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Impact of cardio-renal syndrome on adverse outcomes in patients with Fabry disease in a long-term follow-up

M. Siegenthaler [a,b], U. Huynh-Do [c], P. Krayenbuehl [b], E. Pollock [d], U. Widmer [a], H. Debaix [e], E. Olinger [e], M. Frank [f], M. Namdar [g], F. Ruschitzka [f], A. Nowak [a,*]

[a] Department of Internal Medicine, University Hospital of Zurich, Switzerland
[b] Department of Internal Medicine, Linth Hospital, Uznach, Switzerland
[c] Department of Nephrology, Hypertension and Clinical Pharmacology, University Hospital Bern, Switzerland
[d] Department of Nephrology, University Hospital of Zurich, Switzerland
[e] Institute of Physiology, University of Zurich, Switzerland
[f] Department of Cardiology, University Heart Centre, University Hospital Zurich, Switzerland
[g] Department of Cardiology, Geneva University Hospital, Switzerland

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ABSTRACT

Aims: Fabry disease (FD) is a rare X-linked lysosomal storage disease with a deficiency of α-galactosidase A leading to progressive sphingolipid accumulation in different organs, among them heart and kidney. We evaluated the impact of cardio-renal syndrome (CRS) on the incidence of major cardiovascular complications and death in a prospective FD cohort.

Methods and results: A total of 104 genetically proven FD patients were annually followed at the University Hospitals Zurich and Bern. The main outcome was a composite of incident renal replacement therapy (RRT), hospitalisation due to decompensated Heart Failure, new onset atrial fibrillation, pacemaker/ICD implantation, stroke/TIA and death. Estimated glomerular filtration rate (eGFR) and left ventricular myocardial mass index (LVMMI) where explored as the primary exposure variables. During the median follow-up of 103 [59–155] months, events occurred in 27 patients. In a Cox regression analysis, both higher LVMMI and lower eGFR were independently associated with a greater risk of developing adverse events after adjustment for multiple confounders (HR 1.67 [1.04–2.73] P = 0.03 per SD increase in LVMMI, HR 0.45 [0.25–0.83], P = 0.01 per SD decrease in eGFR). In patients with CRS, the risk to develop events was significantly increased if adjusted for demographics and RRT (HR 4.46 [1.07–18.62], P = 0.04), approaching significance if additionally adjusted for hypertension (HR 4.05 [0.95–17.29], P = 0.06). In Kaplan-Meier-Analysis, the poorest event-free survival was observed among patients with CRS.

Conclusions: CRS was associated with a high risk to develop cardiovascular complications and death, emphasizing the importance of its prevention and early recognition. A focus on cardio-reno-protective therapies is crucial.

1. Introduction

Fabry disease (FD) is an X-linked lysosomal storage disorder in which mutations of the *GLA* gene cause a decreased or absent activity of the enzyme alpha-galactosidase A (α-Gal A) and subsequent progressive intracellular accumulation of globotriaosylceramide (Gb_3) and other sphingolipids in various tissues [1,2]. The initial classic signs and symptoms include acroparesthesias, angiokeratoma, abdominal pain, hypohidrosis, corneal dystrophy and typically appear in childhood. With advancing age, vital organ dysfunction increasingly occurs resulting in cardiovascular disease, renal failure and premature strokes, on average more severely in males [3]. Combined renal and cardiac dysfunction is common in FD and associated with an increased mortality and morbidity risk [4,5].

Cardio-renal syndrome (CRS) is an increasingly recognized clinical entity which refers to the reciprocal association between cardiac and renal dysfunction, whereby injury to one organ directly promotes deterioration of the other [6]. This complex bilateral organ crosstalk can result from a variety of conditions where the primary failing organ may be the heart, the kidney or both. The latter is defined as CRS Type 5 and occurs secondarily to an underlying systemic process. FD is a typical example of the CRS Type 5, as described very recently [7]. CRS has been related to a particularly high morbidity and mortality in several settings [6,8,9], however, little is known about its impact on adverse long-term outcomes in FD patients.

In patients with FD, the indexed left ventricular myocardial mass (LVMMI) has repeatedly been shown to be a reliable parameter reflecting the onset and progression of Fabry disease-related cardiomyopathy [10,11]. The estimated glomerular filtration rate (eGFR) has

* Corresponding author at: Department of Internal Medicine, University Hospital Zurich, Rämistrasse 100, CH-8091 Zürich, Switzerland.
E-mail address: albina.nowak@usz.ch (A. Nowak).

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been used to quantify the renal involvement and shown to be a major risk factor for cardiovascular complications in FD [5]. The aim of this study was to assess the impact of CRS, reflected by LVMMI and eGFR, on clinical outcomes in a large prospective multicentre cohort of genetically confirmed FD patients and a long-term follow-up. The recognition of CRS should lead to specific prevention strategies and therapeutic approaches in order to at least attenuate the organ failure in those patients.

