricular wall thickness >12 mm, with no other cause of hypertrophy such as hypertension. One of the CA patients with AL amyloidosis

TABLE 1 Subject Characteristics, PET, and Echocardiography Data According to Study Group

	AL (n = 15)		ATTR (n = 21)		Hypertrophic Controls (n = 7)		Health Controls (n = 8)		Prospective Group (n = 11)		p Value*	
Male/female	11/4		19/2		5/2		2/6		8/3			
Age (yrs)	69 (10.0)		76 (12.5)		68 (5.0)		53 (11.0)		55 (23.0)			
Positive endomyocardial biopsy	7		17		0		0		2			
Ischemic heart disease	1		6		1		0		0			
Atrial arrhythmia	1		6		3		0		0			
Diuretic use	7		15		1		0		3			
Beta-blocker use	5		12		7		0		0			
ACE-inhibitor/ARB use	6		5		5		0		3			
NYHA functional class I/II/III	3/4/8		2/8/11		2/3/2		-		10/1/0			
NT-proBNP (ng/l)	2,973 (2,485)		3,638 (5,449)		376 (1,089)		ND		77 (605)			
	Reader		Reader		Reader		Reader		Reader		Reader	
PET data	1	2	1	2	1	2	1	2	1	2	1	2
Visually positive	15	15	21	21	0	0	0	0	7	6	**<0.001**	**<0.001**
SUV ratio 10-20 min	2.61 (2.61)	1.60 (1.8)	1.64 (0.62)	1.3 (0.73)	0.88 (0.26)	0.63 (0.12)	0.87 (0.26)	0.94 (0.14)	1.16 (0.40)	1.12 (0.36)	**<0.001**	**<0.001**
Retention index 10-20 min (min⁻¹)	0.086 (0.075)	0.065 (0.063)	0.045 (0.014)	0.045 (0.01)	0.029 (0.005)	0.024 (0.004)	0.033 (0.005)	0.032 (0.006)	0.040 (0.011)	0.039 (0.007)	**<0.001**	**<0.001**
Echocardiography data												
Interventricular septum (mm)	16 (4)		20 (9)		16 (2)		7.5 (4)		10 (2)		**0.048**	
LV ejection fraction	0.61 (0.12)		0.56 (0.16)		0.67 (0.19)		0.67 (0.10)		0.64 (0.09)		0.158	
LV mass (g/m²)	127 (50)		123 (99)		121 (43)		66 (20)		79 (39)		0.819	
LV diastolic diameter (mm)	41 (8)		46 (7)		43 (11)		44 (6)		52 (15)		0.125	
LV global longitudinal strain	13 (8)		11 (3)		14 (6)		21 (4)		15 (8)		0.057	
LA volume (ml/m²)	35 (12)		48 (28)		35 (28)		34 (8)		31 (19)		**0.027**	
TAPSE (mm)	16 (6)		16 (11)		19 (9)		26 (5)		24 (5)		0.418	
E/A ratio	1.8 (2.3)		2.2 (2.6)		0.7 (0.5)		1.2 (0.9)		1.6 (0.7)		**0.015**	
E′ septum	5.0 (3.1)		4.0 (1.7)		4.0 (1.0)		8 (2.5)		9.2 (3.5)		0.173	
E/E′	16 (7)		21 (9)		19 (13)		7.5 (2.8)		8.8 (5.0)		0.187	

Values are, n (%), or median (interquartile range). *Kruskal-Wallis test comparing AL, ATTR, and hypertrophic controls. Statistically significant values (p < 0.05) are in **bold**.

ACE = angiotensin-converting enzyme; AL = light chain amyloidosis; ARB = angiotensin receptor blocker; ATTR = transthyretin amyloidosis; E/A = ratio of the Doppler peak velocity of E- and A-wave; E′ = early relaxation velocity on tissue Doppler; E/E′ = early filling velocity divided by the early relaxation velocity; LA = left atrial; LV = left ventricular; ND = not done; PET = positron emission tomography; SUV = standardized uptake value; TAPSE = tricuspid annular plane systolic excursion.

had a positive fat pad biopsy and typical MRI findings for cardiac amyloidosis, although echocardiography showed no increased wall thickness. Twelve of the 15 AL-CA patients had received cytotoxic therapy before enrollment in the study, and 3 were untreated. Among the hereditary ATTR-CA subjects, 3 had the Danish Leu111Met mutation and 2 had the Swedish His88Arg mutation. In the prospective group, all 6 transthyretin mutation carriers had the Leu111Met mutation. The penetrance of this mutation is known to be 100% and cardiac involvement is usually noted between 40 and 55 years of age (21). The median age of the studied subjects with Leu111Met mutation was 48 years (range 45 to 54 years) in the CA group, and 40 years (range 27 to 55 years) in the prospective group. The His88Arg mutation causes late onset cardiomyopathy (≥50 years of age) (22), and the 2 studied patients with this mutation were 67 and 63 years of age.

The hypertrophic control group comprised of patients with cardiac hypertrophy but without any clinical suspicion of amyloidosis. In 3 of the patients, hypertrophy was caused by hypertension and 4 patients had idiopathic hypertrophic cardiomyopathy.

The healthy controls were volunteers with no medical history of cardiac disease or hypertension. Volunteers older than 65 years were not included because of the increasing incidence of wild-type ATTR amyloidosis with age (23).

All subjects were examined with PIB-PET and a consecutive echocardiogram was performed within 1 to 2 weeks of PET. Except for the healthy controls, subjects also had blood samples, blood pressure, and ECG taken, and were clinically assessed regarding New York Association (NYHA) functional classification. Informed consent was obtained from all subjects and the study was performed with the approval of the local ethics committees (the Regional

FIGURE 1   Examples of Summed PIB-PET Images Taken 10 to 20 Min After Injection of [^{11}C]PIB
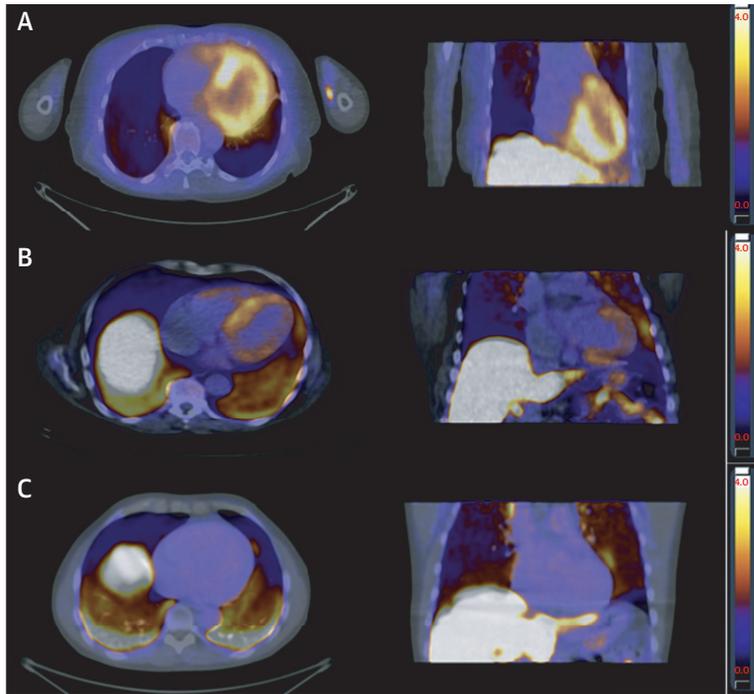
The signal represents myocardium/blood SUV ratio, and the color scale ranges from black (SUV ratio 0) to white (SUV ratio 4.0). Transaxial and coronal view of **(A)** a patient with AL amyloidosis and high degree of [^{11}C]PIB uptake in the left ventricle wall and septum; **(B)** a patient with ATTR amyloidosis and moderate degree of [^{11}C]PIB uptake in the cardiac walls, most apparent in the septum, and **(C)** a healthy volunteer with no myocardial [^{11}C]PIB uptake. All subjects show high signal in the liver, which is due to normal metabolization of [^{11}C]PIB, and in the lungs, which is most likely caused by tracer lipophilicity. ATTR = transthyretin amyloidosis; PET = positron emission tomography; PIB = Pittsburgh compound B; SUV = standardized uptake value.

Ethics Committee in Uppsala and the Central Jutland Regional Committee on Health Research and Ethics). **Table 1** presents an overview and characteristics of the study groups, and additional characteristics of each individual subject is presented in Supplemental Table 1.

PET/CT AND ECHOCARDIOGRAPHY. The study was performed at 2 sites (Uppsala, Sweden, and Aarhus, Denmark) where subjects were examined according to identical protocols. Subjects in Uppsala were scanned with a Discovery ST or MI PET/CT (GE Healthcare, Waukesha, Wisconsin) and subjects in Aarhus with a Biograph Truepoint TrueV 64 PET/CT (Siemens, Erlangen, Germany). After low-dose computed tomography scanning, [^{11}C]PIB (5 MBq/kg)

was injected intravenously, and simultaneously a dynamic emission scan of the heart was started and continued for 35 min. Images were reconstructed according to standard settings provided by the vendors, using all relevant corrections, and divided into 31 timeframes of increasing duration (12 × 5, 6 × 10, 4 × 30, 2 × 60, 2 × 120, and 5 × 300 s).

Image analysis was performed with Carimas software version 2.63 (Turku, Finland) as previously described (24). Regions of interest were manually placed in the left atrial blood pool and left ventricular wall, from which time-activity curves were derived. Images were defined as positive or negative for amyloid presence by qualitative visual inspection. Summed images from 10 to 20 min after [^{11}C]PIB

injection were also evaluated semiquantitatively by calculating a myocardium/blood standardized uptake value ratio (SUVR). For calculation of the [¹¹C]PIB retention index (RI), the mean tissue concentration between 10 and 20 min was divided by the integral of the blood time-activity curve from 0 to 15 min after injection. All PIB-PET examinations were evaluated by 2 independent observers, 1 at each study site, who were blinded regarding the diagnosis of the subjects. The degree of agreement between the 2 different readers was good, with an intraclass correlation coefficient of 0.73 (95% confidence interval [CI]: 0.54 to 0.84) for SUVR and 0.93 (95% CI: 0.88 to 0.96) for RI.

Echocardiography was performed by experienced investigators according to current guidelines (25). Measurements included for example interventricular septal wall thickness (IVST), tricuspid annular plane systolic excursion (TAPSE), left ventricular ejection fraction (LVEF) and left ventricular global longitudinal strain, all of which have been previously associated with outcome in CA (26).

STATISTICAL ANALYSIS. IBM SPSS 23 and SAS JMP 14 were used for statistical analysis. Median was used as the measure of central tendency, and interquartile range (IQR), the difference between 75th and 25th percentiles, for statistical dispersion. The Mann-Whitney U test and Kruskal-Wallis test were used for group comparison. Spearman rank-order test and ordinal logistic regression were used to evaluate correlations of [¹¹C]PIB uptake with clinical and echocardiographic parameters. Receiver operating characteristic (ROC) curves were analyzed to determine optimal cutoff values that could differentiate the study groups with the highest sensitivity and specificity. Positive and negative predictive values as well as accuracy were calculated. The p values <0.05 were considered statistically significant.

RESULTS

VISUAL ASSESSMENT.
At visual inspection of the summed PET images at around 10 to 20 min after [¹¹C]PIB injection, all known CA patients, both AL and ATTR, were [¹¹C]PIB positive. In contrast, none of the hypertrophic or healthy controls showed visible [¹¹C]PIB uptake. **Figure 1** shows examples of PET images used for visual assessment.

STANDARDIZED UPTAKE VALUE.
The myocardium to blood SUV ratio was calculated for all time frames, and was higher in CA patients than in controls from 3.5 min after [¹¹C]PIB injection (**Figure 2**). This is demonstrated by a larger separation between the myocardium and blood curves. The ratio was higher in AL than in ATTR patients (p < 0.001), but with a larger

FIGURE 2 SUV Time Curves for Arterial Blood and LV Wall

Legend: AL LV-Wall, ATTR LV-Wall, Controls LV-Wall, AL Blood, ATTR Blood, Controls Blood

SUV represents median values of the patients with AL and ATTR amyloidosis, and the controls (hypertrophic and healthy together). Time is minutes after injection of [¹¹C]PIB. AL = light chain amyloidosis; LV = left ventricle; other abbreviations as in **Figure 1**.

variability among AL patients. The largest difference between amyloidosis patients and controls was seen at 10 to 20 min after [¹¹C]PIB injection. At this time point, the median SUVR was 2.61 (IQR: 2.61) in AL patients; 1.64 (IQR: 0.62) in ATTR; 0.88 (IQR: 0.26) in the hypertrophic controls; and 0.87 (IQR: 0.26) in the healthy controls, with a significantly higher ratio (p < 0.001) in CA than in controls (**Central Illustration**).

