SUPPLEMENTAL MATERIAL

Reactive vasodilation predicts mortality in primary systemic light chain (AL) amyloidosis

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Methods

Controls’ selection in the prospective cohort

In order to assess differences in vascular characteristics attributed to the disease, AL patients were matched to controls in a 1:k (maximum k=7) ratio by using a greedy matching algorithm of nearest neighbor for age and sex \(^1\). Sex was matched exactly whilst the caliper for age enabled matching within a range of 2 years. Next, renal dysfunction by eGFR-staging (CKD Stage I >90, Stage II 60-90, Stage IIIA 45-60, Stage IIIB 30-45, Stage IV 29-15 and Stage V <15 ml/min/1.73m\(^2\)) was determined within cases and k-control pairs. All eligible controls with the same CKD stage were retained and evaluated in the next matching steps that employed a relevant iterative approach for the following exposure variables in hierarchical order: CAD, diabetes mellitus, smoking and hyperlipidemia. If no matching could be achieved on CKD stage (as well as on subsequent matching factors) for neither of k controls, we retained these pairs and evaluated sequentially the next matching criteria (CAD, DM, smoking and hyperlipidemia). Hypertension was not a matching criterion because low BP is a clinical characteristic of AL, which was part of the main hypothesis of the study. At the final step, if more than one matches for all parameters were available, one was selected at random to remove any potential bias.

Controls were retrospectively recruited from an ongoing FMD registry of subjects examined in our laboratory between 2008-2018 for research purposes. The FMD registry is currently comprised of 5 cohorts as follows 1. patients at high CV risk (n=213), 2. Volunteers at low or moderate risk (n=204) 3. Patients with various levels of renal dysfunction (n=47) 4. Postmenopausal women (n=571) and 5. Young healthy volunteers with ideal CV risk profile (n=36). FMD registry cohorts have been recruited for the purposes of previously published and ongoing clinical research (example publications: \(^2-9\)). FMD in this registry was performed in the same laboratory using the same FMD protocol and analyzed by the same operator (MK).
From the total control pool, we determined 74 subjects without any traditional risk factors (less than 60 years old, diabetes mellitus, hypertension, hyperlipidemia, smoking, sedentary life, obesity) or any disease or treatment. From this cohort, 23 subjects could be exactly matched for age and sex with 23 AL patients and their matched counterparts for risk factors, using the same method as described above.

Aiming to estimate associations between hemoatologic response and post-AL treatment effect FMD changes, we further analyzed data on 15 patients from the derivation cohort (recruited between 12/2007 to 12/2009) with available FMD measurements at baseline (before treatment initiation) and 6 to 12 months post treatment (prospective observational cohort) as described in supplementary methods. FMD re-assessment was performed when treatment regimen should be changed due to disease progression or complete response to therapy or at a maximum of 12 months if treatment was planned to be continued.

Exclusion criteria for the study

Derivation and validation AL cohorts: previous treatment for systemic AL amyloidosis and denial to consent

exhNO and CPT AL cohorts: Denial to consent.

Main analysis, 115 case-control matched pairs and exhNO controls: Control subjects with autoimmune or chronic inflammatory diseases, cancer, active infection, acute renal failure, acute coronary syndrome, acute stroke or any other condition limiting survival to less than 1 year were excluded from the matching process.

Subgroup analysis, 23 age- and sex-matched healthy controls and CPT protocol control subjects: Older than 60 years, diabetes mellitus, hypertension, hyperlipidemia, smoking, sedentary life, obesity, any disease, any treatment.

Clinical parameters
In all participants, a complete documentation of history, clinical and biochemical parameters for the presence of traditional risk factors (diabetes, smoking, dyslipidemia and hypertension), coronary artery disease and renal function was obtained. Renal function and staging was evaluated based on eGFR, calculated by the MDRD formula\(^{10}\). Renal function staging was based on standard guidelines. Hypertension was defined as reported history of hypertension or antihypertensive treatment before diagnosis of AL amyloidosis or office SBP >140 and/or DBP>90mmHg on two different occasions. Dyslipidemia was defined as reported history of hyperlipidemia or hypolipidemic treatment or total cholesterol >200mg/dl. Diabetes was defined as reported history of diabetes or antidiabetic treatment or fasting plasma glucose >126mg/dl on 2 occasions or diagnostic postprandial glycemic curve. Orthostatic hypotension (or postural hypotension) was defined as a systolic blood pressure decrease of at least 20 mm Hg or a diastolic blood pressure decrease of at least 10 mm Hg within 3 minutes of standing\(^{11}\).

**Mayo Staging in AL patients**

We classified AL patients (prospective derivation and validation cohort) into groups of disease prognosis and therapeutic response by using the widely staging system of Mayo Clinic 2004. We used the following combinations of NT-proBNP and cardiac TnT or TnI at presentation of diagnosis: stage I (NT-proBNP <332 ng/L and troponin-T <0.035 μg/L), stage II (NT-proBNP >332 ng/L or troponin-T >0.035 μg/L), stage IIIA (NT-proBNP >332 ng/L and troponin-T >0.035 μg/L) and stage IIIB (NT-proBNP >8500 ng/L and troponin-T >0.035 μg/L)\(^{12,13}\). A stage IIIB subgroup is associated with poorest survival\(^{14}\).

**Vascular studies**

During initial evaluation visit and before initiation of any chemotherapy or steroids, a series of vascular studies were performed. Peripheral vascular studies were performed in all patients and controls as previously described\(^{5,7,15}\). All subjects abstained from food or drink or smoking at least 12 hours before vascular studies. Vaso-active medication, including antihypertensive and hypolipidemic...
treatment, was retained for at least 2 drug half-lives prior to vascular measurements. Reactive vasodilatory was assessed by flow-mediated vasodilation (FMD). FMD was measured using high resolution ultrasonography (14.0-MHz multifrequency linear array probe, Vivid 7 Pro; General Electric Healthcare, Milwaukee, Wisconsin, USA). FMD was measured in the right brachial artery of each subject offline on ECG R wave by acquiring an average of 3 diameter measurements at baseline and the maximum diameter from a series of continuous manual measurements taken every 15 seconds after cuff deflation (post to 5 minutes ischemic inflation at the level of antebrachium) for a time period of 90 seconds\textsuperscript{16}. The intraobserver variability for brachial artery diameter measurements in our laboratory was $0.08 \pm 0.19$ mm, and intraclass correlation coefficient (ICC) for 2 repeated measurements of FMD at 2 consecutive days was 0.743. We normalized brachial artery diameter to body surface index (brachial artery diameter in mm/ body surface index in m$^2$) as previously described \textsuperscript{17}. A dedicated automatic border detection software (Medical Imaging Applications LLC, USA) was made available after initiation of the study\textsuperscript{18}. However, given a. the prospective nature of the study, b. the rarity and high early mortality of the disease under study and c. in order to retain as high as possible dataset consistency, we chose not to exclude the manually measured FMD measurements until that point but rather to complete FMD analyses in all subjects as initially designed. In a subgroup of 23 AL patients and 35 controls, FMD was calculated and compared using both methods. The level of agreement between the 2 methods based on the ICC calculation was satisfactory (ICC=0.881).

In a secondary approach, FMD was allometrically scaled to baseline diameter of right brachial artery as previously described\textsuperscript{19}. In brief, we used a log-linked generalized linear model with the change in diameter as the outcome and logarithmically transformed baseline diameter of right brachial artery as a covariate and obtained percentage values of allometric FMD by dividing the covariate-adjusted mean difference with the antilog value of the coefficient of baseline diameter.

Rationale for not measuring nitrate induced dilation

Nitrate induced dilation (NID) was not measured in this study in order to avoid serious nitroglycerin induced reactions in this group of patients with high prevalence of symptomatic (32.2%) hypotension.
Most importantly, as explained below, other conditions such as autonomic and cardiac dysfunction, commonly found in AL amyloidosis, may mediate serious adverse effects of nitroglycerin. Thus, we decided to avoid nitroglycerin administration because we could not exclude unpredictable severe reactions in some patients from the AL population.

In specific, acute administration of nitroglycerin:

a. may induce an unpredictable syndrome of hypotension and bradycardia,\textsuperscript{20, 21}
b. may induce inhibition of sympathetic system through triggering of alpha 2-adrenoceptors as a consequence of stimulation of peripheral sensory receptors with vagal afferents to the medulla,\textsuperscript{22} and
c. may provoke prolonged post-faint hypotension at least partly through lowering of cardiac output.\textsuperscript{23}

Given that the main reasons for hypotension in AL amyloidosis are believed to be low cardiac output, low oncotic pressure due to hypoalbuminemia and mainly autonomic dysfunction manifested as vascular sympathetic denervation and a generalized dysfunction in the autonomic control of vascular reactivity,\textsuperscript{24} these adverse actions of nitroglycerin on AL patients may jointly and unpredictably exacerbate most of the hypotensive effects with possible severe presentation and even life threatening bradycardic arrhythmias. In support, a recent study indicated that most AL patients with cardiac amyloidosis die due to bradycardic arrhythmias.\textsuperscript{25} Considering this evidence, we deemed that we would not be able to accurately predict an acute exacerbation of severe and possibly life-threatening hypotensive and/or bradycardic reaction by nitroglycerin-induced autonomic dysfunction in this specific group by simply excluding those with hypotension. Indeed, in our population most of AL patients had cardiac involvement with cardiac dysfunction (67%) and signs of autonomic dysfunction (40%) reflected by orthostatic hypotension and/or peripheral nerve involvement.