2. Methods

This is a retrospective analysis of a prospective, multi-centre cohort in Switzerland. The study was conducted in accordance with the principles of the Helsinki Declaration. The patients who could be contacted gave written informed consent. The authors have read and approved the manuscript.

2.1. Study population and treatment

The prospective FD cohort consisted of 104 patients, who were genetically tested (for further information see the Supplementary Table 1). The cohort was established in 2001 when enzyme replacement therapy (ERT) was newly developed and offered to all FD patients. Consecutive FD patients were registered and received routine annual multidisciplinary examinations at two tertiary care hospitals – University Hospitals Zürich (93 patients) and Bern (11 patients). Here, we report on the clinical course and adverse outcomes of 64 females and 40 males.

The baseline clinical evaluation was performed at the time the patients were included in the cohort. For the present analysis, the clinical data and information on hospitalisation were extracted from medical records.

FD patients in Switzerland are personally followed-up and examined at the Fabry Centres, the adverse events were evaluated during annual examinations. All patients had a comprehensive workup, including medical history, cardiac evaluation with echocardiography, renal, and neurological evaluations. A 24- or 48-hours ECG was performed annually and additionally if patients complained of palpitations or chest pain. Hospitalisation was defined as a hospital stay for at least 24 h. Standard transthoracic 2D-echocardiography was routinely performed in all patients.

For the evaluation of diastolic dysfunction, the left atrial volume index (LAVI), the mitral inflow (E/A ratio) and the mitral annular movement with tissue Doppler (e′ septal and lateral as well as an average of E/e′ ratio) was measured according to the current practice guidelines [12,13]. These measurements were performed using continuous recordings of mitral inflow, analysing the pulsed-wave during 10 s: the peak E-wave velocity (cm/s) in early diastole, the A-wave (cm/s) in late diastole at the leading edge of the spectral waveform and the pulsed wave tissue Doppler e′ velocity (cm/s) in early diastole. These measurements were available in the Zurich subgroup ($N = 82$) of patients. Diastolic dysfunction has been graded according to the recent algorithm from the ASE/EACVI recommendations for the evaluation of the left ventricular diastolic function [13] by reviewing and repeating all measurements of the archived echocardiographic studies.

Enzyme replacement therapy was initiated according to the written local guidelines and prescribed at the licensed dose of either 0.2 mg/kg body weight of recombinant α-agalsidase (Replagal) or 1 mg/kg body weight β-agalsidase (Fabrazyme) and given intravenously every 14 days. According to the guidelines, ERT was indicated in all males. In females, ERT was indicated if they had proteinuria of >300 mg per day, Fabry-typical kidney biopsy findings, signs of Fabry cardiomyopathy such as left ventricular hypertrophy or arrhythmia, stroke or transient ischemic attack (TIA), acroparesthesias despite conventional analgesic therapy, and/or gastrointestinal symptoms.

2.2. Endpoint definition and evaluation

As a primary endpoint, we defined the composite of requiring renal replacement therapy (RRT) (kidney transplantation or chronic dialysis), newly diagnosed atrial fibrillation (AF) of any type (paroxysmal/persistent), pacemaker and/or ICD implantation, hospitalisation due to decompensated heart hailure (HF), cerebrovascular events (stroke or TIA), and death, whichever occurred first. The follow-up was censored at the date of the first event to calculate Hazard Ratios. The follow-up time for patients without events was censored at the 1st of July 2015. If the patients died outside of the Fabry Centre, the date of death was evaluated by asking the responsible General Practitioner, the family or the nurse administering ERT in the home care setting.

2.3. Primary exposure variables: cardiac and renal involvement

LVMMI at baseline was used as the primary exposure variable to express cardiac involvement. For this, standard transthoracic 2D echocardiography was routinely performed in all patients. Left ventricular end-diastolic dimension and end-diastolic thickness of the posterior wall and the septum were measured using standard M-mode echocardiographic methods in parasternal long-axis images. LVMMI was calculated using the Devereux formula [14].

Estimated glomerular filtration rate (eGFR) at baseline was used as the primary exposure variable to express renal involvement. The eGFR was derived from serum creatinine and age using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [15]. The CKD-EPI equation is known to have a similarly good statistical performance as previously used equations when the estimated GFR is below 60 ml/min/1.73 m^2, but with better performance in those with higher eGFR levels [16].

2.4. Statistical analysis

We used descriptive statistics for the baseline characteristics and laboratory parameters. Categorical variables were expressed as proportions, continuous variables as means with standard deviations and medians with interquartile ranges (IQR). Normal distribution was assessed by the Kolmogorov-Smirnov-Test.

The cut-off values for LVMMI and eGFR were calculated by maximizing the product of sensitivity and specificity by using receiver operating curve characteristics (ROCs).

Kaplan-Meier analysis was performed for event-free survival and log-rank values to assess statistical significance.