With an SUVR cutoff at 1.09, the area under the curve (AUC) of the ROC curve was 0.98 (95% CI: 0.94 to 1.00), discriminating CA patients (AL and ATTR) from controls (hypertrophic and healthy) with 94% (95% CI: 80% to 99%) sensitivity and 93% (95% CI: 66 to 100) specificity (**Table 2**). The positive predictive value with this cutoff was 97% (95% CI: 83% to 100%) and the negative predictive value 88% (95% CI: 60% to 98%). The method's accuracy was 94% (95% CI: 84% to 99%). A cutoff SUVR of 1.4 completely differentiated AL amyloidosis from controls with 100% (95% CI: 88% to 100%) accuracy. When discriminating ATTR patients from controls, a cutoff value of 1.09 yielded 91% (95% CI: 68% to 98%) sensitivity and 93% (95% CI: 66% to 100%) specificity, AUC of the ROC curve was 0.96 (95% CI: 0.90 to 1.00). The positive predictive value with this cutoff was 95% (95% CI: 73% to 100%) and the negative predictive value 88% (95% CI: 60% to 98%). Accuracy was 92% (95% CI: 78% to 98%).

CENTRAL ILLUSTRATION SUV Ratio (Myocardium to Blood) and Retention Index (min⁻¹) for the Different Study Groups, 10 to 20 min After [11C]PIB Injection

Rosengren, S. et al. J Am Coll Cardiol Img. 2020;13(6):1337–47.

Analysis includes the patients with known CA, but not AL patients or TTR mutants without increased wall thickness. The **red reference line** represents the cutoff SUV ratio 1.09 and retention index 0.037 min⁻¹. **Circles** represent outliers, and **stars** show extreme outliers. AL = light chain amyloidosis; ATTR = transthyretin amyloidosis; CA = cardiac amyloidosis; PIB = Pittsburgh compound B; SUV = standardized uptake value.

TABLE 2 Sensitivity and Specificity Data From ROC Curves, Differentiating the Stated Study Groups

	Cutoff (min^{-1})	Sensitivity (%)	95% CI (%)	Specificity (%)	95% CI (%)	AUC	95% CI
SUV ratio 10-20 min							
Reader 1							
CA: controls	1.09	94	80-99	93	66-100	0.98	0.94-1.00
AL-CA: controls	1.40	100	75-100	100	75-100	1.00	1.00-1.00
ATTR-CA: controls	1.09	91	68-98	93	66-100	0.96	0.90-1.00
Reader 2							
CA: controls	0.95	94	80-99	93	66-100	0.97	0.94-1.00
AL-CA: controls	1.12	100	75-1.00	100	75-100	1.00	1.00-1.00
ATTR-CA: controls	0.95	91	68-98	93	66-100	0.96	0.86-1.00
Retention index 10-20 min							
Reader 1							
CA: controls	0.037	94	80-99	100	75-100	1.00	0.99-1.00
AL-CA: controls	0.040	100	75-100	100	75-100	1.00	1.00-1.00
ATTR-CA: controls	0.035	100	81-100	93	66-100	1.00	0.98-1.00
Reader 2							
CA: controls	0.039	94	80-99	93	66-100	0.97	0.91-1.00
AL-CA: controls	0.039	100	75-100	93	66-100	0.99	0.97-1.00
ATTR-CA: controls	0.039	91	68-98	93	66-100	0.95	0.87-1.00

Analysis includes the patients with known CA, but not AL patients and TTR mutation carriers without increased wall thickness. "Controls" represents both hypertrophic and healthy controls together.

AUC = area under the curve; CA = cardiac amyloidosis; CI = confidence interval; ROC = receiver-operating characteristic; other abbreviations as in **Table 1**.

RETENTION INDEX. The myocardial RI between 10 and 20 min was significantly higher in CA patients than in controls ($p < 0.001$) (**Central Illustration**). The median RI was 0.086 (IQR: 0.075) min^{-1} in AL patients; 0.045 (IQR: 0.014) min^{-1} in ATTR; 0.029 (IQR: 0.005) min^{-1} in the hypertrophic controls, and 0.033 (IQR: 0.005) min^{-1} in the healthy controls. RI could also completely discriminate AL-CA from controls (both hypertrophic and healthy), with the cutoff value of 0.040 min^{-1} (**Table 2**). Combining all CA and control subjects, the cutoff value 0.037 min^{-1} yielded 94% (95% CI: 80% to 99%) sensitivity and 100% (95% CI: 75% to 100%) specificity. The positive predictive value was 100% (95% CI: 87% to 100%) and negative predictive value 88% (95% CI: 62% to 98%). Accuracy was 96% (95% CI: 87% to 100%).

TABLE 3 Characteristics and Results of Each Subject in the Prospective Study Group

Sex	Race	Age (yrs)	Diagnosis	Positive EMB	NYHA Functional Class	NT-proBNP (ng/l)	Troponin T (ng/l)	Visually PET Positive†	SUV Ratio	Retention Index (min^{-1})	Echo IVST (mm)
Male	Caucasian	58	AL	Yes*	II	734	19	‡	0.88	0.032	11
Male	Caucasian	70	AL	No	I	-	-	Yes	1.19	0.04	9
Male	Caucasian	69	AL	No	I	649	20	Yes	1.69	0.074	10
Male	Caucasian	60	AL	No	I	443	62	Yes	1.18	0.044	11
Male	Caucasian	60	AL	No	I	75	18	No	1.36	0.03	10
Male	Caucasian	53	ATTR Leu111Met	No	I	60	14	‡	1.05	0.039	9
Female	Caucasian	55	ATTR Leu111Met	Yes	I	1,070	25	Yes	1.25	0.044	11
Female	Caucasian	27	ATTR Leu111Met	No	I	73	6	No	0.85	0.045	8
Male	Caucasian	30	ATTR Leu111Met	No	I	37	6	No	0.70	0.034	10
Female	Caucasian	37	ATTR Leu111Met	No	I	78	6	Yes	0.78	0.04	8
Male	Caucasian	42	ATTR Leu111Met	No	I	67	6	‡	1.16	0.034	9

*EMB showed amyloid confined to the vessels. †Yes or no indicate positive or negative by both readers. ‡Disagreement between reader 1 and 2.

EMB = endomyocardial biopsy; IVST = interventricular septum thickness; NYHA = New York Heart Association; other abbreviations as in **Table 1**.

TABLE 4 Associations of Semiquantitative Estimates of Myocardial [^{11}C]PIB Uptake With Clinical and Echocardiographic Parameters

Ordinal Logistic Regression	AL				ATTR				Combined Amyloidosis			
	SUV Ratio		RI		SUV Ratio		RI		SUV Ratio		RI	
	Chi-Square	p Value	Chi-Square	p Value	Chi-Square	p Value	Chi-Square	p Value	Chi-Square	p Value	Chi-Square	p Value
NYHA functional class (I/II/III)	0.15	0.69	0.36	0.55	**8.3**	**0.004**	**8.2**	**0.004**	0.67	0.41	0.66	0.42
Spearman rank correlation	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
NT-proBNP	0.33	0.17	0.23	0.35	**0.56**	**0.003**	**0.54**	**0.005**	0.39	0.008	0.36	0.02
Interventricular septal thickness	**0.62**	**0.004**	**0.55**	**0.01**	**0.51**	**0.006**	0.28	0.15	0.38	0.009	0.23	0.12
LV ejection fraction	0.14	0.56	0.17	0.46	**-0.56**	**0.002**	-0.32	0.11	-0.19	0.21	-0.04	0.78
LV mass/m^2	0.32	0.18	0.24	0.31	**0.55**	**0.003**	0.39	0.05	0.40	0.005	0.28	0.06
LV diastolic diameter	**-0.52**	**0.02**	**-0.56**	**0.01**	-0.20	0.32	-0.28	0.16	-0.39	0.008	-0.47	0.001
LV global longitudinal strain	0.18	0.44	0.27	0.26	-0.24	0.23	-0.25	0.21	-0.01	0.93	0.03	0.87
LA volume/m^2	-0.27	0.26	-0.44	0.06	0.44	0.02	0.38	0.05	0.04	0.81	-0.09	0.56
TAPSE	**-0.61**	**0.004**	**-0.55**	**0.01**	**-0.63**	**<0.001**	**-0.51**	**0.007**	**-0.60**	**<0.001**	**-0.55**	**<0.001**
E/A ratio	0.05	0.85	-0.03	0.89	0.20	0.35	0.37	0.08	0.11	0.46	0.14	0.36
E' septum	-0.39	0.09	-0.31	0.18	**-0.67**	**<0.001**	-0.45	0.02	-0.42	0.003	-0.28	0.06
E/E'	0.31	0.18	0.21	0.37	**0.57**	**0.002**	0.42	0.03	0.34	0.02	0.24	0.11

Correlation coefficients (rho) <-0.5 or >0.5 with p values <0.05 are presented in **bold**.

RI = retention index; other abbreviations as in **Tables 1 and 3**.

There was no significant difference between the hypertrophic and healthy controls, either in SUVR (p = 0.694) or in RI (p = 0.054).

PROSPECTIVE GROUP. Among the amyloidosis patients without increased wall thickness, 5 of 11 subjects were unanimously judged as visually [^{11}C]PIB positive by the 2 readers, 2 of 6 transthyretin mutation carriers and 3 of 5 AL patients (**Table 3**). Three of the 11 prospective subjects were classified as [^{11}C]PIB positive by 1 of the readers but not the other. Median RI was higher in the predisposed patients without increased wall thickness compared with controls (p = 0.001) (**Table 1**). In 2 of the prospective patients, endomyocardial biopsies were performed at follow-up after PIB-PET. One PET-positive transthyretin mutation carrier with normal echocardiography and no symptoms had an amyloid-positive endomyocardial biopsy at follow-up. In 1 PET-negative AL patient without increased wall thickness, but with low LVEF and NYHA functional class II, endomyocardial biopsy was positive for amyloid confined to the vasculature, this patient has been described in a case report by Clemmensen et al (27).

ASSOCIATIONS WITH ECHOCARDIOGRAPHY AND CLINICAL PARAMETERS. In the ATTR group, both SUVR and RI were significantly associated with NYHA functional class (both p = 0.004); NT-proBNP levels (p ≤ 0.005) and TAPSE (p ≤ 0.007) (**Table 4**). In addition, SUVR was associated with IVST (p = 0.006), LVEF (p = 0.002), left ventricular mass (p = 0.003), E', and E/E' (p ≤ 0.002). In the AL cohort, both SUVR and RI were significantly associated with

IVST (p ≤ 0.01) left ventricular diastolic diameter (p ≤ 0.02) and TAPSE (p ≤ 0.01). In the combined amyloidosis group, there was an association between TAPSE and both SUVR and RI (p < 0.001), but no correlation to any other clinical or echocardiographic parameter.

DISCUSSION

To date, this dual-site study composes the largest number of CA patients examined with PIB-PET. It is also the first [^{11}C]PIB study with a control group with non-amyloid-induced hypertrophic myocardium. The main finding of this study was the high sensitivity (94%) and specificity (93% to 100%) of PIB-PET for distinguishing cardiac amyloidosis patients from both hypertrophic patients and healthy controls. When viewed by experienced professionals, visual interpretation alone achieved an accuracy of 100% in this cohort. The high accuracy in this study is encouraging, considering that different sites, scanners, and observers were used. This suggests that the use of PIB-PET could be the predominant method for assessing cardiac involvement in drug trials, similar to the same tracer's use in determining Alzheimer disease (28), it also suggests that PIB-PET can be of clinical value for CA diagnosis. Including this study, there are published reports on a total number of 137 unique cardiac PIB-PET examinations. These reports have shown that the method is 89% to 100% accurate in detecting cardiac amyloidosis (16-20). In comparison, a recent meta-analysis on the accuracy of late gadolinium enhancement on cardiac MRI showed a

summary sensitivity of 85% and specificity of 92% (29). This makes PIB-PET probably at least comparable to contrast-enhanced MRI, as a diagnostic tool in CA. Because [¹¹C]PIB binds directly to amyloid, PIB-PET can detect amyloidosis on a molecular level, as opposed to MRI and echocardiography, which mainly show indirect signs of amyloidosis. PIB-PET is thereby able to detect amyloid even when distribution is "patchy," cases in which MRI (and endomyocardial biopsies) may be misleading.

Another finding in this study was the high prevalence of PIB uptake among the amyloidosis patients without increased cardiac wall thickness (both AL and ATTR), indicating that PIB-PET may be able to detect early stages of CA. Similar findings were reported from a study including 10 patients with transthyretin mutations and inconclusive echocardiographic findings, where all patients were [¹¹C]PIB positive (20). Thus, PIB-PET may be useful for early diagnosis and treatment initiation, which is of great importance to counter the dismal prognosis associated with CA. The availability of highly accurate imaging tools for earlier diagnosis appears particularly relevant in the light of the recent recognition of amyloidosis as an underestimated cause of heart failure with preserved ejection fraction (30), and treatment advances both in AL (2–4) and ATTR amyloidosis (5–7).

A recent multicenter study using bone scintigraphy reported a >99% sensitivity and 86% specificity for the diagnosis of ATTR-CA, and specificity increased to 100% when a positive bone scintigraphy was combined with the absence of a monoclonal protein (12).

Unlike bone scintigraphy, PIB-PET detects CA in both AL and ATTR patients, with higher [¹¹C]PIB retention in the AL patients in our study. The method was able to completely distinguish AL amyloidosis from both non-CA hypertrophic patients and healthy volunteers, with 100% accuracy by simple semiquantitative indexes or visual inspection. Thus, in clinical practice, PIB-PET might have its greatest value in diagnosis of AL-CA where bone scintigraphy is not a reliable method. Furthermore, the proposed nonbiopsy diagnosis of ATTR-CA with bone scintigraphy required the suspicion of CA on echocardiography or MRI, both of which show signs of CA quite late in the disease process. A possible clinical use of PIB-PET could thereby be for early detection of cardiac CA, whereas the role of scintigraphy may be to distinguish between amyloid types.