We discarded the option to measure NID in the rest of the patients who did not have cardiac or autonomic dysfunction because 1. this would introduce major selection bias 2. the sample size would be too small (n=32, 27.8%) and would not be representative of the population and 3. safety could not be guaranteed given that these reactions are often unpredictable.
Ultrasound was performed in the carotid and femoral arteries using a 14.0 MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine (14.0-MHz multifrequency linear array probe, Vivid 7 Pro; General Electric Healthcare, Milwaukee, Wisconsin, USA). Carotid segments of interest are described as follows: a. right and left common carotid artery, defined as the segment 1 cm proximal to carotid dilation, b. carotid bulb, defined as the segment between the carotid dilation and carotid flow divider and c. internal carotid artery, defined as 1 cm long arterial segment distal to the flow divider. In each arterial segment of interest, the maximal intima-media thickness (IMT) in the far wall was obtained offline, gated at R-wave, using a dedicated automated border detection software (GE Healthcare). Carotid IMT IOCV ranged between 4 and 10%, depending on the segment. All measurements were obtained by an experienced operator blinded to the medical history of the patients. Carotid and/or femoral plaques were recorded after detailed interrogation of each segment of interest to identify the optimal image showing maximum plaque size with the greatest encroachment into the lumen. Plaques were defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness of ≥1.5 mm.

Shear stress calculation

Hyperemic shear stress is a major correlate of brachial artery flow-mediated dilation. We calculated shear rate as an approximation of shear stress averaged over the whole cardiac cycle by using the equation: Shear stress (in dyn/cm²) = 8 × μ × mean flow velocity/resting diameter, where μ is the viscosity of blood as previously described. Blood viscosity, μ was assumed to be 0.035 dyne×s/cm².

Rationale and description for additional assessment of possible mechanisms associated with increased FMD in AL amyloidosis

Given the difference in FMD between AL patients and controls and the association of its increased values with all-cause mortality, we sought to provide mechanistic insights on the paradoxically increased reactive vasodilatation in this special group of interest.

In specific, we addressed 2 hypotheses:
1. That autonomic dysfunction affecting vascular function, which is commonly found in AL amyloidosis, would be associated with increased FMD. In specific we hypothesized that due to commonly found impaired sympathetic vascular modulation in AL amyloidosis, any vasoconstrictive response to sympathetic stimulation would be attenuated. FMD is known to be augmented in conditions of sympathetic attenuation. For this purpose, we performed cold pressor test (CPT) in a population of AL patients and healthy control subjects without amyloidosis and assessed brachial arterial changes during and after this well validated sympathetic stimulus.

2. Since FMD is known to be at least partly mediated by nitric oxide (NO) availability, we measured markers of the metabolic pathways in our population of AL patients and controls. In specific, we hypothesized that markers of NO bioavailability and associated nitrosative stress would be augmented in AL patients as compared to their controls. To this end, we measured exhaled alveolar NO and circulating levels of nitrites as indirect measurement of NO availability, cGMP as the target molecule of NO in tissues, and 3-nitrotyrosine as an index of nitrosative stress.

Cold pressure test

Nineteen newly recruited AL patients and 10 healthy volunteers underwent brachial artery diameter assessment during and after concomitant sympathetic stimulation. Online Table VIII outlines disease-specific characteristics of AL patients (n=19).

For stimulation of the sympathetic nervous system, immersion of left hand in iced water (4°C) was used. All measurements were performed in a quiet, temperature-controlled room. The hand remained in iced water for 3 minutes (Cold pressure test, CPT). Responses to CPT were analyzed as following: A pre-CPT diameter of right brachial artery was determined from the average 60 s data prior to placing of the subject’s hand in ice water. Subsequently, brachial artery diameter was analyzed a) during the 3 mins of CPT and b) for additional 3 minutes following removal of patient's hand from ice water. The minimal, mean and maximal right brachial diameter and CPT response were recorded as a percent change from the pre-CPT base diameter. High resolution ultrasonography (14.0-MHz multifrequency linear array probe, Vivid 7 Pro; General Electric Healthcare, Milwaukee, Wisconsin, USA) and automatic border detection software (Medical Imaging Applications LLC,
USA) was implemented for CPT in all subjects. Noradrenaline was measured at three pre-specified time points in all participants, at the baseline, 2 minutes post application of the cold stimulus and 15 minutes after removal of the cold stimulus\(^{47}\). At these time points, plasma samples were collected using EDTA-Na2 as an anticoagulant. The samples were centrifuged for 15 minutes at 1000×g at 4°C within 30 minutes of collection. The supernatant was collected and stored at -80°C for the analysis of noradrenaline using a commercially available kit (Noradrenaline Research ELISA, BA E-5200, Rocky Mountain Diagnostics, Inc.) Briefly, 400μL of plasma samples and diluted standards and controls were placed in a cis-diol-specific affinity gel plate for the extraction of noradrenaline. Then, noradrenaline was acetylated and converted enzymatically according to manufacturer’s instructions. The derivatized standards, controls and samples were assigned to the competitive ELISA microtiter plate format and quantification of noradrenaline in the samples was achieved by comparing their absorbance with the standard curve using Graph Pad prism version 7 (Graph Pad Software, Inc.) (assay sensitivity 2pg/mL), The results were expressed in nmol/mL.

Nitric oxide pathway

In a subgroup of AL (n=57) and control patients (n=24) with available blood plasma samples, we measured markers of the NO metabolic pathway. In specific, we quantified circulating levels of nitrites as indices of NO availability\(^{41}\), cGMP as the target molecule of NO in tissues\(^{40, 42, 43}\) and 3-nitrotyrosine an index of nitrosative stress\(^{44}\). In addition, we measured exhaled alveolar NO in a newly recruited group of AL patients (n=26) as compared to a healthy control group without AL (n=30). Descriptive characteristics of the subgroup of AL patients, as well as comparisons with the overall AL population, are depicted in online Tables IX-XI.

Blood measurements

In the subgroup of AL (n=57) and control patients (n=24) plasma samples were collected using EDTA-Na2 as an anticoagulant. The samples were centrifuged for 15 minutes at 1000×g at 4°C within 30 minutes of collection. The supernatant was collected and stored at -80°C for further
analysis. Each sample was assigned a unique identification code and the researcher who performed the blood measurements was blinded.

Circulating nitrite measurement

The concentration of nitrite in blood plasma was determined using Griess reaction with a commercially available kit (Cayman's Nitrate/Nitrite Colorimetric Assay Kit 780001) as we have described previously\textsuperscript{48}. Each plasma sample was ultrafiltered through a 10kDa molecular weight cut-off filter (Pall Nanosep\textsuperscript{®} centrifugal device with Omega membrane, Sigma Aldrich: Z722065). The filters were pre-rinsed with Ultrapure water prior to ultrafiltration of the plasma. Then, 500μL of plasma was centrifuged for 30 minutes at 14,000xg at 4°C. For the determination of nitrites, 40μL of the filtrate was used, the Griess reagents were added and the absorbance of each well was measured at 540nm using the reader Infinite 200 PRO series (Tecan). The concentration of nitrite was determined with a nitrite standard curve using Graph Pad prism version 7 (Graph Pad Software, Inc.) according to the manufacturer’s instructions. The results were expressed in μmol/L and converted to log scale prior to statistical analysis.

Plasma cyclic GMP levels determination

The cGMP content was determined using a commercially available enzyme-linked immunosorbent assay kit following the manufacturer’s instructions (Direct cGMP ELISA kit, Enzo Life Sciences: ADI-900-013) as we have previously described\textsuperscript{49}. Plasma samples were diluted 1:5 to the Assay Buffer provided and 100μL of the diluted samples were used for the non-acetylated version of the assay. All standards and samples were tested in duplicate. The kit uses a polyclonal antibody against cGMP that binds, in a competitive manner, to the cGMP in the standards or samples. Therefore, the intensity of the yellow color was inversely proportional to the concentration of cGMP in the standards/samples. The optical density was determined at 405 nm with the microplate reader Infinite 200 PRO series (Tecan). The concentration of cGMP for each sample was calculated from the standard curve in pmol/mL (assay sensitivity is 0.37pmol/mL) using Graph Pad prism version 7 (Graph Pad Software, Inc.) and converted to log scale prior to statistical analysis.
3-Nitrotyrosine measurement in plasma

3-Nitrotyrosine in plasma samples was determined by competitive ELISA method according to the manufacturers' instructions (3-Nitrotyrosine ELISA Kit, BioVision,#K4158-100). More specifically, the nitrotyrosine standards were carefully prepared by reconstitution of the nitrotyrosine Standard Vial in appropriate volumes of Standard/Sample Dilution Buffer. 50μL of standards/samples were added to the designated wells in duplicates. The microtiter plate provided in this kit is pre-coated with 3-nitrotyrosine. Therefore, the 3-nitrotyrosine in the sample or standard competed with a fixed amount of 3-nitrotyrosine on the solid phase supporter for specific sites on a biotinylated detection antibody. Then, the excess conjugate and unbound sample/standard were washed three times from the plate. HRP-Streptavidin (SABC) was added to each well and incubated. A TMB substrate solution was added to each well and the enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution. The color change was measured spectrophotometrically at 450nm using the reader Infinite 200 PRO series (Tecan). The concentration of 3-nitrotyrosine in the samples was determined by comparing the absorbance of the samples to the standard curve using Graph Pad prism version 7 (Graph Pad Software, Inc.). The results were expressed in ng/mL and converted to log scale prior to statistical analysis (assay sensitivity 1.06ng/mL).