Cox regression analysis was used to examine the risk of adverse events associated with baseline LVMMI and eGFR on a continuous scale and for cardiac, renal and cardio-renal involvement as categorical variables. For the categorization, cardiac involvement was defined as LVMMI above the best calculated cut-off value of 107 (g/m^2), we used this value for both genders because males have a higher LVMMI and a higher risk to develop adverse outcomes due to the hemizygosity; renal involvement was defined as eGFR below the best calculated cut-off value of 90 ml/min/1.73 m^2; CRS was defined as LVMMI >107 (g/m^2) and eGFR <90 ml/min/1.73 m^2.

Multivariable models were applied to adjust for potential confounders using prior knowledge of variables that have been associated with risk in FD patients in previous studies. We hierarchically adjusted for demographics (age, gender) in model 1, RRT (kidney transplant or dialysis at baseline) in model 2 and presence of arterial hypertension (systolic RR > 140 or diastolic RR > 90 mm Hg or intake of antihypertensive drugs) in model 3. These models were used for all multivariable Cox regression analyses.

To evaluate the effect of ERT on the primary endpoint, Cox regression analysis was used, where ERT was expressed as a categorical covariate. In a multivariate Cox regression analysis, an adjustment for age and gender and for cardio-renal involvement was applied to evaluate if the ERT effect remained independent.

The statistical analyses were performed using the SPSS/PC (version 22.0; SPSS Inc., Chicago, IL, USA) software package. All statistical tests were two-sided, and P values <0.05 were considered significant.

3. Results

The baseline characteristics of all patients according to gender and to the presence of CRS are shown in Table 1. In total, 104 patients (40 males and 64 females) with a mean age of 45 ± 16 years were included into the analysis. A baseline echocardiography was available in 91, results on diastolic dysfunction in 82 patients. The left ventricular ejection fraction (LVEF) was normal in all patients. The patients with cardio-renal involvement at baseline were older, had more frequently a Classic disease phenotype, arterial hypertension, echocardiographic signs of diastolic dysfunction, had higher serum NT-proBNP levels and elevated urine protein/creatinine ratios.

Angiotensin converting enzyme inhibitor (ACE-i) or angiotensin receptor blocker (ARB) were used in 17 (16%) of the 104 patients at baseline. During the follow-up time, ACE-i or ARB were started in a further 17 patients.

The NYHA classes at baseline are summarized in Table 1. At the end of the follow-up time, NYHA I/II was present in 23 (22%) patients: 7 (18%) males, 16 (25%) females and 10 (23%) in patients with CRS at baseline; NYHA III/IV was present in 9 (8.6%) patients: 5 (13%) males, 4 (6.3%) females and 5 (12%) patients with CRS at baseline. NYHA class increased in 24 (23%) patients: 11 (28%) males, 13 (20%) females and 9 (21%) CRS patients.

48 patients were treated with α-agalsidase and nine with β-agalsidase throughout the follow-up time. Eight patients were switched from β-agalsidase to α-agalsidase (seven due to shortage of β-agalsidase, one at the discretion of the treating physician), one from α-agalsidase to β-agalsidase (due to patient's priority) an one from β-agalsidase to α-agalsidase (due to shortage) and back to β-agalsidase (due to patient's priority).

3.1. Events

During the median follow-up time of 105 [45–139] months, the first event occurred in 27 (26%) of the patient population: in 11 patients, the first event was stroke or TIA, in 2 kidney transplantation, in 4 chronic

Table 1
Baseline characteristics, laboratory parameters and cumulative ERT dose.