Visual inspection of a PET image obtained around 10 to 20 min after [¹¹C]PIB injection works well for distinguishing CA patients from controls. The reason for the better accuracy of visual inspection is because CA often presents with heterogeneous distribution of [¹¹C]PIB, and uptake in smaller areas might be missed by using a more global LV/blood ratio. In cases where uptake is difficult to assess visually, comparison of the time-activity curves of myocardium and blood (where higher myocardial activity compared with blood at the time interval 10 to 20 min indicates [¹¹C]PIB uptake), as well as semiquantitative measures such as SUVR and RI, can be useful. The results of this study suggest that, at the time interval of 10 to 20 min, cutoff values of 1.09 for the SUVR and 0.037 min⁻¹ for RI can be used. A recent study of [¹¹C]PIB kinetics showed that the simplified measures SUV and RI were highly correlated to results from fully quantitative measurements with arterial blood sampling and metabolite analysis. This suggests that these semiquantitative methods work well, and metabolite correction is not regarded as necessary (31). In clinical practice, we suggest that visual inspection at around 10 to 20 min is used at first hand. As a semiquantitative method, we recommend the simple measure SUVR, using the cutoff 1.09 for PIB-PET positivity.

There is no specific binding of [¹¹C]PIB to myocardium without amyloidosis (16), but [¹¹C]PIB signal was not 0 in the control groups (**Central Illustration**), which can be explained by spill-in of radioactivity from the blood pool and surrounding organs into the cardiac regions of interest.

The production of [¹¹C]PIB requires a cyclotron nearby, which limits utility to larger hospitals. However, [¹¹C]PIB is already available in many facilities worldwide because it is a standard tool for Alzheimer diagnosis. Compared with cardiac biopsy, PET is a noninvasive method without significant patient risks, and with possibly shorter response times compared with the more time-consuming histopathological examination. The cost is generally higher than other imaging modalities, and the added value of early diagnosis with [¹¹C]PIB PET for directing amyloid-targeting therapies should be evaluated in future studies.

A method for grading the CA burden would be of clinical value because there is currently no effective imaging method for follow-up after amyloidosis treatment. Lee et al. (17) found a significantly higher [¹¹C]PIB uptake in chemotherapy-naïve AL patients compared with chemotherapy-treated patients, suggesting that the method could be used to assess disease burden. This could not be reproduced in our study, because most AL patients had been previously treated. In the current study, we found some associations between [¹¹C]PIB uptake and measures of

cardiac function, suggesting that [11C]PIB measurements may be indicative of amyloid burden to some extent. However, the degree of [11C]PIB retention is strongly influenced by the type of amyloid fibrils. This was seen in our study, where [11C]PIB uptake was highest in AL patients. Pilebro et al. (20) reported a difference in [11C]PIB retention in hereditary ATTR amyloidosis depending on the type of amyloid fibrils, with higher uptake in patients with full-length fibrils than in patients with fragmented fibrils. Thus, there seems to be a difference in the binding of [11C]PIB depending on the type of amyloid fibrils; further studies are needed to elucidate the relevance of this finding.

Myocardial blood flow could also theoretically influence the degree of [11C]PIB retention, with possibly lower delivery of [11C]PIB to the tissue in patients with poor myocardial perfusion or scarring. This could possibly contribute to the lower [11C]PIB uptake in ATTR compared to AL because the thicker cardiac walls seen in ATTR could be associated with lower perfusion. However, in a previous small study (16), there was no significant correlation between [11C]PIB retention and myocardial blood flow as assessed by PET. Further studies with follow-up PET examinations after amyloidosis treatment would be needed to evaluate PIB-PET as a tool for evaluating treatment efficacy.

STUDY LIMITATIONS. In our study, only two-thirds of the CA patients had endomyocardial biopsies performed. For the remaining patients, echocardiography was used together with a noncardiac biopsy as reference. Had endomyocardial biopsy been performed on all subjects, there could possibly have been more false-negative patients with PIB-PET.

Furthermore, even though this is the largest PIB-PET study in CA, the patient groups are still small and the confidence intervals around the point estimates for sensitivity and specificity are wide. The prospective group includes only 11 subjects and, although our results complement the findings of Pilebro et al., more studies on [11C]PIB-PET in early CA detection are needed to define the diagnostic accuracy in this setting.

The CA groups in this study are heterogeneous, consisting of patients in different stages of disease, some newly diagnosed and some in late disease stages. Among the AL-CA patients, there are both treatment-naïve and chemotherapy-treated patients, the majority having received amyloidosis treatment. It is likely that if all AL patients were untreated, there would be even higher PIB uptake in this group. There is also an imbalance in terms of sex, with only 6 female CA subjects, which makes it statistically uncertain to control for possible sex differences when it comes to cardiac structure and function parameters.

CONCLUSIONS

[11C]PIB-PET was an excellent method to distinguish both ATTR and AL subtypes of cardiac amyloidosis from healthy and hypertrophied controls, with high accuracy for both simple visual analysis and semi-quantitative measurements. The method performed exceptionally well in AL cardiac amyloidosis, where accuracy was 100%. [11C]PIB uptake was also seen in a subgroup of amyloidosis patients without increased wall thickness, indicating that PIB-PET may be able to detect early stages of cardiac involvement. [11C]PIB uptake was somewhat associated with symptoms and signs of cardiac dysfunction, and may potentially reflect disease burden, although further studies are needed to evaluate the role of PIB-PET in follow-up of CA patients.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: [11C] PIB PET is a sensitive method for visualization of cardiac amyloidosis, with 100% accuracy in AL amyloidosis. The method may be able to detect early stages of cardiac amyloidosis.

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: When investigating patients with suspected cardiac amyloidosis, PIB-PET is a sensitive and accurate, noninvasive method that is especially relevant in detection of early disease stages, and when AL amyloidosis is suspected.

TRANSLATIONAL OUTLOOK: Even though there seems to be associations between [11C]PIB uptake and cardiac function, further studies are needed to examine whether [11C]PIB-PET can be used as a method for follow-up after amyloidosis treatment.

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KEY WORDS diagnosis, heart, nuclear imaging, PET, Pittsburgh compound B

APPENDIX For a supplemental table, please see the online version of this paper.

Paper III ▮

ORIGINAL ARTICLE

Outcome prediction by myocardial external efficiency from ^{11}C-acetate positron emission tomography in cardiac amyloidosis

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Abstract

Aims This study aimed to study the prognostic value of myocardial oxygen consumption (MVO_2) and myocardial external efficiency (MEE) from ^{11}C-acetate positron emission tomography (PET) in cardiac amyloidosis (CA) patients.

Methods and results Forty-eight CA patients, both transthyretin (ATTR) and immunoglobulin light chain (AL) amyloidosis, and 20 controls were included. All subjects were examined with ^{11}C-acetate PET and echocardiography. MVO_2, forward stroke volume (FSV), and left ventricular mass (LVM) were derived from ^{11}C-acetate PET and used to calculate MEE. CA patients were followed for survival and the prognostic impact of clinical, echocardiographic, and ^{11}C-acetate PET parameters was analysed. MVO_2 and MEE were reduced in CA compared with controls, but without significant difference between deceased and surviving CA patients. The ratio of ^{11}C-acetate PET-derived FSV and LVM was also reduced in CA and significantly lowered in deceased patients compared with survivors. In univariate analysis, New York Heart Association class, N-terminal pro-brain natriuretic peptide, and the ^{11}C-acetate PET parameters FSV/LVM and MEE were the strongest prognostic factors. Of the ^{11}C-acetate PET parameters, FSV/LVM was the strongest survival predictor with hazard ratio of 0.56 per 0.1 mL/g (95% confidence interval 0.39–0.81, $P = 0.002$) and independently prognostic in a multivariate model. MEE significantly separated deceased from surviving CA patients with the cut-off of 15.7% ($P = 0.032$). Survival was significantly shorter with FSV/LVM below 0.27 mL/g ($P < 0.001$), also when separating AL- and ATTR-CA.

Conclusions Reduced MEE was associated with shorter survival in CA patients, but FSV/LVM was the strongest survival predictor and the only independently prognostic ^{11}C-acetate PET parameter in multivariate analysis.

Introduction

Cardiac amyloidosis (CA) is a disease where amyloid fibrils of different origin are deposited progressively in the myocardium with increasing wall thickness leading to restrictive ventricular filling and clinical heart failure. The two main subtypes of CA are transthyretin (ATTR), wild-type or hereditary, and immunoglobulin light chain (AL) amyloidosis. CA is a serious condition with poor survival in both ATTR and AL subtypes. In the recent years, great progress has been made in diagnosis and therapy of both AL- and ATTR-CA, including introduction of new and potentially life-prolonging treatments, making strategies to select patients for different treatment options important.

Several different methods of predicting outcome in CA have been proposed. Cardiac biomarkers N-terminal pro-brain natriuretic peptide (NT-proBNP) and troponins are prognostic at diagnosis in both AL- and ATTR-CA and are used in the Mayo staging system for AL amyloidosis.[1,2] Furthermore, NT-proBNP and estimated glomerular filtration rate are used to predict outcome in ATTR-CA in the UK National Amyloidosis Centre staging system.[3] Stroke volume (SV), tricuspid annular plane systolic excursion (TAPSE), and global longitudinal strain (GLS) derived from echocardiography or cardiac magnetic resonance (CMR) are also shown to provide independently prognostic information for survival.[4–6] Left ventricular myocardial work index (LVMWI), a method that measures systolic myocardial deformation in relation to afterload conditions, is prognostic for both major adverse cardiac events and survival.[7] Higher extracellular volume (ECV) and left ventricular mass (LVM) represent greater amyloid burden. Mass together with SV can be used to calculate myocardial contraction fraction (MCF), which is defined as SV divided by the myocardial volume (MV). MV in turn is calculated as LVM divided by the mean density of myocardium (1.05 g/mL). MCF independently predicts survival in both AL- and ATTR-CA.[8,9] The prognostic value was validated by Mayo clinic with echocardiography in AL-CA patients[5] and confirmed in both AL and ATTR patients with CMR.[4,10]

The utilization of positron emission tomography (PET) in CA has mainly been focused on amyloid-binding tracers for diagnosis.[11,12] With the perfusion tracers ^{11}C-acetate and ^{15}O-water, PET can be used to measure myocardial oxygen consumption (MVO_2) and myocardial blood flow (MBF) and was recently validated for measuring FSV and LVM using both ^{11}C-acetate and ^{15}O-water.[13,14] Myocardial external efficiency (MEE) can be derived non-invasively with a high degree of automation and repeatability from ^{11}C-acetate PET as the energetic ratio of stroke work and oxidative energy consumption.[15,16]

MEE was previously used in CA to investigate the role of echocardiography-derived work index based on strain imaging.[7] As MEE incorporates measures of oxygen metabolism and mechanical work, both of which are affected in CA, it is potentially highly prognostic but has never been tested. MEE integrates the components of the MCF parameter, and we wanted to evaluate whether the addition of metabolic data enhances the predictive value of MCF.

Methods

Study population

This retrospective study included 48 patients with known CA, enrolled at Uppsala University Hospital in Sweden and Aarhus University Hospital in Denmark, 25 with ATTR-CA (18 wild-type and 7 hereditary) and 23 with AL subtype. We also included 14 healthy volunteers with normal echocardiograms and 6 patients with increased cardiac wall thickness but without amyloidosis, serving as controls. The hereditary ATTR group comprised four subjects with the Danish Leu111Met mutation and two with the Swedish His88Arg mutation; in one patient, the type of mutation was unknown. The diagnosis of CA was confirmed with endomyocardial biopsy in 33 patients and with abdominal fat pad biopsy together with echocardiography (in one patient with CMR) in 15 patients. In controls with increased cardiac wall thickness, the underlying cause was either hypertensive heart disease ($n = 3$) or idiopathic hypertrophic cardiomyopathy ($n = 3$), without any clinical suspicion of amyloidosis. The healthy controls were volunteers without any history of cardiac disease or hypertension. Volunteers older than 65 years were not selected due to the increasing incidence of wild-type ATTR amyloidosis with age[17] and the risk of subjects having undiagnosed ATTR amyloidosis.

All subjects were examined with ^{11}C-acetate PET and echocardiography at rest. Patients from the Uppsala cohort (15 CA subjects) also underwent ^{15}O-water PET at rest. Subjects were also examined with the amyloid-binding tracer ^{11}C-PIB PET as part of two previous studies,[11,18] in which ^{11}C-acetate and ^{15}O-water PET were performed prospectively to evaluate haemodynamic and metabolic heart function.

The investigation conforms with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all subjects before inclusion, and the study was performed with approval of the local ethics committees in Uppsala and Central Denmark Region.