Alveolar nitric oxide measurement

We evaluated 26 newly – diagnosed patients with AL amyloidosis and 30 healthy volunteers, with no previous history of cardiopulmonary disease and normal lung function, as controls. Disease-specific characteristics of AL patients with assessment of exhaled NO are summarized in online Table XI.

All patients underwent spirometry and single-breath determination of carbon monoxide uptake in the lung, corrected for hemoglobin (carbon monoxide diffusion capacity, DLCO) in a Master screen Body (Jaeger, Germany), according to manufacturer’s instructions and standard European Respiratory Society (ERS) / American Thoracic Society (ATS) guidelines. Exhaled NO was online measured with the single breath technique by achemiluminescence analyzer CLD 88 sp (ECO MEDICS AG, Switzerland), supported with DENOX 88 module to generate NO free air and to integrate adaptive expiratory flow control, according to specific needs and standard ERS / ATS guidelines. Calibration
with syringe volume was daily. Each patient was comfortably seated with the mouthpiece at proper height and position, and breathed normally, inspired to Total Lung Capacity (TLC) and then exhaled for at least 12 sec, against a respiratory resistance with a positive mouth piece pressure. The exhalation phase was followed by a plateau over a 3-second period. This procedure excluded nasal NO leakage by closing the velopharingeal aperture. Applied flow rates were 50, 100, and 250 ml/sec and exhaled NO values were used to calculate alveolar NO\textsuperscript{54}.

The whole population refrained from eating, drinking and smoking before NO analysis, did not suffer from respiratory infections and did not receive inhaled or oral corticosteroids\textsuperscript{53}.

Statistical analysis
Continuous variables are expressed as mean±standard deviation for variables following normal distribution or median (interquartile range) for variables not following normal distribution. Nominal variables are presented in absolute and percentage values (\%). Normal distribution of all continuous variables was tested with the parametric test Shapiro-Wilk and graphically assessed by histograms and P-P plots. Markers of NO pathway are depicted as mean values±standard error in relevant. Respectively, FMD was shown as median values±standard error. Correlation between variables in the original scale or after logarithmic transformation was evaluated using Pearson’s correlation coefficient. The association between dichotomous outcomes and nominal variables (e.g. FMD≥4.5%) was tested with Pearson’s chi-squared test; in exploratory analyses for the association between ordinal and nominal variables in small samples we used the likelihood-ratio chi-squared test or the Kendall’s tau b test.

Differences between controls and cases were assessed by independent samples T-Test for continuous variables and chi-square test for nominal variables. When more than 2 groups were compared for continuous variables, we used one-way Analysis of Variance (ANOVA) with post-hoc comparisons corrected by the Dunnett’s method. FMD did not follow the normal distribution in histograms and quantile-quantile plots and was tested after natural log transformation or inverse rank normalization. For the main outcome (i.e. FMD in logarithmic scale), we used linear regression analysis to further compare the two groups after adjusting for potential confounders: a) traditional risk factors, i.e.
systolic and diastolic BP, hypertension, hyperlipidemia, smoking and diabetes mellitus b) baseline brachial artery diameter and post-occlusion hyperemic flow stimulus c) presence of peripheral plaque and/or coronary artery disease. In a second/confirmatory step, continuous FMD was used as the dependent variable after inverse ranking normalization. For each added confounder, we calculated a) the increase in the adjusted R squared index as an estimate of explained variation and b) we employed a cross validation procedure (leave one observation in an iterative approach) to calculate the predicted adjusted R squared index after fitting the multivariable model in differing data sets and compared observed to predicted R squared index; to avoid overfitting of the multivariable linear regression model, the three sets of confounders (a-c), were sequentially tested. We did not found evidence of collinearity among the confounders by using the variance inflation factor. We used residual analysis to test for goodness of fit of the multivariable regression models. Certain variables with extremely skewed distribution such as NTproBNP and hsTnT were compared between independent samples by the non-parametric Mann Whitney U test. Finally, we assessed the possible difference in changes of noradrenaline levels during the CPT test between AL patients and healthy controls by employing a two-level linear mixed model with random intercept and random slope; noradrenaline was transformed with the natural logarithm and was used as the dependent variable whereas the disease status (i.e. healthy control or AL patient) was inserted as an independent factor. Moreover, we assessed the possible difference in changes of noradrenaline levels during the CPT test between AL patients and healthy controls by employing a two-level linear mixed model with random intercept and random slope; noradrenaline was transformed with the natural logarithm and was used as the dependent variable whereas the disease status (i.e. healthy control or AL patient) was inserted as an independent factor. Finally, in the FMD registry (n=1,071), we used multivariable linear regression analysis to derive independent determinants of FMD. A pre-specified set of exposure variables was included in the model, i.e. age, sex, hypertension, hyperlipidemia, diabetes mellitus, eGFR stage and CAD.

Analysis of the prospective survival data
Cox proportional-hazards models were used to examine the association between baseline predictors and main endpoints of our study; data were censored at the time of the last visit. For patients lost during follow-up, their survival data were censored at the last date they were known to be alive. All cause death was flagged as the primary endpoint in survival analysis. Early mortality at 6 months was assessed as a secondary endpoint. The proportional hazard assumption of Cox model was assessed using the appropriate graph and statistical test (Schoenfeld residuals). Associations are presented as Hazard Ratio (HR) with 95% confidence intervals (CI). Multivariable survival models (i.e. best predictive model) for main endpoints were built as previously described. After reviewing potential prognostic variables of overall survival in AL amyloidosis from well-designed studies with adequate sample size and complete phenotyping, we tested all proposed predictors in univariate Cox regression models. We did not apply exclusion criteria in terms of prognostic variables; thus, variables that were not shown previously to affect prognosis but were biologically plausible (e.g. age and sex) were tested as well under univariate settings. Those presenting a signal or significant association (p<0.1) in univariate models were tested in the multivariable Cox models to identify independent parameters for survival endpoints. Predictors that were tested in univariate models were age, sex, Mayo Stage, renal-, liver- and nerve involvement, levels of dFLC, SBP≤100 mmHg, SBP as continuous variable, FMD<or ≥4.5%, CAD and presence of atherosclerotic plaque. Three multivariable models were used: a) Mayo Stage, nerve involvement, SBP≤100 mmHg and FMD<or ≥4.5% b) model A plus shear stress, baseline brachial diameter and hyperemic flow and c) model A plus age, sex, presence of coronary artery disease and presence of subclinical atherosclerosis. The interaction between FMD subgroups and potential effect modifiers in association with the endpoint of interest was statistically tested by introducing interaction-terms into the Cox regression models, and none of them were found to be statistically significant at P< 0.05 level.

In order to investigate the potential non-linear association between the continuous FMD and all-cause mortality in AL patients (n=115), we implemented restricted cubic splines (RCS) (i.e. natural splines) with 3 knots fixed at the 10th, 50th and 90th percentile of the FMD distribution and produced a smooth curve versus logHR in the y axis. Selection of the number of knots was data-driven. In specific, we fitted models with different number of knots in a non-parsimonious approach and
compared the Bayesian information criterion (BIC); among models with different number of knots, the one with smaller BIC was selected. In case of trivial differences in BIC, we favored less complex models (3 knots: 406.1, 4 knots: 405.5 and 5 knots: 410.1, respectively). In contrast, knot placement was prespecified according to fixed percentiles of FMD’s marginal distribution, given that there was no prior knowledge in literature of possible change points. However, knots’ placement is not as crucial as their number for adequate fit to observed data\textsuperscript{56-59}. Cox regression models with FMD modeled as a continuous term or in the form of RCS were compared in terms of information criteria (AIC, Akaike's information criterion).

After visual inspection of the dose-response curve between all-cause mortality and FMD that suggested the existence of a possible change point in the phenomenon, we aimed to analyze the prognostic value of FMD using a clinically relevant cutoff. For this purpose, we implemented the method by Contal and O’Quigley\textsuperscript{60} and we identify a cutoff point (i.e. 4.5 %) in the association between FMD and all-cause mortality in AL patients under survival settings. This method is based on the log-rank test and estimates the value of a continuous predictor that maximizes the difference in survival between the two groups defined by the optimal cutoff point. We further confirmed the existence of the cut off point for FMD by ROC analysis and selection of the maximum product of sensitivity and specificity (Liu method)\textsuperscript{61}, which indicated the FMD cutoff value best predicting mortality among AL amyloidosis patients.