	All patients (n = 104)	Males (n = 40)	Females (n = 64)	Patients with cardio-renal involvement[a] (n = 29; males n = 14)
Age (years)	45 ± 16	48 ± 14	44 ± 17	60 ± 10
Phenotype				
Classic n (%)	89 (86)	33 (83)	56 (88)	28 (97)
Later-onset n (%)	15 (14)	7 (17)	8 (12)	1 (3)
BMI, (kg/m^2)	22 (20–25)	22 (19–23)	22 (20–26)	23 (22–25)
ERT n (%)[b]	65 (63)	35 (88)	31 (48)	25 (86)
Cumulative dose α-agalsidase, (g)[c]	3.0 (1.4–4.4)	3.4 (2.1–4.6)	2.2 (0.9–3.2)	3.4 (1.9–4.5)
Cumulative dose β-agalsidase, (g)[c]	13.2 (5.1–19.3)	13.2 (6.5–21.8)	10.6 (4.2–16.9)	17.7 (10.2–20.6)
Mainz Severity Score Index	15 ± 11	21 ± 11	11 ± 8	25 ± 10
Systolic BP (mm Hg)	120 (110−131)	124 (115–133)	120 (110−130)	129 (120–138)
Diastolic BP (mm Hg)	80 (70–82)	80 (75–85)	78 (70–80)	80 (73–90)
Arterial hypertension n (%)	27 (26)	15 (38)	12 (19)	16 (55)
On ACE-i/ARB (%)	17 (16)	12 (30)	5 (8)	9 (31)
Diabetes mellitus n (%)	1 (1)	0	1 (2)	0
Cholesterol (mmol/l)	4.6 (4.1–5.6)	4.3 (3.9–5.4)	4.9 (4.2–5.7)	5.2 (4.3–6.1)
Smoker n (%)	37 (36)	21 (53)	16 (25)	12 (41)
NYHA I/II n (%)	13 (13)	5 (13)	8 (13)	9 (21)
NYHA III/IV n (%)	5 (4.8)	1 (2.5)	4 (6.3)	3 (7)
NT-proBNP, ng/l	435 ± 1222	683 ± 1938	305 ± 614	1083 ± 2131
Creatinine (μmol/l)	75 (67–91)	93 (82–132)	71 (64–76)	94 (75–131)
Protein/creatinine-ratio in urine (m g/mmol)	9 (6–24)	13 (7–39)	8 (4–17)	18 (9–42)
Chronic dialysis[d] n (%)	7 (7)	7 (18)	0	3 (10)
Renal transplantation n (%)	8 (8)	8 (20)	0	4 (14)
LVMMI (g/m^2)	89 (64–127)	109 (80–147)	75 (58–116)	147 (124–190)
LAVI (ml/m^2)	28 ± 11	30 ± 13	27 ± 10	39 ± 11
E/e (cm/s)	8 ± 6	8 ± 3	8 ± 7	12 ± 8
Ejection fraction, %	65 ± 7	65 ± 7	64 ± 6	66 ± 7
Atrial fibrillation n (%)	1 (1)	1 (2.5)	0 (0)	0 (0)
Pacemaker	1 (1)	0 (0)	1 (1.6)	0 (0)

Abbreviations: ACE-i = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; BMI = body mass index; BP = blood pressure ERT = enzyme-replacement therapy; HR = Hazard Ratio;; LVMMI = left ventricular myocardial mass index, NYHA New York Heart Association functional class; TIA = transient ischemic attack.
Data are given as mean ± standard deviation, median (interquartile range) or numbers (%).
[a] Patients with concurrent cardiac (LVMMI > 107 g/m^2) and renal (eGFR < 90 ml/min/1.73 m^2) involvement at baseline.
[b] Four males were untreated due to mild phenotype, one due to compliance issue.
[c] Only patients on ERT included in this analysis.
[d] At baseline or before kidney transplantation.

dialysis, in 2 hospitalisation due to decompensated HF, in 8 death. 15 (56%) of the 27 patients who developed an event during the follow-up time had cardio-renal involvement at baseline. A pacemaker implantation or AF did not occur as a first event.

Overall, 57 events occurred: 4 patients had a new kidney transplantation, of whom 3 (75%) had cardio-renal involvement at baseline; 4 began chronic dialysis, of whom 3 (75%) had cardio-renal involvement at baseline; 2 patients were hospitalized due to decompensated HF, both (100%) with cardio-renal involvement at baseline; 11 had at least one stroke or TIA, 8 (73%) of whom had cardio-renal involvement at baseline; 7 had new-onset AF, of whom 6 (86%) had cardio-renal involvement at baseline; 6 underwent pacemaker and/or ICD implantation (with one appropriate shock secondary to sustained ventricular tachycardia in one patient during the entire follow-up period), all of whom had cardio-renal involvement at baseline. Of these 6 patients, 3 received a pacemaker due to symptomatic second- or third-degree atrioventricular block and one due to sinus dysfunction; 3 had an ICD implanted due to recurrent sustained and hemodynamically not tolerated ventricular tachycardia. 11 patients died: 2 due to cardiac arrhythmia, 2 malignancy, 1 infection, 1 suicide, 5 unknown; 6 (55%) of the deceased had cardio-renal involvement at baseline. The two male patients (59 and 47 years old) who died from cardiac arrhythmia, (one ventricular tachycardia and one asystole with pulseless electrical activity) suffered from cardiomyopathy, had kidney transplantation and a moderate infection.

The prognostic accuracy of baseline LVMMI and eGFR to predict major adverse clinical events using ROC curves was moderate to high as shown in the Supplementary Fig. 1. We calculated an area under the curve of 0.81 for LVMMI and 0.86 for GFR. The best calculated cut-off for the events development was 107 g/m^2 for LVMMI and 90 ml/min/1.73 m^2 for eGFR.

When examining continuous variables using Cox regression analysis, both higher LVMMI (Table 2A) and lower eGFR (Table 2B) at baseline were associated with a greater risk of developing an adverse clinical event in the crude model, after adjustment for possible confounders in model 1, model 2 and in the fully adjusted model 3. Of note, LVMMI and eGFR were associated with adverse events independently from each other if included into the same fully adjusted model (Table 2B).

Protein/creatinine ratio in a random urine sample and cholesterol levels were not associated with the adverse events in the crude model. These data are not shown. Smoking was associated with adverse events in the crude Cox regression analysis (HR 2.28 [1.03–5.03], P = 0.04) but lost its independent effect on event occurrence after adjustment for male gender (HR 2.05 [0.91–4.63], P = 0.09).