Positron emission tomography

PET scans were performed with a Discovery ST or MI PET/CT (GE Healthcare, Waukesha, Wisconsin) in Uppsala and with a Biograph TruePoint TrueV 64 PET/CT (Siemens, Erlangen, Germany) in Aarhus according to the same protocol. After a low-dose computed tomography (CT) scan, ^{15}O-water (400 MBq) and/or ^{11}C-acetate (5 MBq/kg) was injected intravenously as a standardized bolus, and list mode data were collected for 6 and 27 min, respectively. ^{11}C-acetate was injected 12 min after ^{15}O-water to allow for isotopic decay. Heart rate and systemic blood pressures were collected after at least 15 min of rest and immediately before tracer injections. Images were reconstructed to dynamic time-series with standard vendor settings. Images were analysed with highly automated software developed in-house as previously described and validated kinetic models were used to calculate MVO_2 from ^{11}C-acetate PET.[15,16] Cardiac output was measured from first-pass images using indicator dilution techniques,[19] and forward stroke volume (FSV) was calculated as cardiac output divided by heart rate. LVM was

assessed by segmentation of the left ventricle using pixel-wise images created from the most relevant kinetic model parameters.[13,14] MEE was calculated, as previously described,[20] by the following equation:

$$MEE = \frac{EW \times 1.33 \times 10^{-4}}{LVM \times MVO_2 \times 20} \times 100.$$

EW (external work) was calculated as the product of PET-derived FSV, heart rate, and mean arterial blood pressure. The constants in the equation are used for conversion to joules. Echocardiography was performed according to current guidelines,[21] including measurement of interventricular septal wall thickness (IVST), left ventricular ejection fraction (LVEF), and TAPSE.

Statistics

Statistical analyses were performed with IBM SPSS 28. Distribution was assessed with the Shapiro–Wilk test, and parameters were not normally distributed. Numerical values are presented as median with interquartile range (IQR). The Mann–Whitney U test and the Kruskal–Wallis test were used for group comparison. Correlations were assessed using the Spearman rank correlation or linear regression (association of MEE towards FSV/LVM). Receiver operating characteristic (ROC) curves were used to establish the optimal cut-off values separating deceased CA patients from survivors. The Kaplan–Meier estimate was used for survival analysis, and the log-rank test was used to compare survival curves. Factors potentially influencing survival were first explored in uni-variate Cox regression analyses, and the significant parameters of interest were then analysed in multivariate Cox proportional hazard analysis. P-values < 0.05 were regarded as statistically significant.

Results

Patient characteristics

Characteristics of the study groups including echocardiography data are presented in *Table 1*. Comparing ATTR and AL groups, ATTR patients were significantly older (73 vs. 67 years) and had thicker IVST (18 vs. 16 mm).

[11]C-acetate positron emission tomography results in cardiac amyloidosis patients and controls

Figure 1 shows the ^{11}C-acetate PET results for deceased CA patients compared with survivors and controls. MVO$_2$ was significantly reduced in CA (0.08 mL/min/g, IQR 0.06–0.1) compared with healthy controls (0.10 mL/min/g, IQR 0.08–0.12), with no significant difference between deceased and surviving CA patients. MVO$_2$ was not significantly different between AL and ATTR, median 0.08 mL/min/g (IQR 0.06–0.1 mL/min/g) vs. 0.07 mL/min/g (IQR 0.06–0.1 mL/min/g), and did not differ significantly between deceased and surviving patients in either of the CA subtypes. MEE was significantly lowered in CA, in both deceased (13%, IQR 9–17) and surviving (16%, IQR 10–19) patients, compared with both hy-

Table 1 Subject characteristics and echocardiography data according to study group

	AL	ATTR	Hypertrophic	Healthy controls	P-value[a]
Number of subjects	23	25	6	14	—
Male [n (%)]	18 (78)	22 (88)	4 (67)	6 (43)	—
Age (years)	67 (60–70)[b]	73 (64–79)[b]	69 (62–72)	56 (51–62)	0.004
Positive EMB [n (%)]	11 (48)	21 (84)	—	—	—
NYHA class I/II/III/IV	3/7/12/1	3/8/14/0	2/3/1/0	—	—
NT-proBNP (ng/L)	3315 (1758–10 088)	2962 (1125–6348)	229 (76–1077)	—	0.007
Atrial fibrillation [n (%)]	3 (13)	3 (12)	0 (0)	—	—
Echocardiography					
IVST (mm)	16 (13–18)[b]	18 (14–22)[b]	16 (14–16)	8.0 (7.0–11)	<0.001
LVEF	0.58 (0.44–0.62)	0.55 (0.44–0.61)	0.67 (0.51–0.71)	0.66 (0.59–0.69)	0.031
LVEDd (mm)	4.3 (3.9–4.8)	4.6 (4.2–4.9)	4.1 (3.7–4.8)	4.5 (4.2–4.8)	0.58
LVGLS ($-$%)	13 (9.0–17)	11 (8.8–12)	14 (12–18)	21 (18–22)	<0.001
LA volume (mL/m^2)	38 (33–48)	43 (33–68)	31 (23–54)	34 (28–36)	0.047
TAPSE (cm)	1.6 (1.2–1.8)	1.6 (1.2–2.3)	1.8 (1.5–2.3)	2.6 (2.3–3.0)	0.001
E/A ratio	1.9 (1.6–3.2)	2.6 (1.3–3.9)	0.7 (0.6–1.2)	1.2 (0.9–1.6)	<0.001
E/e'	17 (15–21)	19 (16–26)	20 (12–28)	7.0 (5.5–9.0)	<0.001

AL, light chain amyloidosis; ATTR, transthyretin amyloidosis; E/A, ratio of the Doppler peak velocity of E- and A-wave; E/e', early filling velocity divided by the early relaxation velocity; EMB, endomyocardial biopsy; IVST, interventricular septum thickness; LA, left atrium; LVEDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; TAPSE, tricuspid annular plane systolic excursion.
Values are n (%) or median (interquartile range).
[a]Kruskal–Wallis test comparing all groups.
[b]$P < 0.05$ comparing AL and ATTR.

Figure 1 ^{11}C-acetate positron emission tomography parameters: (A) myocardial oxygen consumption (MVO$_2$), (B) myocardial external efficiency (MEE), and (C) forward stroke volume/left ventricular mass (FSV/LVM) for deceased and surviving cardiac amyloidosis (CA) patients [light chain (AL) and transthyretin (ATTR) amyloidosis], hypertrophic controls, and healthy controls. The *P*-value is derived from the Kruskal–Wallis test comparing the groups.

Figure 2 Correlation between forward stroke volume/left ventricular mass (FSV/LVM) and myocardial external efficiency (MEE) in deceased (black) and surviving (white) cardiac amyloidosis (CA) patients. R_s, Spearman's rho.

nated deceased from surviving CA (0.24 mL/g, IQR 0.20–0.36 vs. 0.35 mL/g, IQR 0.28–0.46; *P* = 0.009). FSV/LVM was significantly lower in ATTR (0.26 mL/g, IQR 0.16–0.35) than in AL (0.36 mL/g, IQR 0.25–0.44) and differed significantly between deceased and surviving ATTR, but not AL, patients.

There was a strong correlation between FSV/LVM and MEE in CA patients (R_s = 0.76, *P* < 0.001; *Figure 2*). Both MEE and FSV/LVM were correlated with standard echocardiographic indicators of left ventricular (LV) diastolic (E/e'), LV systolic (GLS), and right ventricular systolic function (TAPSE). As shown in *Figure 3*, both MEE and FSV/LVM were reduced in CA compared with healthy controls. Furthermore, E/e', GLS, and TAPSE were all abnormal in CA patients as compared with healthy controls. FSV index (FSVI) was negatively associated with heart rate (R_s = −0.6, *P* < 0.001).

FSV measured with ^{11}C-acetate PET correlated well with FSV from ^{15}O-water PET (R_s = 0.76, *P* < 0.001). There were also strong correlations for LVM and FSV/LVM between the two PET scans (R_s = 0.94, *P* < 0.001 and R_s = 0.92, *P* < 0.001). Figures showing correlations between ^{11}C-acetate and ^{15}O-water PET parameters are presented in the Supporting Information, *Figure S1*.

pertrophic (28%, IQR 26–31) and healthy (27%, IQR 21–34) controls. MEE did not differ significantly between AL and ATTR, median 13% (IQR 11–18%) vs. 14% (IQR 8–18%), and there was no significant difference between deceased and surviving patients in AL or ATTR. ^{11}C-acetate PET-derived FSV was significantly lower, and LVM was higher in CA patients compared with both hypertrophic and healthy controls, and the ratio between FSV/LVM significantly discrimi-

Outcome prediction by ^{11}C-acetate positron emission tomography

Median time from PET to death or end of follow-up was 41 (IQR 22–135) months for CA patients. Total mortality during the follow-up period was 54.2% (*n* = 26).

ROC analysis was performed on ^{11}C-acetate PET parameters to determine the cut-off values that best discriminated

Figure 3 Associations of myocardial external efficiency (MEE) and forward stroke volume/left ventricular mass (FSV/LVM) from ^{11}C-acetate positron emission tomography towards echocardiographic indicators of systolic and diastolic function in cardiac amyloidosis. Subjects enrolled in dedicated study with near-simultaneous positron emission tomography and echocardiography [*n* = 19 light chain (AL), 23 transthyretin (ATTR) amyloidosis, and 9 healthy controls]. E/e', early filling velocity divided by the early relaxation velocity; GLS, global longitudinal strain; R_s, Spearman's rho; TAPSE, tricuspid annular plane systolic excursion.
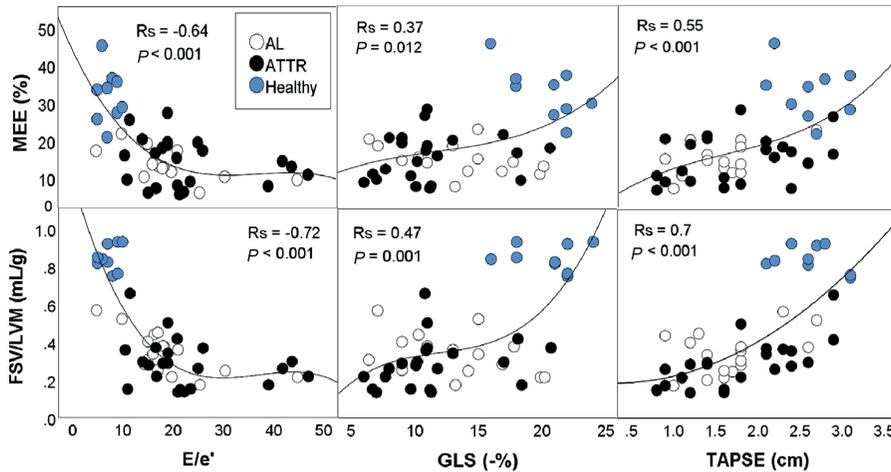
between deceased and surviving CA patients. For MEE, the optimal cut-off was 15.7% with area under the curve (AUC) of 0.63 [95% confidence interval (CI) 0.47–0.79]. FSV/LVM best discriminated deceased from survivors in all CA patients (cut-off 0.27 mL/g; AUC 0.72, 95% CI 0.57–0.87), as well as in AL and ATTR separately. The optimal prognostic cut-off value of FSV/LVM in AL patients was 0.39 mL/g, and that in ATTR was 0.27 mL/g.

Survival was significantly shorter in patients with low vs. high MEE, using the cut-off value of 15.7%, with median survival of 35 (IQR 7–71) vs. 135 (IQR 29–135) months (*P* = 0.032; *Figure 4*). When analysing AL and ATTR groups separately, statistically significant hazard ratios were not reached using FSV/LVM as a continuous variable. With FSV/LVM lower than the cut-off of 0.27 mL/g, survival was significantly impaired with median survival of 23 (IQR 6–40) vs. 135 (IQR 41–135) months (*P* < 0.001). The survival difference between FSV/LVM below and above the cut-off point was significant also for AL and ATTR separately (*P* = 0.011 and *P* = 0.004).

In univariate Cox regression analysis, New York Heart Association (NYHA) class, NT-proBNP, LVEF, and left atrium (LA) volume from echocardiography, as well as the ^{11}C-acetate PET parameters heart rate, FSVI, FSV/LVM, and MEE, were all significantly associated with overall survival in CA (*Table 2*). FSV/LVM remained independently significant compared with all other univariate predictors in bivariate analysis.

The ^{11}C-acetate PET parameters FSVI, FSV/LVM, and MEE were selected for multivariate analysis, where FSV/LVM was the only independent predictor for survival (hazard ratio 0.52 per 0.1 mL/g, 95% CI 0.28–0.96, *P* = 0.038).

When separated into CA subtypes and using the continuous ^{11}C-acetate PET variables and not cut-off values, the significant prognostic factors were NYHA class, FSVI, and FSV/LVM in AL and NT-proBNP, LVEF, and TAPSE in ATTR. All results of the univariate Cox regression analysis in AL and ATTR separately are included in Supporting Information, *Tables S1* and *S2*.

Discussion

The key findings of the study were the following:

1. MEE, MVO_2, and FSV/LVM are reduced in CA compared with controls.
2. MEE and FSV/LVM derived from ^{11}C-acetate PET are significant outcome predictors.
3. FSV/LVM is the only independent predictor of survival.