In addition, we sought to assess the incremental value of FMD over established risk factors in terms of reclassification improvement for the pre-specified endpoint by implementing the continuous NRI (NRI), a category-free version of the NRI\textsuperscript{62}, and the integrated discrimination improvement (IDI), which integrates the NRI over all possible cutoffs and is equivalent to the difference in discrimination slopes (all measures of reclassification)\textsuperscript{62}. Correct reclassification was defined as new risk estimates for all-cause death showing increased concordance to actual mortality, i.e. higher risk for AL patients who eventually died and lower risk for participants who survived until the end of the follow-up period by adding increased FMD over the core prognostic model.

Sample size calculations
In terms of survival analysis, the final sample size of 115 subjects and 48 events provided over 85% power to establish a 2.5-fold alteration in HR (two-sided) towards the primary endpoint, assuming a ratio of control to experimental group of 1.35 and a survival rate for the control group (i.e. with FMD<4.5%) of 71.2% at the end of the follow up under the Freedman method for the disparity of two survivor functions. For independent samples comparisons (n=230), the sample size of 115 subjects per arm (patients with amyloidosis and matched controls) yielded over 95% power to detect a significant difference of 1% or more in FMD between the two subgroups. Measures of dispersion for FMD were calculated post-hoc from our study and Type I error was predefined at 0.05. Statistical analysis and power calculations were performed by STATA package, version 11.1 (StataCorp, College Station, Texas USA). The package "survMisc" in R version 3.4.0 was used to implement the method of Contal and O’Quigley for the identification of the optimal cutoff point of the association of continuous FMD with all-cause mortality. Regarding our secondary hypothesis exploring the association of increased FMD in AL patients with markers of NO metabolism, P-values were corrected by the Holm-Bonferroni method\(^63\) to control the family-wise error rate. We deemed statistical significance at a=0.05.
Supplemental References

1. Deen JF, Krieger EV, Slee AE, Arslan A, Arterburn D, Stout KK, Portman MA. Metabolic syndrome in adults with congenital heart disease. *J Am Heart Assoc*. 2016;5

2. Stamatelopoulos K, Sibbing D, Rallidis LS, Georgiopoulous G, Stakos D, Braun S, Gatsiou A, Sopova K, Kotakos C, Varounis C, Tellis CC, Kastritis E, Alevizaki M, Tselepis AD, Alexopoulos P, Laske C, Keller T, Kastrati A, Dimmeler S, Zeiher AM, Stellos K. Amyloid-beta (1-40) and the risk of death from cardiovascular causes in patients with coronary heart disease. *Journal of the American College of Cardiology*. 2015;65:904-916

3. Stamatelopoulos K, Georgiou S, Kanakakis I, Papamichael C, Oikonomidis N, Mantzou A, Samoulidou E, Loizos S, Zakopoulos N, Sfikakis PP. Circulating levels of TNF-like cytokine 1a correlate with reflected waves and atherosclerosis extent and may predict cardiac death in patients with stable coronary artery disease. *Cytokine*. 2015;72:102-104

4. Georgiopoulous GA, Lambrinoudaki I, Athanasouli F, Armeni E, Rizos D, Papamichael C, Protogerou A, Stellos K, Stamatelopoulos K. Prolactin as a predictor of endothelial dysfunction and arterial stiffness progression in menopause. *J Hum Hypertens*. 2017

5. Lambrinoudaki I, Georgiou GAS, Athanasouli F, Armeni E, Chatzidou S, Koutli E, Makris N, Kanakakis I, Stamatelopoulos K. Free androgen index as a determinant of arterial stiffness in menopause: A mediation analysis. *Menopause*. 2017

6. Georgiopoulous GA, Lambrinoudaki I, Athanasouli F, Armeni E, Rizos D, Kazani M, Karamanou M, Manios E, Augoulea A, Stellos K, Papamichael C, Stamatelopoulos K. Free androgen index as a predictor of blood pressure progression and accelerated vascular aging in menopause. *Atherosclerosis*. 2016;247:177-183

7. Georgiopoulous GA, Stamatelopoulos KS, Lambrinoudaki I, Lykka M, Kyrkou K, Rizos D, Creatsa M, Christodoulakos G, Alevizaki M, Sfikakis PP, Papamichael C. Prolactin and preclinical atherosclerosis in menopausal women with cardiovascular risk factors. *Hypertension*. 2009;54:98-105

8. Karatz K, Georgiou GAS, Yannakoula M, Efthimiou E, Voidonikola P, Mitrikou A, Manios E, Alevizaki M, Papamichael C, Stamatelopoulos K. Eating frequency predicts new onset hypertension and the rate of progression of blood pressure, arterial stiffness, and wave reflections. *J Hypertens*. 2016;34:429-437; discussion 437

9. Karatz K, Yannakoula M, Psaltopoulou T, Voidonikola P, Kollias G, Sergentanis TN, Retzas T, Alevizaki M, Papamichael C, Stamatelopoulos K. Meal patterns in healthy adults: Inverse association of eating frequency with subclinical atherosclerosis indexes. *Clin Nutr*. 2015;34:302-308

10. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med*. 1999;130:461-470

11. Kasper DL, Fa, Hauser SL, Longo DL, James JL, Loscalzo J. *Harrison's principles of internal medicine*. 2 (19th ed.). New York: McGraw Hill Education Medical, [2015]; p. 2639.

12. Dispenzieri A, Gertz MA, Kyle RA, Lacy MQ, Burritt MF, Therneau TM, Greipp PR, Witzig TE, Lust JA, Rajkumar SV, Fonseca R, Zeldenrust SR, McGregor CG, Jaffe AS. Serum cardiac troponins and n-terminal pro-brain natriuretic peptide: A staging system for primary systemic amyloidosis. *J Clin Oncol*. 2004;22:3751-3757

13. Dispenzieri A, Gertz MA, Kyle RA, Lacy MQ, Burritt MF, Therneau TM, McConnell JP, Litzow MR, Gastineau DA, Tefferi A, Inwards DJ, Micallef IN, Ansell SM, Porrata LF, Elliott MA, Hogan WJ, Rajkumar SV, Fonseca R, Greipp PR, Witzig TE, Lust JA, Zeldenrust SR, Snow DS, Hayman SR, McGregor CG, Jaffe AS. Prognostication of survival using cardiac troponins and n-terminal pro-brain natriuretic peptide in patients with primary systemic amyloidosis undergoing peripheral blood stem cell transplantation. *Blood*. 2004;104:1881-1887

14. Wechalekar AD, Schonland SO, Kastritis E, Gillmore JD, Dimopoulos MA, Lane T, Foli A, Foad D, Milani P, Rannigan L, Hegemont U, Hawkins PN, Merlini G, Palladini G. A
15. Stamatelopoulos KS, Kitas GD, Papamichael CM, Chryssohou E, Kyrkou K, Georgiopoulos G, Protogerou A, Panoulas VF, Sandoo A, Tentolouris N, Movrikakis M, Sfikakis PP. Atherosclerosis in rheumatoid arthritis versus diabetes: A comparative study. *Arterioscler Thromb Vasc Biol*. 2009;29:1702-1708

16. Heiss C, Laufer T, Dejam A, Kleinborg P, Hamada S, Rassaf T, Matern S, Feelisch M, Kelm M. Plasma nitroso compounds are decreased in patients with endothelial dysfunction. *J Am Coll Cardiol*. 2006;47:573-579

17. Chambers JC, Fusi L, Malik IS, Haskard DO, De Swiet M, Kooner JS. Association of maternal endothelial dysfunction with preeclampsia. *JAMA*. 2001;285:1607-1612

18. Vita JA, Keaney JF, Jr., Larson MG, Keyes MJ, Massaro JM, Lipinska I, Lehman BT, Fan S, Osypiuk E, Wilson PW, Vasan RS, Mitchell GF, Benjamin EJ. Brachial artery vasodilator function and systemic inflammation in the framingham offspring study. *Circulation*. 2004;110:3604-3609

19. Atkinson G, Batterham AM, Thijssen DH, Green DJ. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *J Hypertens*. 2013;31:287-291

20. Khan AH, Carleton RA. Nitroglycerin-induced hypotension and bradycardia. *Arch Intern Med*. 1981;141:984

21. Viti JA, Keaney JF, Jr., Larson MG, Keyes MJ, Massaro JM, Lipinska I, Lehman BT, Fan S, Osypiuk E, Wilson PW, Vasan RS, Mitchell GF, Benjamin EJ. Brachial artery vasodilator function and systemic inflammation in the framingham offspring study. *Circulation*. 2004;110:3604-3609

22. Wuerz R, Swope G, Meador S, Holliman CJ, Roth GS. Safety of prehospital nitroglycerin. *Ann Emerg Med*. 1994;23:31-36