When examining categorical variables in a Cox regression analysis where (i) cardiac involvement is defined as LVMMI above the best calculated cut-off (>107 g/m^2), (ii) renal involvement as eGFR below the best calculated cut-off (<90 ml/min/1.73 m^2) and (iii) CRS as both simultaneously, the risk to develop events was higher in patients with CRS as compared to all patients with cardiac and to all patients with renal involvement (Table 3) in a crude model. The Cox regression model for CRS remained significant after adjusting for possible confounders in model 1 and model 2, and approached significance in the fully adjusted model.

The cumulative dose of ERT did not influence the occurrence of clinical events for α-agalsidase, β-agalsidase and the sum of both in patients with cardiac (HR 0.88 [0.68–1.15], P = 0.36 for α-agalsidase; HR 1.04 [0.97–1.11], P = 0.24 for β-agalsidase; HR 1.04 [0.96–1.12], P = 0.36 for sum), renal (HR 0.89 [0.73–1.08], P = 0.24 for α-agalsidase; HR

Table 2A
Hazard Ratios (and 95% CIs) for occurrence of primary endpoint[a] according to baseline LVMMI.

Parameter	Crude			Model 1			Model 2			Model 3		
	HR	CI	P	HR	CI	P	HR	CI	P	HR	CI	P
LVMMI (g/m^2)[b]	2.14	1.52–3.01	<0.001	1.65	1.06–2.58	0.03	1.67	1.04–2.73	0.03	1.67	1.04–2.73	0.03
Age				1.03	1.00–1.07	0.045	1.03	1.00–1.07	0.06	1.02	0.98–1.06	0.26
Gender				1.56	0.63–3.87	0.34	1.68	0.66–4.32	0.28	1.55	0.60–3.97	0.37
Kidney transplant							0.47	0.07–3.18	0.44	0.42	0.07–2.66	0.36
Dialysis							2.36	0.41–13.68	0.34	2.05	0.11–3.21	0.40
Hypertension										1.92	0.71–5.21	0.20
eGFR										0.32	0.16–0.61	0.001

Abbreviations: LVMMI = left ventricular myocardial mass index; eGFR = estimated glomerular filtration rate; HR = Hazard Ratio; CI = confidence interval.
Model 1: adjusted for age and male gender; model 2: additionally adjusted for renal replacement - transplantation and dialysis at baseline; model 3: additionally adjusted for presence of hypertension at baseline.
[a] Primary endpoint was defined as a composite of first occurrence of renal replacement therapy requirement (kidney transplant or chronic dialysis), new onset of atrial fibrillation, pacemaker and/or ICD implantation, hospitalisation due to decompensated Heart Failure, cerebrovascular events (stroke or TIA), death.
[b] Per SD decrease.

1.06 [1.00–1.13], P = 0.07 for β-agalsidase, HR 1.04 [0.97–1.12], P = 0.23 for sum) and cardio-renal (HR 0.85 [0.64–1.12], P = 0.24 for α-agalsidase; HR 1.05 [0.98–1.12], P = 0.20 for β-agalsidase; HR 1.04 [0.87–1.12], P = 0.35 for sum) involvement (all per 1 g increase and in fully adjusted model).

If defining only RRT, hospitalisation due to decompensated HF, cerebrovascular events (stroke or TIA), and death as the endpoint for the Cox regression model, excluding AF as an event of a less clinical severity, higher LVMMI and lower eGFR at baseline remained associated with a greater risk of developing an adverse clinical event in the crude model (HR 2.21 [1.43–3.42], P < 0.001 for LVMMI, HR 0.41 [0.27–0.62], P < 0.001 for eGFR), and remained significant for the eGFR approaching the significance for LVMMI after adjustment for possible confounders in model 1, (HR 1.65 [0.91–2.97], P = 0.10 for LVMMI, HR 0.41 [0.24–0.71], P = 0.001 for eGFR) model 2, (HR 1.74 [0.94–3.20], P = 0.08 for LVMMI, HR 0.37 [0.20–0.70], P = 0.002 for eGFR) and in the fully adjusted model 3 (HR 1.76 [0.98–3.18], P = 0.06 for LVMMI, HR 0.39 [0.20–0.76], P = 0.006 for eGFR) (all models per SD increase in LVMMI and SD decrease in eGFR).

When examining the effects of ERT on event occurrence, patients on ERT were at higher risk in the crude model (HR 11.62 [1.57–86.05], P = 0.02) and after adjustment for age and gender (HR 9.37 [1.21–72.47], P = 0.03). However, this "negative" ERT effect was attenuated after adjustment for cardio-renal involvement at baseline (HR 6.09 [0.76–49.06], P = 0.09).

In the Kaplan-Meier-Analysis, the poorest event-free survival was observed among patients with baseline cardio-renal involvement (Fig. 1, A–C). This effect outweighed the gender-related difference in outcome within the cohort (Fig. 1, D).