FSV/LVM was superior to MEE and echocardiography parameters for prediction of outcome in CA. FSV/LVM resembles MCF (SV/MV), which was previously shown, with both echocardiography and CMR, to be an independent predictor

Figure 4 Survival with myocardial external efficiency (MEE) and forward stroke volume/left ventricular mass (FSV/LVM) above or below the cut-off levels in (A) all cardiac amyloidosis (CA) patients, (B) light chain-CA (AL-CA), and (C) transthyretin-CA (ATTR-CA).
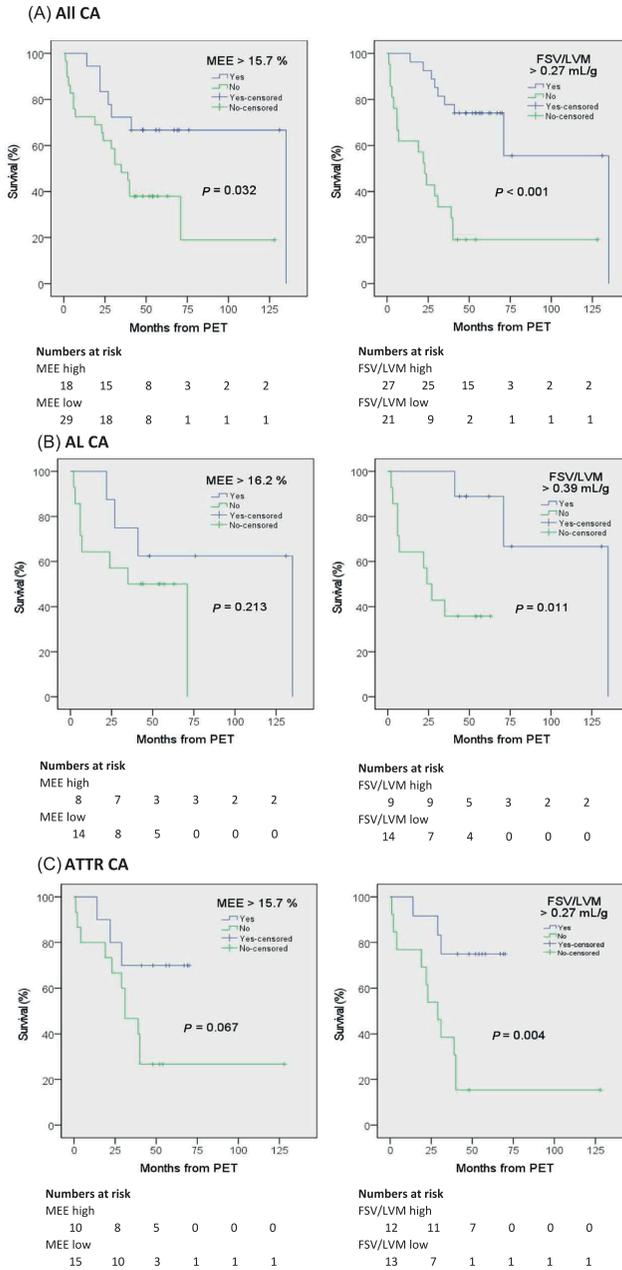
Table 2 Univariate and multivariate survival analysis

Univariate Cox regression	Hazard ratio	95% CI	*P*-value
Gender	0.16	0.02–1.18	0.072
Age	1.03	0.99–1.07	0.16
NYHA class	4.19	1.76–10.0	<**0.001**
Log NT-proBNP	1.49	1.07–2.08	**0.017**
Echocardiography			
IVST	1.06	0.96–1.16	0.27
LVEF	0.96	0.93–0.99	**0.005**
LVEDd	1.28	0.69–2.38	0.44
LA volume	1.03	1.01–1.05	**0.015**
TAPSE	0.93	0.87–1.01	0.09
E/A ratio	1.28	0.95–1.72	0.11
E/e′	1.02	0.98–1.06	0.30
[11]C-acetate PET			
HR	1.04	1.01–1.07	**0.019**
MAP	1.02	0.98–1.05	0.36
MVO$_2$ (per 0.01 mL/min/g)	1.02	0.87–1.20	0.83
FCO index	0.95	0.87–10.4	0.27
FSV index	0.93	0.88–0.98	**0.007**
LVM index	1.01	1.00–1.02	0.06
FSV/LVM (per 0.1 mL/g)	0.56	0.39–0.81	**0.002**
MEE	0.92	0.85–1.00	**0.042**
Multivariate Cox regression	Hazard ratio	95% CI	*P*-value
[11]C-acetate PET			
FSV index	0.97	0.89–1.05	0.43
FSV/LVM (per 0.1 mL/g)	0.52	0.28–0.96	**0.038**
MEE	1.08	0.95–1.23	0.26

CI, confidence interval; E/A, ratio of the Doppler peak velocity of E- and A-wave; E/e′, early filling velocity divided by the early relaxation velocity; FCO, forward cardiac output; FSV, forward stroke volume; HR, heart rate; IVST, interventricular septum thickness; LA, left atrium; LVEDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; MAP, mean arterial pressure; MEE, myocardial external efficiency; MVO$_2$, myocardial oxygen consumption; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PET, positron emission tomography; TAPSE, tricuspid annular plane systolic excursion. Bold indicates significant *P*-values.

of survival in CA and having a progressively increasing likelihood of being abnormal with increasing amyloid burden.[4]

FSV/LVM measured with PET was reproducible across different scanners and across two different perfusion tracers (^{11}C-acetate and ^{15}O-water), which means that it might be useful in a clinical routine. MCF can be measured with echocardiography and CMR but is typically constructed using a geometric total SV (end-diastolic volume–end-systolic volume) instead of FSV. The use of FSV for MCF assessments might have incremental value over the geometric volume because total SV disregards valvular insufficiencies often seen in CA.

FSV/LVM appears to have a very high accuracy for outcome prediction, suggesting that this index is at the core of CA pathology. In proportion to overall amyloid infiltration, LVM increases, leading to reduced filling capacity, stiffness, and lowered FSV, making the ratio FSV/LVM a sensitive measure of disease progression and can explain why FSV/LVM is a strong prognostic marker. The cut-off FSV/LVM of 0.27 mL/g might represent a 'point-of-no-return' where amyloid burden is increased, and systolic and diastolic function decreased to the extent that adequate systemic circulation can no longer be maintained. As an attempt to compensate for a low FSV, heart rate is increased, and higher heart rate was also significantly associated with worse survival. Higher LVM is associated with lowering of resting MBF, which can lead to further worsening of the contractile capacity in a vicious cycle.

In this study, the standard echocardiographic parameters used for evaluation of prognosis were all inferior to FSV/LVM. The best echocardiographic predictors were LVEF and LA volume, considered indicators of late-stage disease, supported by the high prevalence of NYHA class III patients in the CA cohort. The multivariate outcome might have been different in a cohort with earlier disease. However, there was a distinctly lowered FSV/LVM also in non-fatal CA, compared with hypertrophic controls, suggesting that low FSV/LVM can be indicative of CA diagnosis as opposed to non-CA causes of hypertrophy. In addition, FSV/LVM was correlated with echocardiographic indicators of systolic and diastolic dysfunction but appears to be lowered earlier in the disease process, suggesting that FSV/LVM could be a useful parameter in the pursuit of early CA diagnosis.

MEE was highly different between controls and CA patients independent of outcome. The generally lowered MEE in all CA subjects suggests that poor energetic efficiency is an early pathophysiologic response to amyloid infiltration. MEE incorporates FSV/LVM but the measure also includes MVO$_2$. MVO$_2$ was not significantly different in deceased and surviving CA patients, suggesting that this parameter

does not improve the prognostic value of MEE in CA generally. MVO_2 was in fact reduced in CA compared with healthy controls leading to elevation of MEE. Reduced MVO_2 indicates an altered oxygen metabolism in CA, which can be caused by factors such as amyloid affecting blood vessels, different levels of myocardial oxidative stress and mitochondrial function,[22] and the direct energy-sparing effect of drugs such as beta-blockers.[23]

MEE in CA is also confounded by the degree of amyloid burden because both myocytes and ECV were integrated in LVM calculation, and MVO_2 measured by ^{11}C-acetate PET is probably a reflection of oxidative metabolism in viable myocytes only. In CA survivors, septal thickness, LVM index, and MVO_2 were comparable with hypertrophic controls, while MEE was dramatically lowered in CA. In contrast, MEE in hypertrophic and healthy controls was similar. This could reflect the fact that ECV is higher in CA patients than in hypertrophic controls, because the latter is characterized by hypertrophy of myocytes and not expansion of the extracellular space. There is currently no method by which ECV can be determined by ^{11}C-acetate PET. Further studies of myocardial oxidative metabolism in CA might be relevant for research regarding novel therapeutics but would potentially require combined PET/magnetic resonance studies to correct the PET signal for differences in ECV.

PET-FSV has been validated against both gold standards right heart catheterization (RHC) and CMR[19,24] and was, as previously shown and repeated here, highly reproducible across tracers. Similarly, ^{11}C-acetate PET LVM estimates have high accuracies compared with CMR.[20,25] Different measurement techniques are required for different tracers but produce comparable LVM estimates in both normal and hypertrophied hearts. In theory, FSV/LVM or MCF measurements are available with any cardiac perfusion tracer and might be useful for early detection of concentrically hypertrophied hearts with altered haemodynamics, including CA, and in patients evaluated for heart-related symptoms. MEE correlates well to FSV/LVM, but the addition of oxygen consumption does not seem to add prognostic value to FSV/LVM in CA.

Given the increasing availability of targeted therapies and the known diagnostic delays in CA, detection of an FSV/LVM below the normal range in a subject with early symptoms or signs of heart failure with preserved LVEF should potentially raise a diagnostic red flag and further CA-specific work-up should be considered. FSV/LVM can also be measured with more generally available imaging methods than ^{11}C-acetate PET, and if reported from echocardiography or CMR, it can give an indication of possible CA. However, low FSV/LVM is not specific for CA and more targeted diagnostic methods such as PET with amyloid-binding tracers or scintigraphy with bone-seeking agents are then required. FSV/LVM is a strong survival predictor and might be used for patient stratification in clinical trials either to focus on early CA treatment in patients with relatively preserved contractile function or to aim at reducing mortality in CA subjects with severely affected contractile function. There is also a need for methods to monitor the CA disease and treatment response. FSV/LVM could be a parameter of interest in evaluation of cardiac response to amyloid-specific treatment.

As AL and ATTR are separate diseases where different underlying pathogeneses are involved, it is reasonable to evaluate the two groups separately. However, when divided into subgroups of CA, the patient numbers in this study were small. We did see some differences in the prognostic value of the ^{11}C-acetate PET parameters between AL and ATTR. In univariate analysis, FSVI and FSV/LVM, but not LVM itself, had significant impact on survival in AL, suggesting that FSV is a more important prognostic factor than LVM in AL. In ATTR, FSV/LVM was significantly lower in the deceased patients compared with the survivors. However, in univariate Cox regression analysis where the aspect of survival time is included, FSV/LVM only reached borderline significance ($P = 0.07$) for predicting survival in ATTR. These findings could indicate differences in prognostic parameters between AL and ATTR, but larger patient numbers would be needed to elucidate potential differences between subtypes of CA.

Limitations of the study include the relatively small number of subjects, which reduced the number of parameters that was possible to include in multivariate analysis. Especially when separated in amyloid subtypes, the study groups were small. The study population also included patients at different disease stages, and both patients who were untreated and those undergoing treatment for amyloidosis, factors that could influence outcome.

We conclude that MEE is prognostic for survival in CA, but the strongest and only independently prognostic parameter derived by ^{11}C-acetate PET was FSV/LVM. The finding is in line with previous research that has identified the ratio of SV and LV volume (MCF) as an important prognostic factor in CA.

Further studies are indicated to explore the underlying mechanisms of altered oxidative metabolism in CA, and the inclusion of ECV measurement using PET–magnetic resonance imaging could be beneficial in this aspect. Larger and more uniform patient cohorts would be needed to investigate potential differences in oxidative metabolism and the prognostic value in the different subgroups of CA.

Conflict of interest

Funding

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Correlations between 11C-acetate and 15O-water PET measuring FSV (A), LVM (B) and FSV/LVM (C). R: Spearman rho.
Table S1. Univariate survival analysis in AL patients.
Table S2. Univariate survival analysis in ATTR patients.

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Paper IV

Population-based incidence, prevalence and survival of systemic AL amyloidosis in Sweden.

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Abstract

Background and aim: There are few population-based epidemiological studies in AL amyloidosis. We aimed to determine the incidence, prevalence and survival of AL amyloidosis in the general population of defined geographic regions of Sweden, and to analyse trends over time.

Methods: We identified all new cases of systemic AL amyloidosis from Uppsala and Stockholm County of Sweden with a biopsy verified diagnosis during year 2000-2020, that were registered in databases at the pathology departments of Uppsala and Karolinska University Hospitals in Sweden. Patient records were reviewed for all identified cases, and only patients assessed as systemic AL amyloidosis were included.

Results: During the studied time period, a total of 302 new patients were diagnosed with systemic AL amyloidosis, 76 were residents in Uppsala and 226 in Stockholm County. The age- and sex-adjusted incidence rate was 12.0 (95% CI 9.3-14.7) per million person-years for Uppsala County, where the identification of AL cases was assessed as complete. When analysing the trend over time, there was no significant change in the incidence during the study period (p = 0.153). The 5-year limited duration prevalence in Uppsala County increased from 9.9 cases per million inhabitants in 2004 to 38.6 in 2020, but the trend was not statistically significant (p = 0.434). The 20-year prevalence for 2020 was 48.9 (95% CI 31.3-76.4) per million inhabitants. Median survival from diagnosis for patients from Uppsala and Stockholm County was 21 months (95% CI 14.5-27.5), and was significantly longer for patients diagnosed during the later time period, with a median survival of 28 compared to 13 months (p < 0.001). Early mortality within 6 months from diagnosis decreased significantly from the earlier to the later part of the time period (from 38.2 to 25.6%, p = 0.003).