23. Stamatelopoulos KS, Papamichael CM, Chryssohou E, Kyrkou K, Georgiopoulos G, Protogerou A, Panoulas VF, Movrikakis M, Sfikakis PP. Atherosclerosis in rheumatoid arthritis versus diabetes: A comparative study. *Arterioscler Thromb Vasc Biol*. 2009;29:1702-1708

24. Mitrovic O, Pasic M, Ristic G, Vucicevic S, Jovanovic M, Djordjevic J. Increased flow-mediated vasodilation in cirrhotic patients with ascites: Relationship with renal resistive index. *Liver Int*. 2008;28:1396-1401
Endothelial function and peripheral vasomotion in the brachial artery in neurally mediated syncope. Clin Cardiol. 2000;23:820-824

Park KH, Han SJ, Kim HS, Jo SH, Kim SA, Park WJ. Endothelial function and cardiovascular autonomic activity in neurally mediated syncope. Cardiology. 2016;134:65-71

Galetta F, Franzoni F, Plantinga Y, Ghiadoni L, Merico G, Tocchini L, Braccini L, Rossi M, Carpi A, Taddei S, Santoro G. Endothelial function in young subjects with vaso-vagal syncope. Biomed Pharmacother. 2006;60:448-452

Thijssen DH, de Groot P, Kooijman M, Smits P, Hopman MT. Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. Am J Physiol Heart Circ Physiol. 2006;291:H3122-H3129

Dyson KS, Shoemaker JK, Hughson RL. Effect of acute sympathetic nervous system activation on flow-mediated dilation of brachial artery. Am J Physiol Heart Circ Physiol. 2006;290:H1446-H1453

Corretti MC, Plotnick GD, Vogel RA. The effects of age and gender on brachial artery endothelium-dependent vasoactivity are stimulus-dependent. Clin Cardiol. 1995;18:471-476

Lind L, Johansson K, Hall J. The effects of mental stress and the cold pressure test on flow-mediated vasodilation. Blood Press. 2002;11:22-27

Velasco M, Gomez J, Blanco M, Rodriguez I. The cold pressor test: Pharmacological and therapeutic aspects. Am J Ther. 1997;4:34-38

Carlström M, Liu M, Yang T, Zollbrecht C, Huang L, Pelesi M, Borniquel S, Kishikawa H, Hezel M, Persson AEG. Cross-talk between nitrate-nitrite-no and no synthase pathways in control of vascular no homeostasis. Antioxidants & redox signaling. 2015;23:295-306

Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov. 2008;7:156-167

Moncada S. Nitric oxide: Physiology, pathology, and pharmacology. Pharmacol Rev. 1991;517:317-326

Parker BA, Smithmyer SL, Jarvis SS, Ridout SJ, Pawelczyk JA, Proctor DN. Evidence for reduced sympatholysis in leg resistance vasculature of healthy older women. Am J Physiol Heart Circ Physiol. 2007;292:H1148-H1156

Pascualy M, Petrie EC, Brodkin K, Peskind ER, Wilkinson CW, Raskind MA. Hypothalamic pituitary adrenocortical and sympathetic nervous system responses to the cold pressor test in alzheimer’s disease. Biological psychiatry. 2000;48:247-254

Bibil SI, Papapetropoulos A, Iliodromitis EK, Papadimitris EK, Daiber A, Randriamoavonjy V, Steven S, Brouckaert P, Chatzianastasiou A, Kypreos KE, Hausenloy DJ, Fleming I, Andreadou I. Nitroglycerin limits infarct size through s-nitrosation of cyclophilin d: A novel mechanism for an old drug. Cardiovasc Res. 2018

Parissis JT, Andreadou I, Markantonis SL, Bistola V, Louka A, Pyriochou A, Paraskevaidis I, Filippatos G, Iliodromitis EK, Kremastinos DT. Effects of levosimendan on circulating markers of oxidative and nitrosative stress in patients with advanced heart failure. Atherosclerosis. 2007;195:e210-e215

Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. General considerations for lung function testing. Eur Respir J. 2005;26:153-161

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas
D. Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J.* 2005;26:319-338

52. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, Gustafsson P, Hankinson J, Jensen R, McKay R, Miller MR, Navajas D, Pedersen OF, Pellegrino R, Wanger J. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J.* 2005;26:720-735

53. Ats/ers recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med.* 2005;171:912-930

54. Högman M, Drca N, Ehrstedt C, Meriläinen P. Exhaled nitric oxide partitioned into alveolar, lower airways and nasal contributions. *Respiratory medicine.* 2000;94:985-991

55. Adam MA, Pura J, Goffredo P, Dinan MA, Reed SD, Scheri RP, Hyslop T, Roman SA, Sosa JA. Presence and number of lymph node metastases are associated with compromised survival for patients younger than age 45 years with papillary thyroid cancer. *J Clin Oncol.* 2015;33:2370-2375

56. Harrell F. Regression modeling strategies: With applications to linear models, logistic regression, and survival analysis. *New York, NY, Springer-Verlag.* 2001

57. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med.* 2010;29:1037-1057

58. Greenland S. Dose-response and trend analysis in epidemiology: Alternatives to categorical analysis. *Epidemiology.* 1995:356-365

59. Saraiya M, Kottiri BJ, Leadbetter S, Blackman D, Thompson T, McKenna MT, Stallings FL. Total and percent free prostate-specific antigen levels among u.S. Men, 2001-2002. *Cancer Epidemiol Biomarkers Prev.* 2005;14:2178-2182

60. Contal C, O’Quigley J. An application of changepoint methods in studying the effect of age on survival in breast cancer. *Computational statistics & data analysis.* 1999;30:253-270

61. Liu X. Classification accuracy and cut point selection. *Stat Med.* 2012;31:2676-2686

62. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med.* 2011;30:11-21

63. Marcus R, Eric P, Gabriel KR. On closed testing procedures with special reference to ordered analysis of variance. *Biometrika.* 1976;63:655-660
Additional Relevant References

1. Ando Y, Suhr OB. Autonomic dysfunction in familial amyloidotic polyneuropathy (fap). *Amyloid*. 1998;5:288-300

2. Jacob G, Costa F, Shannon J, Robertson D, Biaggioni I. Dissociation between neural and vascular responses to sympathetic stimulation: Contribution of local adrenergic receptor function. *Hypertension*. 2000;35:76-81

3. Vogel R. A comparison of the assessment of flow-mediated brachial artery vasodilation using upper versus lower arm arterial occlusion in subjects with and without coronary risk factors. *Clin Cardiol*. 2000;23:571-575

4. Thambyrajah J, Landray MJ, McGlynn FJ, Jones HJ, Wheeler DC, Townend JN. Abnormalities of endothelial function in patients with predialysis renal failure. *Heart*. 2000;83:205-209

5. Nagai K, Shibata S, Akishita M, Sudoh N, Obara T, Toba K, Kozaki K. Efficacy of combined use of three non-invasive atherosclerosis tests to predict vascular events in the elderly; carotid intima-media thickness, flow-mediated dilation of brachial artery and pulse wave velocity. *Atherosclerosis*. 2013;231:365-370

6. Yeboah J, Crouse JR, Hsu F-C, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: The cardiovascular health study. *Circulation*. 2007;115:2390-2397

7. Dorbala S, Vangala D, Bruyere J, Jr., Quarta C, Kruger J, Padera R, Foster C, Hanley M, Di Carli MF, Falk R. Coronary microvascular dysfunction is related to abnormalities in myocardial structure and function in cardiac amyloidosis. *JACC Heart Fail*. 2014;2:358-367

8. Atkinson G, Batterham AM. The clinical relevance of the percentage flow-mediated dilation index. *Curr Hypertens Rep*. 2015;17:4

9. Inoue T, Matsuoka H, Higashi Y, Ueda S-i, Sata M, Shimada K-e, Ishibashi Y, Node K. Flow-mediated vasodilation as a diagnostic modality for vascular failure. *Hypertension Research*. 2008;31:210
10. Ricci F, Fedorowski A, Radico F, Romanello M, Tatasciore A, Di Nicola M, Zimarino M, De Caterina R. Cardiovascular morbidity and mortality related to orthostatic hypotension: A meta-analysis of prospective observational studies. *Eur Heart J.* 2015;36:1609-1617

11. Guivernau B, Bonet J, Valls-Comamala V, Bosch-Morato M, Godoy JA, Inestrosa NC, Peralvarez-Marin A, Fernandez-Busquets X, Andreu D, Oliva B, Munoz FJ. Amyloid-beta peptide nitrotyrosination stabilizes oligomers and enhances nmdar-mediated toxicity. *J Neurosci.* 2016;36:11693-11703

12. Choi S, Won JS, Carroll SL, Annamalai B, Singh I, Singh AK. Pathology of nnos-expressing gabaergic neurons in mouse model of alzheimer's disease. *Neuroscience.* 2018;384:41-53

13. Johansson M, Rundqvist B, Eisenhofer G, Friberg P. Cardiorenal epinephrine kinetics: Evidence for neuronal release in the human heart. *Am J Physiol.* 1997;273:H2178-2185
**Supplementary Tables and supporting information**