The associations remained unchanged if patients with the mutations c.376A>G/p.S126G and c.416A>G/p.N139S were excluded from the analysis, due to genetic variants of unknown significance.

3.2. Diastolic dysfunction

At baseline, 22 of 82 (27%) patients had a diastolic dysfunction. Of them, six (27%) showed an abnormal relaxation pattern - corresponding to diastolic dysfunction grade 1; twelve (55%) pseudonormal filling dynamics - grade 2; three (14%) restrictive filling dynamics – grade 3; one (4.5%) was inconclusive.

When analysing the parameters of diastolic dysfunction on a continuous scale, E/e` was a significant predictor for the occurrence of adverse clinical events in a crude (HR 1.90 [1.44–2.50], P < 0.001) model and remained an independent predictor in model 1 (HR 1.63 [1.3–2.35], P = 0.01), model 2 (HR 1.68 [1.15–2.46], P = 0.007) and model 3 (HR 1.85 [1.23–2.78], P = 0.003]) (per SD increase). In contrast, LAVI predicted event occurrence in the crude (HR 1.85 [1.33–2.57], P < 0.001) but not in the adjusted models (HR 1.23 [0.80–1.88], P = 0.35 for model 1, HR 1.31 [0.81–2.12], P = 0.27 for model 2 and HR 1.49 [0.89–2.52], P = 0.13 for model 3, (all per SD increase)).

3.3. Hospitalisations

All-cause hospitalisations. Overall, 28 of 104 patients were hospitalised during the follow-up period: 15 of 29 (52%) patients with and 13 (17%) of 75 without cardio-renal syndrome at baseline were ever hospitalised.

From a total of 79 hospitalisations (15 due to cardiac event or cardiac-related procedure; 8 TIA or stroke, 10 related to RRT, 4 due to pain, 23 infection, 2 malignancy, 17 other), 57 (72%) occurred in patients with and 22 (28%) without cardio-renal syndrome at baseline.

3.4. Cardiac hospitalisations

Overall, 9 of 104 patients were hospitalised due to a cardiac event or procedure. All of these patients had cardio-renal syndrome at baseline.

Table 2B
Hazard Ratios (and 95% CIs) for occurrence of primary endpoint[a] according to baseline eGFR.

Parameter	Crude			Model 1			Model 2			Model 3		
	HR	CI	P	HR	CI	P	HR	CI	P	HR	CI	P
eGFR[b]	0.42	0.28–0.61	<0.001	0.45	0.27–0.74	0.002	0.42	0.24–0.74	0.003	0.45	0.25–0.83	0.01
Age				1.01	0.97–1.04	0.76	1.00	0.97–1.04	0.85	1.00	0.97–1.04	0.98
Gender				1.30	0.56–3.02	0.54	1.32	0.51–3.38	0.57	1.23	0.47–3.21	0.67
Kidney transplant							0.97	0.21–4.59	0.97	1.06	0.23–4.94	0.94
Dialysis							0.75	0.16–3.68	0.73	0.79	0.16–3.78	0.76
Hypertension										1.52	0.58–3.99	0.40

Abbreviations: LVMMI = left ventricular myocardial mass index; eGFR = estimated glomerular filtration rate; HR = Hazard Ratio; CI = confidence interval.
Model 1: adjusted for age and male gender; model 2: additionally adjusted for renal replacement - transplantation and dialysis at baseline; model 3: additionally adjusted for presence of hypertension at baseline.
[a] Primary endpoint was defined as a composite of first occurrence of renal replacement therapy requirement (kidney transplant or chronic dialysis), new onset of atrial fibrillation, pacemaker and/or ICD implantation, hospitalisation due to decompensated Heart Failure, cerebrovascular events (stroke or TIA), death.
[b] Per SD decrease.

Table 3
Hazard Ratios (and 95% CIs) for occurrence of primary endpoint[a] according to baseline cardiac, renal and cardio-renal involvement.

	Cardiac involvement[b] (N = 38)			Renal involvement[c] (N = 49)			Cardio-renal involvement[d] (N = 29)		
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Crude	4.28	1.58–11.56	0.004	6.38	1.91–21.33	0.003	7.42	2.73–20.23	<0.001
Model 1	1.79	0.55–5.82	0.37	4.30	1.06–17.52	0.04	4.10	1.05–15.99	0.04
Model 2	1.83	0.56–5.97	0.32	4.88	1.12–21.24	0.04	4.46	1.07–18.62	0.04
Model 3	1.75	0.54–5.69	0.35	4.41	0.97–20.06	0.06	4.05	0.95–17.29	0.06

Model 1: adjusted for age and male gender; model 2: additionally adjusted for renal replacement - transplantation and dialysis at baseline; model 3: additionally adjusted for presence of hypertension at baseline.

[a] Primary endpoint was defined as a composite of first occurrence of renal replacement therapy requirement (kidney transplant or chronic dialysis), new onset of atrial fibrillation, pacemaker and/or ICD implantation, hospitalisation due to decompensated Heart Failure, cerebrovascular events (stroke or TIA), death.