Conclusion: This population-based study with only confirmed cases of systemic AL amyloidosis determined the standardized incidence to 12.0 (95% CI 9.3-14.7) per million person years for Uppsala County of Sweden, and the 20-year prevalence to 48.9 (95% CI 31.3-76.4) per million inhabitants. There was no significant change in the incidence rate during the years 2000 to 2020. The 5-year prevalence increased numerically but the trend did not reach statistical significance. Prolonged overall survival and decreased early mortality was seen over time.

Keywords: AL amyloidosis, Incidence, Prevalence, Survival, Population-based

Introduction

In AL amyloidosis mis-folded immunoglobulin light chains produced from a pathologic B-cell clone are deposited in organs, where they cause tissue disruption and toxic effects leading to progressive organ dysfunction (1). The disease is uncommon, symptomatology is varying and specific tests are required for diagnosis. These factors contribute to the diagnostic delay that is often observed in AL amyloidosis (2). Prognosis has historically been dismal, with median survival without effective treatment of approximately 6 months (3). During the latest 20-year time period progress has been made in several areas. Awareness of the diagnosis has increased, diagnostic methods have evolved and treatment possibilities have increased. Improvement of survival over time has been reported from specialized amyloidosis centres, with median survival increasing to around 4.5 years in patients diagnosed after 2010 (4, 5).

There is a lack of population-based data on the incidence and prevalence of AL amyloidosis, and it is not known whether the improvements in diagnostics and treatment have resulted in prolonged survival in patients not treated at highly specialized centres. The aim of this study was to determine the incidence, prevalence and survival of AL amyloidosis in Uppsala and Stockholm County of Sweden, as well as to examine trends over time.

Methods

The diagnosis of AL amyloidosis requires histopathological detection and typing of amyloid. We identified all unique cases that received the code for amyloidosis in the databases at the pathology departments of Uppsala and Karolinska University Hospitals from the start of year 2000 until the end of year 2020, and that were registered inhabitants of Uppsala and Stockholm County according to the Swedish population registry.

The identified pathology reports were reviewed and cases with other subtypes of amyloidosis than AL were excluded. For all new identified AL cases, and all cases with an unclear diagnosis from the pathology reports, a review of the patient records was performed. Cases where the diagnosis remained uncertain (8 cases) were excluded, as were subjects diagnosed with amyloidosis at autopsy, if the amyloid was not typed (34 cases). Patients with localized AL amyloidosis, including nodular amyloidosis of the lungs, were excluded (55 cases). According to the IMWG (International Myeloma Working Group) definition of systemic AL amyloidosis (6), subjects with verified AL amyloidosis but without any organ involvement were also excluded (3 cases).

In all included cases, amyloid was detected through positive Congo red staining and amyloid typing was performed with immunohistochemical or western blot analysis. Cases with an uncertain amyloid typing, but with the

presence of a monoclonal protein or a diagnosis of multiple myeloma, were regarded as AL.

The majority of fat pad biopsies (including the majority from Stockholm County) were examined and typed at the specialized amyloid laboratory in Uppsala. Renal and cardiac biopsies, including those from Uppsala County, were in most cases initially evaluated at the pathology department of Karolinska University Hospital in Stockholm, and in unclear cases sent to Uppsala for review. That is, for residents in Uppsala County, all amyloid diagnostics was performed either at the pathology department in Uppsala or Karolinska and therefore included in this study. In Stockholm County, there are smaller pathology departments that might perform amyloid diagnostics, and these potential cases were not captured in this study. We therefore included only the cases from Uppsala County for the incidence and prevalence calculations, while the patients from Stockholm County were included in the treatment and survival analyses.

Since about half of the patients with AL amyloidosis have > 10% plasma cells (5, 7), co-existing myeloma was defined as myeloma diagnosis together with CRAB-criteria (hypercalcemia, renal insufficiency, anaemia or bone lesions attributable to myeloma).

Definition of organ involvement was based on the original consensus criteria (8). In cases where test results regarding organ involvement were not available for review, the assessment of the clinician described in the patient records was used for determining organ involvement. Since we did not have access to cardiac biomarkers and free light chain results for the majority of patients, the Mayo stage of the patients is not reported, neither were FISH results available.

The study was performed with ethical approval from the Swedish Ethical Review Authority.

Statistics

Statistical analyses were performed with IBM SPSS 28 and R Software. Descriptive measures are presented as numbers with percentages, or medians with range or interquartile range (IQR). For group comparisons, the Mann–Whitney U test and the Kruskal–Wallis test were used for continuous variables, and Pearson Chi-Square test for categorical variables.

Incidence rate was calculated as the number of AL amyloidosis cases each year, divided by the population count of the county for the corresponding year. 5-year limited duration prevalence was calculated for each year from 2004-2020 as the number of patients diagnosed during 5 years back in time and still alive at the end of the year of interest, divided by the population of the county for the corresponding year. 20-year prevalence was calculated for year 2020 using the same method. The age- and sex-adjusted incidence rate was

calculated through standardization to the 2010 Swedish population. Poisson regression was used to analyse trends in incidence rate and prevalence over time.

The Kaplan–Meier estimate was used for survival analysis, and the log-rank test was used to compare survival curves. Factors potentially influencing survival were examined using univariable and multivariable cox regression analyses, and presented as hazard ratios (HR) with 95% confidence intervals (CI). P-values < 0.05 were regarded as statistically significant.

In order to compare different time periods, the study period was divided into an early time period from the start of year 2000 to the end of June 2010, and a late period from July 2010 until the end of 2020.

The patients diagnosed at autopsy were excluded from prevalence calculation, survival analyses and from the descriptive statistics regarding organ involvement, IgM subtype and associated diagnosis.

Results

From January 1, 2000 to December 31, 2020, a total of 302 new patients were diagnosed with AL amyloidosis in Uppsala (n = 76) and Stockholm County (n = 226). The crude incidence rate was 10.8 (95% CI 8.6-13.5) per million person-years for Uppsala County. The incidence was higher in males than in females (incidence risk ratio 1.3 for males, 95% CI 1.1-1.7, p = 0.013). The age- and sex-adjusted incidence rate was 12.0 (95% CI 9.3-14.7) per million person-years.

Comparing the time periods 2000-2010 and 2011-2020, the standardized incidence rate for Uppsala County was 11.6 versus 12.3 per million person-years, and when analysing the trend over time using Poisson regression, there was no significant change during the study period (p = 0.153, figure 1A).

The 5-year limited duration prevalence for Uppsala County was calculated for each year from 2004-2020 and increased numerically during the time period from 9.9 (95% CI 3.4-29.2) cases per million inhabitants in 2004 to 38.6 (95% CI 23.4-63.7) in 2020, but the trend was not statistically significant (p = 0.434, figure 1B). 20-year prevalence for 2020 was 48.9 (95% CI 31.3-76.4) per million inhabitants for Uppsala County.

The majority (292 patients, 97%) were of Caucasian origin. 167 (55%) were males and 135 (45%) were females. Median age at diagnosis was 70 years (range 23-94), the highest incidence was seen in the age span 60-80 years for both sexes, figure 2. Diagnosis was made on autopsy in 15 cases (5.0%). Among the rest of the patients, 121 (40%) were diagnosed using fat pad biopsy, 77 (26%) kidney biopsy, 22 (7.3%) bone marrow biopsy, 21 (7.0%) cardiac biopsy, 20 (6.6%) gastrointestinal biopsy and 8 (2.6%) liver biopsy. In 18 patients (6.0%) diagnosis were made on other tissue biopsies (soft tissue, lungs, lymphatic tissue and skin). In 3 patients tissue biopsies showed both AL and transthyretin amyloidosis. In Uppsala County, more

patients were diagnosed on fat pad biopsy than in Stockholm County (64.5% vs 31.9%, p < 0.001). Whereas in Stockholm County more patients were diagnosed on kidney biopsy (28.8% vs 15.8%, p = 0.025) and bone marrow (9.3% vs 1.3 %, p = 0.021) than in Uppsala.

Organ involvement at diagnosis (patients diagnosed at autopsy excluded) were cardiac in 71%, renal in 59%, liver in 12%, gastrointestinal in 14% and nervous system in 8%. In Stockholm County more patients had renal involvement compared to in Uppsala County (62.4% vs 43.4%, p = 0.004), whereas in Uppsala more patients had cardiac involvement, 82.9% compared to 69.0% in Stockholm, p = 0.019.

51 patients (17.8%) had an associated hematologic diagnosis, including myeloma in 39 patients (13.6%), lymphoplasmacytic lymphoma in 10 patients (3.5%) chronic lymphocytic leukaemia and marginal zone lymphoma in 1 patient (0.3%) respectively. In total, 17 patients (5.9%) had IgM subtype.

First-line treatment after the diagnosis of AL amyloidosis included 28 different treatment regimens, the most common being bortezomib/dexamethasone (24.4%), melphalan/dexamethasone or prednisolone (15.7%) and cyclophosphamide/bortezomib/dexamethasone (13.2%). A significant proportion of patients (14.3%) did not receive amyloid specific treatment, but were only given symptomatic treatment or corticosteroids only. The reasons for this involved poor general condition, rapidly deteriorating disease, the diagnosis of AL amyloidosis not being clearly established or recognised, and the patient´s own wish.

High dose melphalan with autologous stem cell support was given as part of first line treatment to 37 patients (12.9%), and to one patient after disease progression. Among the patients treated with high dose therapy first line, 23 (62.2%) received induction therapy and 14 (37.8%) received high dose melphalan up-front. A significantly higher proportion of patients received induction therapy in the later time period (94.4%) compared to the early (31.6%), and the induction treatment differed between time periods with vincristine/doxorubicin/dexamethasone regimen being the most common in the early time period (83.3% of cases) and bortezomib combinations (88.2%) in the late time period. There was no significant difference in the frequency of high dose therapy between the early and the late time period (15.4% vs 11%, p = 0.263), or between Stockholm and Uppsala County (10.6% vs 18.4%, p = 0.076).

Organ transplantation was performed in 7 patients (2.4%), and included heart transplantation in 3 patients, kidney transplantation in 3 patients and liver transplantation in 1 patient.

When comparing the early and the late part of the studied time period, significantly fewer patients were diagnosed at autopsy in the late time period (1.8% in the late period vs 8.9% in the early period, p = 0.005), and more patients were assessed as having cardiac involvement at diagnosis (77% in the late period vs 62.6% in the early period, p = 0.009). Less patients received chemotherapy based treatment (high dose melphalan excluded) in the late

period (4.9 vs 52%, p < 0.001), instead more patients received proteasome inhibitor (PI), immunomodulatory drug (IMiD) and CD38 antibody based treatments (79.9% all together vs 12.2%, p < 0.001). Only symptomatic treatment was less common in the late time period, 10.4% compared to 20.3% in the early period (p = 0.029). In one patient, information on treatment could not be obtained. Table 1 shows the baseline and treatment characteristics in total and for the two time periods separately.

Median follow-up for surviving patients was 5.6 years (IQR 3.6-10.9 years). Follow-up was ended on September 5th 2023. Overall survival from the time of diagnosis (patients diagnosed at autopsy excluded) was 21 months in median (95% CI 14.5-27.5). The most frequent causes of death were cardiac failure (28.5%), other/general organ progression (14.9%), sudden cardiac death (11.9%) and infection (10.9%). Survival was longer in the later time period compared to the earlier, 28 months (95% CI 18.3-37.7) compared to 13 months (95% CI 7.6-18.4), p < 0.001. There was no significant difference in survival between Uppsala and Stockholm County (median survival 20 vs 21 months, p = 0.47).

Table 2A shows univariable and multivariable cox regression analysis of factors influencing survival. Shorter survival was seen in patients with age above 70 years at diagnosis (HR 1.54, 95% CI 1.16-2.06, p = 0.003) and in patients with cardiac involvement (HR 2.04, 95% CI 1.41-2.93, p < 0.001). Patients who underwent high dose chemotherapy had better outcome (HR 0.30, 95% CI 0.17-0.53, p < 0.001), median survival in patients receiving high dose therapy was 266 months (95% CI 77.5-454.5) or 22.2 years (95% CI 6.5-37.9). Figure 3 shows overall survival according to time period of diagnosis, age group, cardiac involvement and high dose therapy.

Early death (within 6 months from diagnosis) was less frequent in the later time period compared to the earlier, 25.6% versus 38.2%, p = 0.022. Heart involvement was associated with higher risk of early death in univariable and multivariable cox regression analysis (HR 2.52, 95% CI 1.39-4.58, p = 0.002), and treatment with high dose therapy was associated with lower risk (HR 0.15, 95% CI 0.04-0.60, p=0.008), table 2B. Causes of early death were predominantly heart related (cardiac failure 38.2% and sudden cardiac death 21.3%).

Among patients receiving high dose chemotherapy, 4 out of 38 (10.5%) died within the first 100 days from stem cell infusion. Before 2010 mortality was 15.8% and after 2010 5,3%, there were no deaths within 100 days of stem cell infusion after 2016.