**Online Table I.** Baseline characteristics of the AL amyloidosis cohort, the risk factors matched control population and the whole FMD registry from which the risk factors matched control populations was derived.

| Variable                          | AL patients | Age-, sex-and RFs-matched controls | FMD registry |
|-----------------------------------|-------------|------------------------------------|--------------|
| N                                 | 115         | 115                                | 1,071        |
| Age, years [mean(SD)]             | 64.4(10.2)  | 64.3(10.1)                         | *56.2(10.6)  |
| Sex, (male) n(%)                  | 62(53.9)    | 62(53.9)                           | *314(29.3)   |
| Diabetes mellitus, n(%)           | 14(12.2)    | 21(18.3)                           | 75(7.00)     |
| Hyperlipidemia, n(%)              | 50(43.5)    | *69(60.0)                          | 535(50.0)    |
| Smoking, n(%)                     | 18(15.7)    | 23(20.0)                           | *327(30.5)   |
| Arterial hypertension, n(%)       | 45(39.1)    | *68(59.1)                          | 352(32.9)    |
| GFR stage, n(%)                   |             |                                    |              |
| Stage 1                           | 36 (31.3)   | 26 (22.6)                          | *340(30.8)   |
| Stage 2                           | 27 (23.5)   | 33 (28.7)                          | 650(60.7)    |
| Stage 3A                          | 18 (15.7)   | 15 (13.0)                          | 50(3.83)     |
| Stage 3B                          | 13 (11.3)   | 5 (4.35)                           | 14(1.31)     |
| Stage 4                           | 8 (6.96)    | 6 (5.22)                           | 11(1.03)     |
| Stage 5                           | 13 (11.3)   | 6 (5.22)                           | 6(0.560)     |
| History of coronary artery disease, n(%) | 12(10.4) | 14(12.17) | *223(20.8) |
| FMD, (%) [median(IQR)]           | 4.00(1.92-6.06) | *2.32(0.961-4.55) | 4.46(2.63-6.52) |
* indicates significant difference from the reference category (i.e. AL patients) by independent samples T test or the chi squared test. FMD was transformed with the natural logarithm prior to comparison.

FMD did not differ between AL cohort and FMD registry neither univariately nor after adjustment for age, sex, diabetes, hyperlipidemia, smoking, hypertension, GFR stage and CAD.

AL patients were matched 1:1 with subjects from the FMD registry using a hierarchical model by age, sex and GFR stage and subsequently diabetes mellitus, coronary artery disease, smoking and hyperlipidemia. Matching was not performed for hypertension because low BP is a manifestation of AL amyloidosis.

Independent determinants of FMD levels in the FDM registry were age (β=-0.027, 95% CI -0.047/-0.007) and eGFR stage (β=-0.41, 95% CI -0.702/-0.118) by multivariable regression analysis.

Abbreviations: AL; amyloidosis, RF; risk factors, FMD; flow mediated dilatation, SD; standard deviation, CAD; coronary artery disease; IQR; interquartile range, GFR; glomerular filtration rate, BP; blood pressure.
Online Table II. Characteristics of the derivation AL population and of the temporal validation AL cohort

| Parameters                                           | Derivation cohort (n=115) | Validation cohort (n=55) | P value |
|------------------------------------------------------|---------------------------|--------------------------|---------|
| Age (years)                                          | 64.4(10.2)                | 64.6(8.93)               | 0.888   |
| Sex (male), n (%)                                    | 62(53.9)                  | 23(41.82)                | 0.600   |
| Smoking, n (%)                                       | 18(15.7)                  | 13(23.64)                | 0.207   |
| Arterial Hypertension, n (%)                         | 46(39.1)                  | 23(41.82)                | 0.821   |
| Hyperlipidemia, n (%)                                | 50(43.5)                  | 32(58.18)                | 0.091   |
| Diabetes, n (%)                                      | 14(12.2)                  | 6(10.91)                 | 0.811   |
| eGFR stage                                           |                           |                          | 0.312   |
| Stage 1                                              | 36 (31.3%)                | 16(29.09)                |         |
| Stage 2                                              | 27 (23.5%)                | 17 (30.91)               |         |
| Stage 3A                                             | 18 (15.7%)                | 10(18.18)                |         |
| Stage 3B                                             | 13 (11.3%)                | 5(9.09)                  |         |
| Stage 4                                              | 8 (6.96%)                 | 6(10.91)                 |         |
| Stage 5                                              | 13 (11.3%)                | 1(1.82)                  |         |
| History of Coronary artery disease, n (%)            | 12 (10.4%)                | 7(12.73)                 | 0.657   |
| SBP, mmHg                                             | 123.7±22.5                | 111(16.8)                | 0.003   |
| DBP, mmHg                                             | 72.2±10.2                 | 71(12.7)                 | 0.487   |
| Orthostatic hypotension, n (%)                       | 37 (32.2)                 | 21(38.18)                | 0.921   |
| Heart involvement, n (%)                             | 77 (70)                   | 43(78.18)                | 0.163   |
| Nerve involvement, n (%)                             | 22 (19.1)                 | 10(18.87)                | 0.968   |
| FMD (%)                                               | 4.00 (1.92-6.06)          | 3.81(1.53-6.23)          | 0.890   |
| Mayo Stage, n (%)                                     |                           |                          | 0.160   |
| I                                                     | 24(20.9)                  | 5(9.09)                  |         |
| II-IIIA                                               | 78(67.8)                  | 42(85.45)                |         |
| IIIB                                                  | 13(11.3)                  | 8(14.55)                 |         |
| AL-specific treatment, n(%)                          |                           |                          | 0.077   |
| Alkylator/steroid-based regimens, n (%)              | 17(14.8)                  | 3(5.45)                  |         |
| Proteasome inhibitors or Lenalidomide-based regimens, n (%) | 98(85.2)                  | 52(94.5)                 |         |
All continuous and dichotomous variables are described as mean±SD (except than FMD where median (interquartile range) is provided) and n (%), respectively

* indicates previous history of hypertension before diagnosis of AL amyloidosis

P-values are derived from independent samples T test or the chi-squared test for nominal variables.

FMD was transformed with the natural logarithm prior to comparison

Abbreviations: AL; amyloidosis, SD; standard deviation, eGFR; estimated glomerular filtration ratio, SBP; systolic blood pressure, DBP; diastolic blood pressure, FMD; flow-mediated vasodilatation
Table III. Characteristics of the 3 matched populations and the young healthy subjects with ideal CV risk profile

| Variable                  | AL patients | Age- and sex-matched healthy controls | Age-, sex- and RFs matched controls | Healthy subjects < 40 years |
|---------------------------|-------------|----------------------------------------|-------------------------------------|-----------------------------|
| N                         | 23          | 23                                     | 23                                  | 32                          |
| Age, years [mean(SD)]     | 53.2(6.58)  | 52.3(5.85)                             | 53.3(7.1)                          | *25.6(4.98)                 |
| Sex, male [n(%)]          | 13(56.5)    | 13(56.5)                               | 13(56.5)                           | *26(81.3)                   |
| Smoke, n(%)               | 7(30.4)     | 0(0)                                   | 8(34.8)                            | 0(0)                        |
| Arterial Hypertension, n(%)| 3(13.0)    | 0(0)                                   | *9(39.1)                           | 0(0)                        |
| Hyperlipidemia, n(%)      | 12(52.2)    | 0(0)                                   | 13(56.5)                           | 0(0)                        |
| Diabetes mellitus, n(%)   | 3(13.0)     | 0(0)                                   | 3(13.0)                            | 0(0)                        |
| CAD, n(%)                 | 1(4.35)     | 0(0)                                   | 1(4.35)                            | 0(0)                        |
| FMD, (%) [median(IQR)]    | 4.35(2.04)  | 4.17(3.46)                             | *2.17(0.82)                        | *5.86(4.65)                 |
| eGFR stage, n(%)          |             |                                        |                                     |                             |
| Stage 1                   | 13(56.5)    | 12(52.2)                               | 15(65.2)                           | *32(100)                    |
| Stage 2                   | 5(21.7)     | 11(48.8)                               | 5(21.7)                            |                             |
| Stage 3                   | 3(13.0)     | 1(4.35)                                | 1(4.35)                            |                             |
| Stage 4                   | 1(4.35)     | 1(4.35)                                | 1(4.35)                            |                             |
| Stage 5                   | 1(4.35)     | 1(4.35)                                | 1(4.35)                            |                             |

* indicates significant difference from the reference category (i.e. AL patients) by the independent samples T test or the chi squared test. FMD was transformed with the natural logarithm prior to comparison.

AL patients were matched 1:1 with subjects from the FMD registry using a hierarchical model by age, sex and eGFR stage and subsequently diabetes mellitus, coronary artery disease, smoking and hyperlipidemia. Matching was not performed for hypertension because low BP is a manifestation of AL amyloidosis.