[b] All patients with cardiac (LVMMI > 107 g/m^2) involvement at baseline.

[c] All patients with renal (eGFR < 90 ml/min/1.73 m^2) involvement at baseline.

[d] Patients with concurrent cardiac (LVMMI > 107 g/m^2) and renal (eGFR < 90 ml/min/1.73 m^2) involvement at baseline.

In total, 15 cardiac hospitalisations occurred: 8 due to pacemaker/ICD implantation and related complications, 2 acute coronary events, 2 aortic valve replacements, 2 decompensated heart failure, 1 arrhythmic event.

4. Discussion

In this prospective multicentre cohort of 104 Swiss Fabry patients, CRS was associated with a high risk of complications such as death, stroke/TIA, hospitalisation due to decompensated HF and requirement for renal replacement therapy. Cardiac and renal disease independently increased the risk for major clinical events. Interestingly, cardio-renal involvement was a more important independent risk factor than male gender, despite the fact that FD is an X-linked inherited disorder.

Concurrent cardiac and renal involvement may reflect an advanced Gb3 deposition in the vital organs. Indeed, studies have demonstrated that patients with advanced stages of FD tend to experience disease progression and benefit less from enzyme replacement therapy [17,18]. Moreover, cardio-renal involvement was often associated with the more severe classic FD phenotype; in contrast, single-organ manifestations seemed to correspond to the later-onset phenotype, which is known for a milder vascular risk profile [19]. Furthermore, renal disease itself has been shown to be a major risk factor for cardiovascular complications in FD [5]. Similarly, left ventricular hypertrophy was a significant predictor for cardiovascular events in a large-scale registry-based long-term study [20].

Additional pathophysiological mechanisms beyond glycosphingolipid deposition have been proposed to enhance the cardio-renal problem in patients with Fabry disease. On the cellular level, the lysosomal dysfunction may trigger a damaging cascade of events such as cell apoptosis, energy depletion [21], increased oxidative stress [22], inflammation [23] and finally cardiac [24,25] and renal tissue fibrosis [26,27]. On the organ level, left ventricular hypertrophy, diastolic dysfunction and arrhythmia can lead to a reduced cardiac output and a consecutive arterial underfilling resulting in reduced renal perfusion as well as renal venous congestion. On the other hand, reduced glomerular filtration rate causes salt and water retention, azotemia and acid base imbalance, further increasing the cardiac preload and predisposing to left ventricular and diastolic dysfunction. These haemodynamic derangements likely result in an activation of the renin-angiotensin-aldosterone-system and a