Discussion

We calculated the age- and sex-adjusted incidence rate of AL amyloidosis in Uppsala County of Sweden to be 12.0 (95% CI 9.3-14.7) per million person-years, which is consistent with the incidence reported in a previous similar study from Olmsted County, Minnesota (9).

Since the diagnosis of AL amyloidosis requires a tissue biopsy, using pathology reports should identify the vast majority of patients. One possible exception could be patients with symptomatic myeloma and clinically suspected amyloidosis in whom the clinician might refrain from biopsy. This study captures all the biopsy proven AL amyloidosis cases from Uppsala County, and includes only verified cases of AL amyloidosis. The presence of a specialized amyloid laboratory in Uppsala, might also lead to less underdiagnosis in this region.

In this study, we chose not to present the incidence and prevalence for Stockholm County. The reason for this is that in Uppsala there is only one pathology laboratory to which specimens can be sent (except from certain biopsies that are primarily analysed in Stockholm), whereas in Stockholm there are several small departments, including some privately driven, except from the large unit at Karolinska University Hospital. Even though amyloid typing should be performed at a specialized unit, and most specimens probably are sent to the Karolinska or Uppsala department, there is still a possibility that smaller laboratories in Stockholm County attempt to perform amyloid diagnostics. However, we believe that the cases from Stockholm County still represent a population-based unselected cohort based on the finding that there was no difference in survival between Stockholm and Uppsala County. We therefore included this cohort in the treatment and survival analyses.

Table 3 summarizes data from previously published incidence studies in AL amyloidosis. Apart from the present study, there are three population-based reports from the last two decades (9-11), the other listed studies are not population-based or presents merely estimates of the incidence. The incidence rate in our study is similar to that of 12 (95% CI 8-16) per million person-years reported from Olmsted County, Minnesota (9). Like our study, the study from Olmsted only included confirmed cases, but compared to that study, our cohort included twice as many identified AL patients, which should increase the accuracy. Hua et al reported a lower incidence rate of around 6 cases per million person-years from Taiwan (10). The different results can be influenced by methodological differences between the studies, but there could also be variations in the incidence of AL amyloidosis depending on ethnicity. The incidence rate of multiple myeloma is reported to be lower in Asia compared to Western Europe (12), and this might be true for other plasma cell disorders as well.

Using diagnose coding to identify AL cases, as in the Taiwanese and previous Swedish studies (10, 11), is hampered by the risk of mis-classification, especially since the code for AL amyloidosis is grouped under the code E85.8 for "other amyloidosis" in the ICD (international classification of diseases) system, and did not have a specific code in the Swedish version of ICD-10 before 2015.

In our study, 5% of the cases were diagnosed at autopsy, suggesting that there is still an underdiagnosis. However, the proportion of diagnoses made

on autopsy decreased significantly from 8.9 in the earlier time period to 1.8% in the later period, and there were no diagnoses made on autopsy from 2016 and onwards. We did not observe any significant increase in the incidence over time, which could have been expected due to the improved diagnostics. Kyle et al (9) have analysed trends in the incidence of AL amyloidosis from 1950 to 2015 including their previous data (13), and found a numerically higher but not statistically different incidence rate during that time period.

With regards to the prolonged survival that is reported in our and other studies, an increase in prevalence would be expected over time. In our study we could not show any statistically significant increase in the 5-year prevalence during the studied time period. However, the previous Swedish study (11) reported a significant increase in 5-year prevalence during 2011-2019. In a study of the global epidemiology of AL amyloidosis, estimations of the prevalence was performed from previously published incidence and survival data (14). An increase in the estimated prevalence was found in all of the analysed countries, and the estimated 20-year prevalence for Sweden in that study was 56.7 per million.

We observed that patients from Stockholm County had a higher proportion of renal involvement, whereas cases from Uppsala had more cardiac involvement. However, there was no significant difference in survival between Uppsala and Stockholm County, that would indicate a selection of patients with less cardiac involvement in the Stockholm cohort. The rate of organ involvement might be difficult to compare between sites due to differences in clinical routines regarding for example cardiac imaging methods and reporting. We also found a higher frequency of heart involvement during the later time period of the study, which is probably due to increased use of cardiac biomarkers and better cardiac imaging methods.

We believe that our study includes a truly population-based cohort of patients, in opposite to other studies in AL amyloidosis that mostly are from highly specialized centres. A recently published "real-world" observational study from Europe (EMN23) included 4480 patients diagnosed 2004-2018, however these were largely reported from referral centres (15). Median age in that study was 65 years compared to 70 years in our study, indicating that the oldest patients were not included in the EMN cohort. In our study 14.6% of patients only received palliative/symptomatic treatment, the most common reasons being poor general condition and advanced or rapidly deteriorating disease, as compared to 0,5% receiving only steroids and none reported as not treated in the EMN report. The relatively large proportion of patients in our study not receiving amyloid specific treatment could reflect a patient population with more advanced age and comorbidities, but could also indicate that patients are diagnosed in late disease stages, and assessed as not having a chance of improvement. The proportion of patients not being treated decreased from 20.3% in the earlier time period to 10.4% in the later time period suggesting that less patients were diagnosed with advanced disease.

Median survival was 21 months in our study, which is shorter than in EMN23 (48.8 months), which could reflect that we had a truly population-based cohort. This is also indicated in comparison with the previous Swedish study (11) that was based on diagnose coding, were survival was 56 months in patients diagnosed 2011-2019 (compared to 28 months during the late time period in our study).

We found a significant improvement in survival over time, with an increase in median survival from 13 to 28 months comparing the early and late time period of the study. There was a significantly lower number of patients being diagnosed on autopsy and receiving only palliative treatment in the later time period compared to the early, which indicates that patients are diagnosed earlier in the disease process. Time to diagnosis has previously been shown to be an important prognostic factor (16). During the studied time period there has also been diagnostic progress with the introduction of the free light chain analysis, improved amyloid typing and cardiac diagnostics. Furthermore, the treatment pattern has changed between the two time periods, with more patients treated with chemotherapy based regimens (not high dose) in the early time period, and more patients receiving PI, IMiD and CD38-antibody based treatment in the late time period, which probably has had an impact on survival. However, daratumumab was not widely used in first line treatment in Sweden before the publication of the Andromeda study in 2021, and only 9 patients (5.5%) in our study received daratumumab upfront.

Early deaths have also decreased from the early to the late time period, but is still high at 25.6% with the predominating causes of death being related to cardiac involvement. Other reports from specialized centres in the US have also reported a reduction in early mortality to 13 and 24% respectively in two separate studies (4, 5), whereas the EMN23 could not report any improvement in mortality within 3 months of starting treatment (15).

Heart involvement was the most important risk factor for both total and early death in this study. Prognosis in AL amyloidosis is dependent mainly on the degree of cardiac involvement (17, 18). Other important prognostic factors are the depth of treatment response (19, 20) and the time to response (21). Limitations of our study, include the lack of information on cardiac stage and treatment responses.

In this study, high dose therapy in first line treatment was associated with better prognosis, both long term and within 6 months from diagnosis. This may partly be due to a selection of patients with lower degree of organ dysfunction. The frequency of high dose therapy of 12.9% was relatively low compared to the approximately 30% that has been reported from the Mayo clinic (5), but higher than the frequency of 5-6% reported from European studies (15, 22). Median survival among high dose treated patients was 22.2 years (95% CI 6.5-37.9). Previous studies have reported prolonged overall survival after high dose therapy over time, with median OS from diagnosis of 9.8 years in patients treated 2003-2011 reported from Boston (23), 11.4 years in patients

treated in 2007-2012 from the UK (22) and median survival for patients treated during later time periods not reached in either study.

We can describe a reduction in the treatment related mortality with high dose therapy from 15.8% to 5.3% between the two studied time periods. There was no significant difference in the proportion of patients receiving high dose therapy between the early and the late time period, but if patients generally were diagnosed earlier during the late time period, the patients that were selected for high dose therapy during the later period could have less advanced disease.

There was also a significantly higher proportion of patients receiving induction therapy in the later time period, as well as more effective induction regimens being used in the later time period, the majority receiving PI based induction as opposed to chemotherapy based in the early period. Bortezomib based induction therapy has previously been associated with better outcome after high dose therapy (23).

Regarding first-line treatment, a total of 28 different regimens were used during the whole study period, which reflects the variability of the disease and also the development over time with introduction of new treatments. Until recently, there has also been a lack of randomised studies and treatment guidelines, which might have contributed to the diversity in treatment choices. The latest additions to the treatment arsenal such as daratumumab and venetoclax have not contributed significantly to the improved outcome in this patient population, since these treatments were not widely used yet during the studied time period. Further improvement in the prognosis of AL amyloidosis is therefore probably to be expected.

Limitations of the study include the homogenous ethnicity of the patients with the vast majority having Caucasian origin, which might reduce the generalizability of the incidence rate to other populations. Also, given the retrospective nature of the study, factors such as organ involvement might not have been assessed uniformly during the studied time period and information is missing regarding staging, treatment responses and time to diagnosis, factors that are known to influence survival. Furthermore, the incidence calculations are based on a relatively small number of patients, even though the cohort is larger than in previous comparable studies.

In conclusion, this population-based study reports an incidence rate of 12.0 (95% CI 9.3-14.7) per million person-years for systemic AL amyloidosis in Uppsala County of Sweden, which is consistent with what has been found in previous similar studies. The incidence did not change significantly during the studied time period (2000-2020). The 20-year prevalence was 48.9 (95% CI 31.3-76.4) per million inhabitants. A numerical increase in the 5-year prevalence was seen, but the trend was not statistically significant. The observed decrease in patients diagnosed at autopsy, and in patients receiving palliative treatment can indicate that patients are being diagnosed earlier in the disease process. More effective treatments and earlier diagnosis have led to increased

survival over time, and there is also a decrease in the early mortality which is encouraging.

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Table 1: Baseline and treatment characteristics in total and for the early and late time period respectively.

	Total	Early period (2000-mid 2010)	Late period (Mid 2010-2020)	p-value*
Number of subjects [n (%)]	302	135	167	
Uppsala County [n (%)]	76 (25.2)	34 (25.2)	42 (25.1)	0.994
Male [n (%)]	167 (55,3)	74 (54.8)	93 (55.7)	0.879
Age [median years (IQR)]	70 (62-77)	70 (63-77)	70 (61-77)	0.804
Diagnosis on autopsy [n (%)]	15 (5.0)	12 (8.9)	3 (1.8)	**0.005**
Heart involvement [n (%)]	203 (70.7)	77 (62.6)	126 (77)	**0.009**
0-1 organ [n (%)]†	152 (53.0)	60 (48.8)	92 (56.1)	0.219
2 organs [n (%)]†	103 (35.9)	48 (39.0)	55 (32.9)	0.337
3-4 organs [n (%)]†	32 (11.1)	15 (12.2)	17 (10.4)	0.626
IgM subtype [n (%)]	17 (5.9)	8 (6.5)	9 (5.5)	0.718
Associated diagnosis [n (%)]	51 (17.8)	28 (22.8)	23 (14)	0.055
First line treatment				
High dose therapy [n (%)]	37 (12.9)	19 (15.4)	18 (11.0)	0.263
Induction therapy [n (%)]	23 (62.2)	6 (31.6)	17 (94.4)	**< 0.001**
Chemotherapy[1] (not HD) [n (%)]	72 (25.1)	64 (52.0)	7 (4.3)	**< 0.001**
PI based[2] [n (%)]	110 (38,3)	10 (8.1)	100 (61.0)	**< 0.001**
IMiD based[3] [n (%)]	27 (9.4)	5 (4.1)	22 (13.4)	**0.015**
CD38-Ab based[4] [n (%)]	9 (3.1)	0 (0)	9 (5.5)	**0.016**
CD20-Ab based[5] [n (%)]	13 (4.5)	5 (4.1)	8 (4.9)	0.486
Palliative treatment[6] [n (%)]	42 (14.6)	25 (20.3)	17 (10.4)	**0.029**

*Pearson Chi-Square test comparing early and late time period, Kruskal-Wallis test for age. †Out of heart, kidney, liver and gastrointestinal tract. Statistically significant values (p<0.05) are indicated in bold. HD; high dose. PI; proteasome inhibitor. IMiD; immunomodulatory drug. Ab; antibody. [1]Includes low dose melphalan, cyclophosphamide and doxorubicin. [2]Includes bortezomib, carfilzomib, bortezomib/cyclophosphamide/dexamethasone and bortezomib/melphalan/dexamethasone regimens. [3]Includes thalidomide, lenalidomide, pomalidomide, cyclophosphamide/thalidomide/dexamethasone and bortezomib/lenalidomide/dexamethasone regimens. [4]Includes combinations of daratumumab with bortezomib/dexamethasone and bortezomib/cyclophosphamide/dexamethasone. [5]Includes combinations of rituximab with bortezomib, chemotherapy and dexamethasone. [6]Only steroids included.

Table 2: Uni- and multivariable analysis of A) overall mortality and B) death within 6 months from diagnosis. Patients diagnosed at autopsy are excluded.