Abbreviations: AL; amyloidosis, RF; risk factors, FMD; flow mediated dilatation, SD; standard deviation, CAD; coronary artery disease; IQR; interquartile range, eGFR; estimated glomerular filtration rate.
filtration rate, BP; blood pressure, NA; not available
Online Table IV. Univariate analysis of factors affecting survival in 115 patients with AL

|                      | Univariate | P-Value |
|----------------------|------------|---------|
|                      | HR (95%CI) |         |
| **Age**              | 1.00       | 0.793   |
|                      | (0.976 - 1.03) |       |
| **Sex**              | 1.08       | 0.801   |
|                      | (0.610 - 1.90) |       |
| **Disease specific parameters** |            |         |
| **Mayo Stage**       |            |         |
| Stage I              | Reference  |         |
| Stage II-III A       | 4.30       | 0.007   |
|                      | (1.49-12.4) |         |
| Stage IIIB           | 8.83       | <0.001  |
|                      | (2.62-29.7) |         |
| **Renal involvement**| 0.635      | 0.130   |
|                      | (0.353 - 1.14) |         |
| **Liver involvement**| 1.27       | 0.541   |
|                      | (0.591 - 2.73) |         |
| **Nerve involvement**| 3.23       | <0.001  |
|                      | (1.77 - 5.87) |         |
| **Dflc**             |            |         |
| > or ≤40             | 2.13       | 0.112   |
|                      | (0.839 - 5.38) |         |
| < or ≥180 mg/L       | 0.883      | 0.673   |
|                      | (0.496-1.57) |         |
| **SBP (≤100 mmHg)**  | 2.51       | 0.005   |
|                      | (1.32-4.77) |         |
| **SBP (as continuous variable)** | 0.980 | 0.006 |
|                      | (0.965 - 0.994) |         |
| **FMD specific parameters** |          |         |
| FMD≥4.5% or <4.5%    | 2.57       | 0.001   |
|                      | (1.44 - 4.59) |         |
| **Shear stress**     | 0.978      | 0.614   |
|                      | (0.896- 1.07) |         |
| **Baseline diameter**| 0.772      | 0.401   |
|                      | (0.515-1.16) |         |
| **Hyperemic flow**   | 0.990      | 0.473   |
|                      | (0.964- 1.02) |         |
| **Atherosclerosis parameters** |       |         |
| Coronary artery disease | 1.31     | 0.535   |
|                      | (0.555 - 3.11) |         |
| Presence of atherosclerotic plaque | 1.09 | 0.762   |
|                      | (0.611 - 1.96) |         |

Abbreviations: AL; amyloidosis, HR; Hazard Ratio, CIs; confidence intervals, dFLC; difference between involved and uninvolved serum free light chain concentration, SBP; systolic blood pressure, FMD; flow-mediated vasodilatation
Online Table V. Univariate and multivariable analysis of factors affecting early mortality (6 months) in 115 patients with AL

|                          | Univariate       | Multivariate     |
|--------------------------|------------------|------------------|
|                          | HR (95% CI)      | P-Value          | HR (95% CI)      | P-Value          |
| Age                      | 1.00 (0.958-1.05)| 0.896            |                  |                  |
| Sex                      | 1.02 (0.392-2.63)| 0.975            |                  |                  |
| Mayo Stage               |                  |                  |                  |                  |
| Stage I                  | Reference        |                  |                  |                  |
| Stage II-IIIA            | 4.00 (0.520-30.8)| 0.183            | 2.64 (0.334-20.9)| 0.358            |
| Stage IIIB               | 9.22 (1.03-82.6) | 0.047            | 3.13 (0.962-10.2)| 0.058            |
| Renal involvement        | 0.577 (0.220-1.52)| 0.265          |                  |                  |
| Liver involvement        | 1.43 (0.466-4.38)| 0.533            |                  |                  |
| Nerve involvement        | 2.45 (0.905-6.62)| 0.078            | 2.86 (1.02-8.1)  | 0.047            |
| dFLC                     |                  |                  |                  |                  |
| > or ≤40                 | 1.00 (0.288-3.48)| 0.999            |                  |                  |
| < or ≥180 nmol/L         | 1.33 (0.513-3.45)| 0.557            |                  |                  |
| SBP (≤100 mmHg)          | 1.74 (0.567-5.33)| 0.334            |                  |                  |
| SBP (as continuous variable) | 0.977 (0.954-1.00)| 0.061            | 0.987 (0.964-1.01)| 0.267            |
| FMD(≥4.5% or <4.5%)     | 4.75 (1.55-14.6) | 0.006            | 4.36 (1.41-13.5) | 0.010            |
| Coronary artery          | 2.36 (0.673-8.30)| 0.180            |                  |                  |
Presence of atherosclerotic plaque

Multivariate analysis was performed by adjusting for the best prognostic model for early mortality i.e. Mayo Stage (stage I vs II-IIIA vs IIIB) and nerve involvement

Abbreviations: AL; amyloidosis, HR; Hazard Ratio, CIs; confidence intervals, dFLC; difference between involved and uninvolved serum free light chain concentration, SBP; systolic blood pressure, FMD; flow-mediated vasodilatation
Online Table VI. Comparison of amyloidosis related organ involvement between patients with flow-mediated vasodilatation (FMD) <4.5% and those with FMD≥4.5%.

| Parameters | FMD<4.5% | FMD≥4.5% | P-value |
|------------|----------|----------|---------|
| **Traditional cardiovascular risk factors and CVD** | | | |
| Age, years | 64.4±10.4 | 64.4±10.01 | 0.995 |
| Sex, male (%) | 37 (56.1) | 25 (51.0) | 0.592 |
| Smoking, n(%) | 11 (16.6) | 7 (14.3) | 0.702 |
| Arterial Hypertension, n(%) | 30 (45.5) | 15 (30.6) | 0.093 |
| Hyperlipidemia, n(%) | 28 (42.4) | 22 (44.90) | 0.846 |
| Diabetes, n(%) | 9 (13.6) | 5 (10.20) | 0.558 |
| History of coronary artery disease, n(%) | 5 (7.57) | 7 (14.3) | 0.256 |
| eGFR stage | | | 0.370 |
| Stage 1-2 | 40 (60.6) | 23 (46.9) | |
| Stage 3a | 10 (15.2) | 8 (16.3) | |
| Stage 3b | 5 (7.58) | 8 (16.3) | |
| Stage 4 | 3 (4.55) | 5 (10.2) | |
| Stage 5 | 8 (12.1) | 5 (10.2) | |
| Presence of plaques, n(%) | 41(62.1) | 28(57.1) | 0.590 |
| Number of arterial plaques, n | 1(0-3) | 1(0-2) | 0.195 |
| **Specific disease characteristics** | | | |
| Heart involvement, n (%) | 38 (57.6) | 39 (79.6) | 0.013 |
| Renal involvement, n (%) | 51(77.3) | 29(59.2) | 0.037 |
| hsTnT, nmol/L | 33 (17-57.3) | 58.5 (41-94) | 0.007 |
| NTproBNP, nmol/L | 1414 (236-3823) | 2683 (841-7600) | 0.023 |
| Mayo stage (=III), n (%) | 12 (18.2) | 18 (36.7) | 0.033 |
|-------------------------|-----------|-----------|-------|
| Mayo stage (=IIIB), n (%) | 5 (7.58) | 8 (16.3) | 0.143 |
| SBP, mmHg                | 128.6±21.6 | 118±22.3 | 0.011 |
| DBP, mmHg                | 73.2±10.3 | 70.9±9.9 | 0.247 |
| Albumin, mmol/L          | 3.1(2.2-4) | 3.5(2.7-4.1) | 0.249 |

All continuous and dichotomous variables are described as mean±SD and n(%), respectively, except for not normally distributed variables which are described as median (IQR). P-value is derived from independent samples T test or Mann-Whitney test for continuous variables and chi-squared test for nominal variables.

Abbreviations: CVD; cardiovascular disease, eGFR; estimated glomerular filtration ratio, hsTnT; high sensitivity troponin T, NTproBNP; N-terminal pro b-type natriuretic peptide, SBP; systolic blood pressure, DBP; diastolic blood pressure, SD; standard deviation, IQR; interquartile range.
Online Table VII. Changes in noradrenaline levels during concomitant sympathetic stimulation (cold pressure test, CPT)

|                | Baseline (T0) | T1       | T2       | P-value for interaction |
|----------------|---------------|----------|----------|-------------------------|
| AL patients    | 2.08±0.734    | 2.31±0.773| 2.23±0.790| 0.606                   |
| (n=19)         |               |          |          |                         |
| Controls       | 1.96±0.783    | 2.55±0.816| 2.02±0.796|                         |
| (n=10)         |               |          |          |                         |

Levels of noradrenaline are presented in nmol/mL.

P-value for interaction is derived from linear mixed model analysis for repeated measurements of noradrenaline after transformation with the natural logarithm.