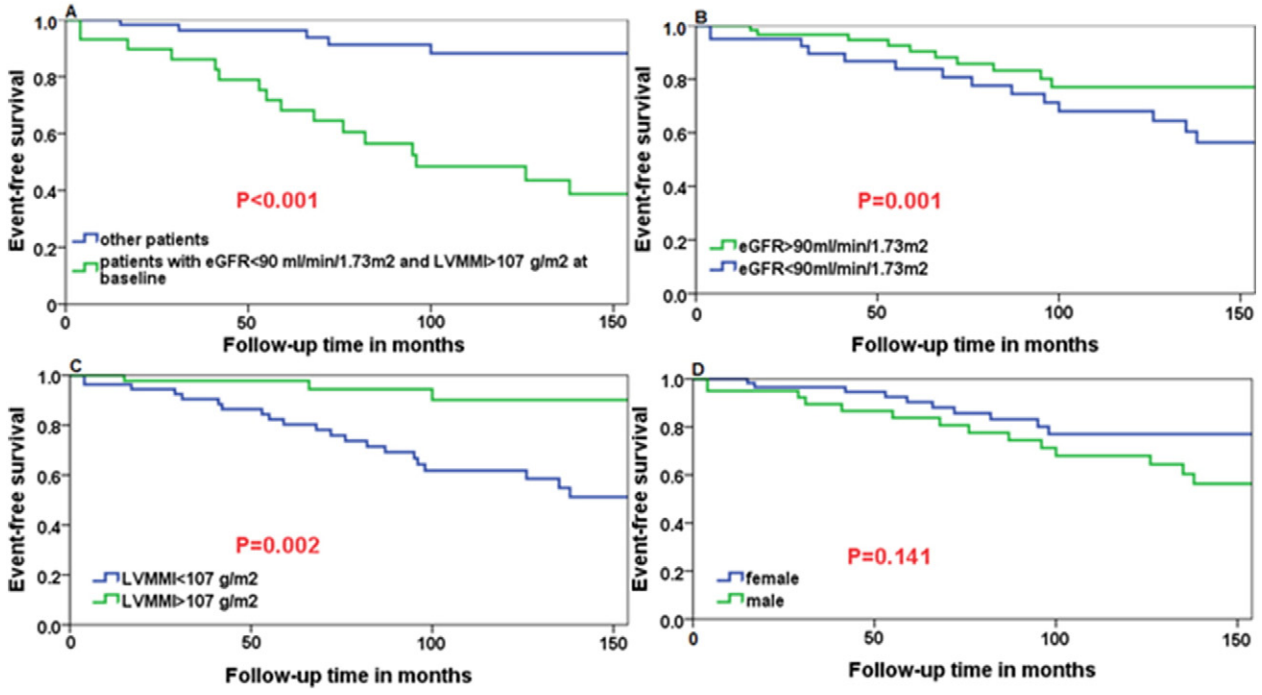

Fig. 1. Time to first complication in Fabry patients presented in groups using the best calculated cut-offs for LVMMI and eGFR at baseline (A), eGFR at baseline (B), LVMMI at baseline (C) and according to gender (D).

release of vasoactive mediators responsible for systemic vasoconstriction. Along the same lines, arterial hypertension has repeatedly been shown as an important risk factor in Fabry disease progression [20].

As shown in this study, diastolic dysfunction plays an important prognostic role in FD. Diastolic dysfunction is known to be an early sign of cardiac involvement in FD occurring before LVH[28]. Our results are in line with a study by Boyd et al., in which left atrial enlargement and reduced atrial compliance in particular were shown as crucial early signs of Fabry cardiomyopathy [28]. This left atrial enlargement as a result of chronic left atrial pressure elevation may lead to renal venous congestion, initiating the vicious cycle of CRS.

Cardiac and renal deterioration should consequently be prevented as far as possible in order to reduce morbidity and mortality. Within our cohort, patients on ERT were still at an increased risk of developing adverse clinical events, which continued to occur independently of the cumulative ERT dose, even after adjustment for possible confounders. The most likely reason for this observation is that ERT was indicated in patients with more severe phenotypes and was initiated relatively late. Importantly, we studied an "old" FD population where a large proportion of the patients had advanced disease, ERT having been initiated and offered to all FD patients from 2001. In this cohort, a high proportion of females (48%) was on ERT but could have been at lower risk of having major clinical events due to the heterozygosity. Therefore, in order to avoid bias by gender, adjustment for gender as a possible confounder has been introduced already in Model 1 and maintained in all multivariate models.

Of note in this study, we adopted a definition of CRS leading to a focus on patients with relatively "advanced "disease. Importantly, we studied an "older" adult FD population where a large proportion of the patients had advanced disease at baseline, because the cohort was established when ERT was initiated and offered to all FD patients in 2001. In the future, it is important to study the association of early disease involvement with clinical events because early ERT initiation benefits the clinical long-term outcome [17,29].

Because Gb3 accumulation is known to occur at early stages of Fabry nephropathy [30,31] and cardiomyopathy [32], early initiation of enzyme replacement therapy prior to overt kidney and heart disease may lead to prevention of CRS. Studies suggest that maximal treatment effect can be achieved at early stages of heart and kidney involvement [33,34].

To slow disease progression, more intensive therapies such as increased ERT dose or additional pharmacological chaperone therapy could enhance the therapeutic outcomes [35–37]. Fabry patients with advanced cardio-renal disease might additionally benefit from general therapies used to control cardiac and renal disease by RAAS inhibition using angiotensin-converting enzyme inhibitors or angiotensin blockers in the chronic and diuretic therapy in the acute setting. Patients with advanced disease should undergo a routine organ staging at regular intervals [38] and be treated according to current guidelines [39].

We included five patients with mutations c.376A>G/p.S126G and c.416A>G/p.N139S, reported in literature as genetic variants of unknown significance [40–43]. Three males, 55, 45 and 23 year old, and one female, 53 year old, with the mutation c.376A>G/p.S126G, had no cardiac or renal involvement at baseline and at 4 years follow-up, which confirms a rather benign nature of this mutation. Similarly, the 43 year old female with the mutation c.416A>G/p.N139S had no cardiac or renal involvement at baseline and at 2 years follow-up. Importantly, the associations with the clinical outcomes remained unchanged if patients with these mutations were excluded from the analysis."

Several limitations merit consideration. Firstly, due to a relatively small cohort size, we had to define a composite outcome in order to increase the power of this study. Secondly, cardiac MRI was not available at cohort initiation. Thirdly, we cannot conclude from this study to what extent patients with cardio-renal involvement benefited from enzyme replacement therapy.

The strengths of this study are its long observational period for hard clinical outcomes in a multicentre cohort in a single country, where patients are treated similarly and exposed to similar environmental conditions, and its negligible loss to follow-up. The latter is due to Swiss regulation that the ERT prescription and patient's follow-up remains reserved for the Fabry Centres.

In conclusion, cardio-renal involvement in Fabry disease is a potential marker of disease progression bearing an increased risk of mortality and cardiovascular events. Multidisciplinary care provided by cardiologists, nephrologists, general physicians and other specialists is important in treating the Type 5 cardio-renal syndrome seen in Fabry disease.

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Potential conflicts of interest

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