A)	Univariable Cox analysis			Multivariable Cox analysis		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Male	1.28	0.98-1.68	0.069	-	-	-
Age at diagnosis ≥ 70 y	2.08	1.58-2.74	**<0.001**	1.57	1.18-2.09	**0.002**
Time period						
Jan 2000-Jun 2010	Reference					
Jul 2010-Dec 2020	0.65	0.49-0.84	**0.001**	0.56	0.43-0.74	**<0.001**
Heart involvement	1.93	1.42-2.64	**<0.001**	1.85	1.36-2.53	**<0.001**
>1 organ involved	1.23	0.95-1.61	0.122	-	-	-
High dose therapy	0.23	0.13-0.40	**<0.001**	0.29	0.16-0.52	**<0.001**
IgM subtype	1.38	0.83-2.30	0.218	-	-	-
Co-existing myeloma with CRAB-criteria	1.13	0.77-1.67	0.525	-	-	-

B)	Univariable Cox analysis			Multivariable Cox analysis		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Male	1.35	0.88-2.08	0.166	-	-	-
Age at diagnosis ≥ 70 y	1.45	0.95-2.22	0.084	-	-	-
Time period						
Jan 2000-Jun 2010	Reference					
Jul 2010-Dec 2020	0.63	0.42-0.96	**0.031**	0.55	0.36-0.84	**0.006**
Heart involvement	1.99	1.16-3.42	**0.013**	1.95	1.13-3.36	**0.017**
>1 organ involved	0.92	0.61-1.40	0.698	-	-	-
High dose therapy	0.13	0.03-0.53	**0.004**	0.14	0.03-0.56	**0.005**
IgM subtype	0.84	0.34-2.07	0.703	-	-	-
Co-existing myeloma with CRAB-criteria	1.25	0.71-2.22	0.437	-	-	-

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Table 3. Previously published incidence studies in AL amyloidosis.

Author, year	Population	Time period	Method	Number of cases	Incidence*	Survival
Kyle, 1992(13)	Olmsted County, Minnesota, USA	1950-1989	Population based. Patient records, autopsy reports	21	8.9 (95% CI 5.1-12.8)†	2.3 y
Hemminki, 2012(24)	Sweden	2001-2008	Estimation from in- and outpatient diagnose registry	-	3.2	3 y
Pinney, 2013(25)	National Amyloidosis Centre, UK	2000-2008	Estimation from referrals to NAC and death certificates	-	3	-
Aguirre, 2016(26)	Buenos-Aires and surroundings, Argentina	2006-2014	Prospective cohort from the Italian Hospital Medical Care Program	12	4.54 (95% CI 1.77-7.31)†	-
Quock, 2018(27)	USA	2007-2015	Insurance claims data, diagnosis of amyloidosis plus AL specific treatment	2207	10.8-15.2†	-
Kyle, 2019(9)	Olmsted County, Minnesota, USA	1990-2015	Population based. Patient records, autopsy reports and death certificates	35	12 (95% CI 8-16)†	-
Hou, 2022(10)	Taiwan	2016-2019	Population based, diagnose code from national health insurance research database	841	5.26-6.55‡	-
Mellqvist, 2023(11)	Sweden	2011-2019	Population based, diagnose code combined with registries	846	5.4-6.8‡	4.7 y

*Cases per million person-years; †age- and sex-adjusted; ‡age-adjusted

Figure 1: Yearly (dots) and trend (solid line) of A) Incidence rate and B) 5-year prevalence per million inhabitants in Uppsala County.

(A)
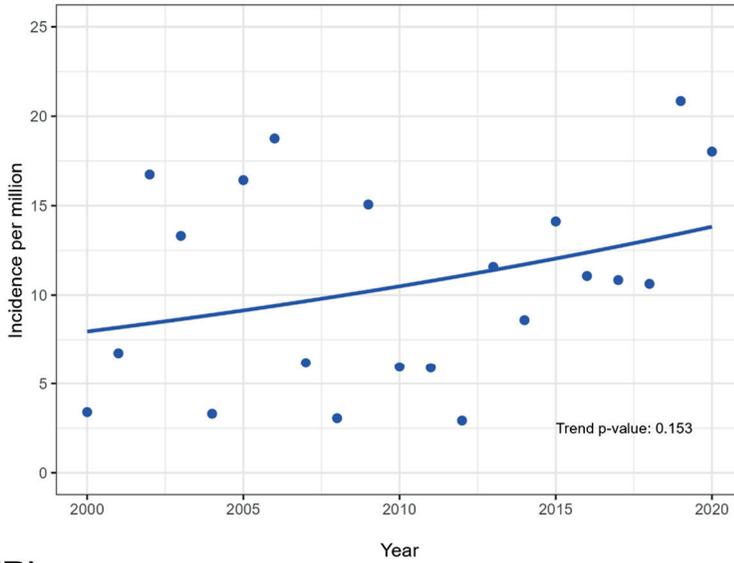
(B)
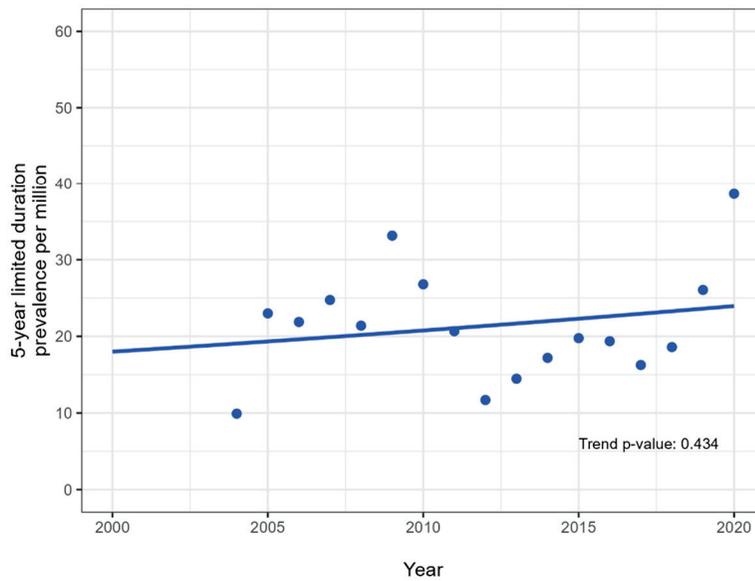
Figure 2: Number of patients diagnosed per age group and gender.
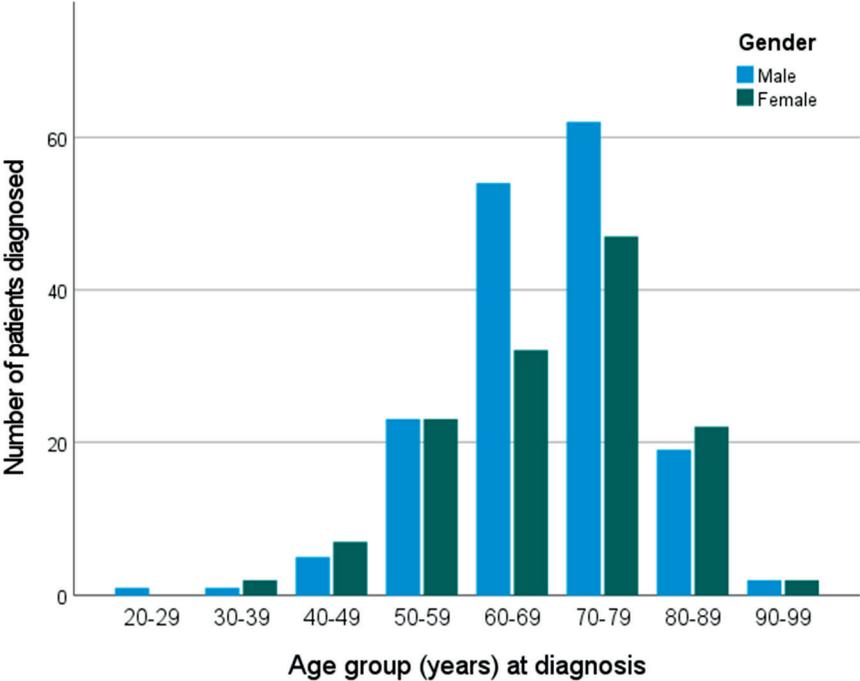
Figure 3: Overall survival according to A) time period of diagnosis, B) age, C) cardiac involvement and D) high dose therapy.

(A)

No. at risk						
Jul 2010-Dec 2020	164	45	18	4		
Jan 2000-Jun 2010	123	35	17	11	6	2

(B)

No. at risk						
<70 years at diagnosis	142	55	29	14	6	2
>70 years at diagnosis	145	25	6	1		

(C)

Survival proportion vs. Months from diagnosis

Log rank p < 0.001

No cardiac involvement

Cardiac involvement
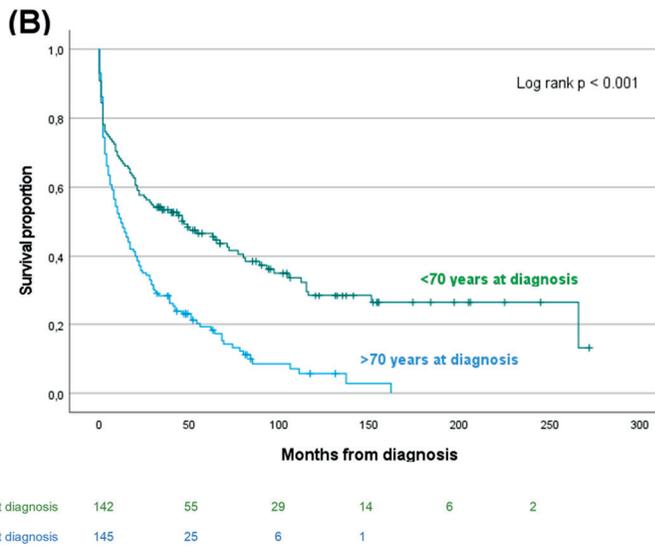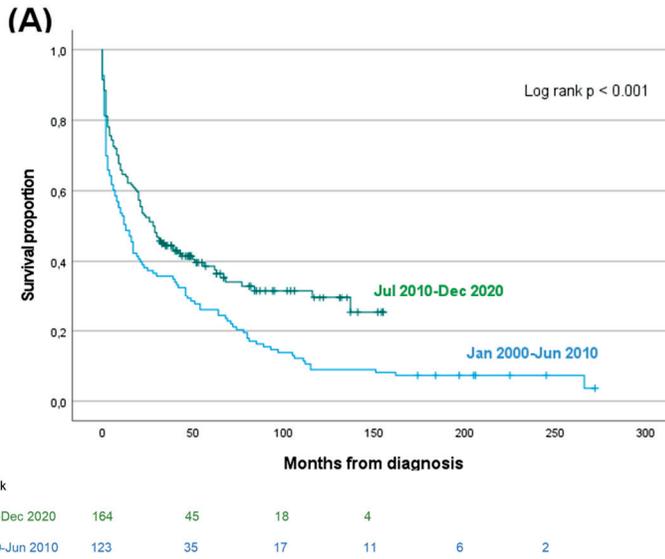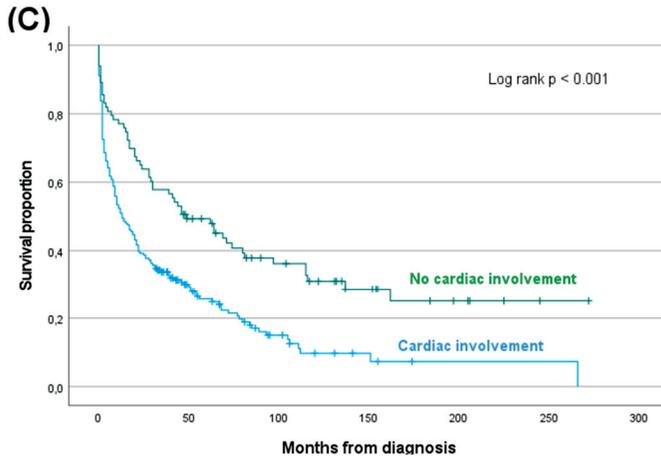
No. at risk

No cardiac involvement	83	38	22	11	5	1
Cardiac involvement	204	42	13	4	1	1

(D)

Survival proportion vs. Months from diagnosis

Log rank p < 0.001

High dose therapy

No high dose therapy
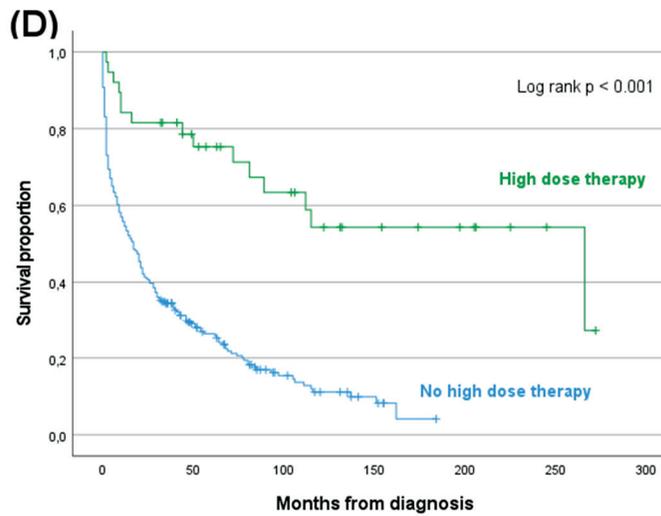
No. at risk

High dose therapy	38	24	16	9	6	2
No high dose therapy	249	56	19	6		

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