Abbreviations: T0; baseline, T1; immersion of hand in iced water, T2; 3 minutes after removal of cold stimulus AL; amyloidosis.
Online Table VIII. Disease characteristics of AL patients who underwent brachial artery diameter assessment during and after concomitant sympathetic stimulation (cold pressure test, CPT)

| Parameter                     | AL patients (n=19) |
|-------------------------------|--------------------|
| Age, (years)                  | 63.9(6.71)         |
| Sex (male), n (%)             | 14(73.7)           |
| Renal Involvement, n (%)      | 10(52.6)           |
| Heart Involvement, n (%)      | 16(84.2)           |
| Hypotension, n (%)            | 9(47.4)            |
| Mayo Stage (=III), n (%)      | 13(68.4)           |
| Performance status ≥2, n (%)  | 3(15.8)            |

Abbreviations: AL; amyloidosis
### Online Table IX. Comparison of AL patients according to availability of blood samples for assessment of the NO pathway

| Parameter                        | Non available NO measurements | Available NO measurements | P-Value |
|----------------------------------|-------------------------------|---------------------------|---------|
| Age, years (mean ±SD)            | 65.6±9.54                     | 63.1±10.8                 | 0.201   |
| BMI, kg/m² (mean ±SD)            | 27±4.18                       | 26.6±6.56                 | 0.716   |
| SBP, mmHg (mean ±SD)             | 125±20.9                      | 122±24                    | 0.433   |
| DBP, mmHg (mean ±SD)             | 72.9±10.6                     | 71.5±9.7                  | 0.442   |
| Sex, male n (%)                  | 24(41.4)                      | 29(50.9)                  | 0.307   |
| Arterial Hypertension, n (%)     | 25(43.1)                      | 20(35.1)                  | 0.338   |
| Hyperlipidemia, n (%)            | 25(43.1)                      | 25(43.9)                  | 0.999   |
| T2DM, n (%)                      | 6(10.3)                       | 8(14.0)                   | 0.568   |
| Smoking, n (%)                   | 11(19.0)                      | 7(12.3)                   | 0.304   |
| Orthostasic hypotension, n (%)   | 18(31.0)                      | 19(33.3)                  | 0.659   |
| Nerve Involvement, n (%)         | 11(19.0)                      | 11(19.3)                  | 0.964   |
| Heart Involvement, n (%)         | 40(69.0)                      | 37(64.9)                  | 0.944   |
| Mayo Stage (=III), n (%)         | 14(24.1)                      | 16(28.1)                  | 0.631   |
| FMD ≥4.5, n (%)                  | 20(34.5)                      | 29(50.9)                  | 0.075   |
| Presence of plaques, n (%)       | 37(63.8)                      | 36(63.2)                  | 0.644   |
| eGFR, (%) [median (IQR)]         | 72.4(52.9)                    | 65.3(60.1)                | 0.476   |
| FMD, (%) [median (IQR)]          | 3.45(4.72)                    | 4.55(3.68)                | 0.324   |
| Baseline brachial diameter, mm/m² (mean ±SD) | 2.21±0.45                    | 2.24±0.38                 | 0.683   |
| Post-occlusion hyperemic flow, cm/s (mean ±SD) | 19.2±11.3                    | 22.3±10.6                 | 0.139   |
| hsTnT, nmol/L [median (IQR)]     | 42.1(52.9)                    | 46.3(61.4)                | 0.583   |
| NTproBNP, nmol/L [median (IQR)]  | 1400(3500)                    | 2500(4900)                | 0.212   |

*P*-values are derived by the independent samples T test or the chi squared test. FMD was transformed with the natural logarithm prior to comparison. High sensitivity Troponin T and NTproBNP are compared by the non-parametric Mann Whitney U test.

Baseline brachial artery diameter is normalized for body surface area

Abbreviations: AL; amyloidosis, NO; nitric oxide, SD; standard deviation; BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, T2DM; type 2 diabetes mellitus, FMD; flow-mediated vasodilatation, eGFR; glomerular filtration ratio, IQR; interquartile range, NTproBNP; N-terminal pro B-type natriuretic peptide
Online Table X. Comparison of characteristics between AL patients and controls with available markers of NO pathway

|                                   | Controls (n=24) | AL patients (n=57) | p-value |
|-----------------------------------|----------------|-------------------|---------|
| Age, years (mean ±SD)             | 61.6±7.4       | 63.1±10.8         | 0.521   |
| Sex, male n (%)                   | 9 (37.5)       | 29 (50.9)         | 0.271   |
| BMI, kg/m² (mean ±SD)             | 28.6±4.75      | 26.6±6.56         | 0.195   |
| SBP, mmHg (mean ±SD)              | 126±18.3       | 122±24            | 0.499   |
| DBP, mmHg (mean ±SD)              | 76±10.9        | 71.5±9.7          | 0.072   |
| Arterial Hypertension, n (%)      | 11 (45.8)      | 20 (35.1)         | 0.364   |
| Hyperlipidemia, n (%)             | 11 (45.8)      | 25 (43.9)         | 0.87    |
| T2DM, n (%)                       | 6 (25.0)       | 8 (14.0)          | 0.233   |
| Smoking, n (%)                    | 9 (37.5)       | 7 (12.3)          | 0.01    |
| Presence of plaques, n (%)        | 14 (58.3)      | 36 (63.2)         | 0.683   |
| CAD, n (%)                        | 0              | 3 (5.26)          | 0.252   |
| eGFR stage, n (%)                 |                |                   | 0.007   |
| Stage 1                           | 12 (50.0)      | 16 (28.1)         |         |
| Stage 2                           | 11 (45.8)      | 14 (24.6)         |         |
| Stage 3                           | 1 (4.17)       | 17 (29.8)         |         |
| Stage 4                           |                | 5 (8.77)          |         |
| Stage 5                           |                | 5 (8.77)          |         |
| SBP<100mmHg, n (%)                | 2 (8.33)       | 10 (18.2)         | 0.262   |
| FMD, (%) [median (IQR)]           | 1.52 (3.80)    | 4.55 (3.67)       | <0.001  |
| FMD (natural log transformation), mean±SD | 0.82±0.754   | 1.58±0.524        | <0.001  |
| FMD (inverse ranking normalization), mean±SD | -0.43±0.682  | 0.185±1.05        | 0.01    |
| eGFR, (%) [median (IQR)]          | 88 (21)        | 65.3 (60.1)       | 0.005   |

Abbreviations: AL; amyloidosis, NO; nitric oxide, SD, standard deviation; BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, T2DM; type 2 diabetes mellitus, CAD; cardiovascular disease, eGFR; glomerular filtration ratio, FMD; flow-mediated vasodilatation, IQR; interquartile range
| Parameter                        | AL patients |
|---------------------------------|-------------|
| Age, (years)                    | 62.7(8.82)  |
| Sex, male n (%)                 | 11(42.3)    |
| Renal Involvement, n (%)        | 17(65.4)    |
| Heart Involvement, n (%)        | 19(73.1)    |
| Hypotension, n (%)              | 13(50.0)    |
| Mayo Stage (=III), n (%)        | 13(50.0)    |
| Performance status ≥2, n (%)    | 5(19.2)     |

Abbreviations: AL; amyloidosis, NO; nitric oxide
Online Table XII. Increased reactive vasodilation in two repeated measurements is associated with increased all-cause mortality in patients with AL amyloidosis (n=15)

| All-cause mortality |       |       |       |
|---------------------|-------|-------|-------|
|                     | No    | Yes   | P-value |
| Baseline and follow-up FMD<4.5% | 5 (62.5) | 1 (14.3) |       |
| Baseline and/or follow-up FMD≥4.5% | 3 (37.5) | 6 (85.7) | 0.026 |

P-value is derived from Kendall’s tau b test

Abbreviations: FMD, flow-mediated dilatation
Supplementary Figures and Figure Legends

Online Figure I. Dose-response curve between FMD and all-cause mortality in 115 AL patients. Continuous FMD was modeled as a restricted cubic spline with 3 knots and plotted versus the log hazard ratio (HR). Predicted HRs were estimated from a proportional hazards Cox regression model for all-cause mortality that controlled for low systolic blood pressure, Mayo Stage and Nerve Involvement. The knots were placed at 0%, 4% and 7.51% nodes (corresponding to the 10th, 50th and 90th percentile of the distribution of FMD). The upper- and lower-most dashed curves represent the 95% CIs around the predicted HRs (middle solid line). Hollow circles in the trajectory of the solid line in each plot represent the location of the knots. FMD; flow-mediated vasodilatation, AL; amyloidosis.
Online Figure II. Association of baseline and follow-up FMD values with hematologic response in a cohort of 15 AL patients. FMD; flow-mediated vasodilatation, AL; amyloidosis.
Online Figure III. Kaplan-Meier curves for the cumulative probability of survival in 15 AL patients with repeated measurements of vascular function (pre- and post-AL specific treatment) according to FMD values above or below 4.5%. Kaplan Meier curves show a steep reduction in survival in patients with increased FMD ($\geq 4.5\%$) during at least one measurement as compared to those with lower levels ($<4.5\%$) at both time instances. Due to the small number of patients ($n=15$) log-rank test was not calculated. The figure is presented for descriptive purposes due to the large deviation of the curves until the end of the follow-up. AL; amyloidosis, FMD; flow-mediated vasodilatation.
Graphical Abstract:

Certain items on this figure have been adapted from Servier medical arts website (https://smart.servier.com/).