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Cyclin dependent kinase inhibitor 1 C is a female-specific marker of left ventricular function after acute myocardial infarction☆

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ABSTRACT

Background: A significant proportion of patients develop left ventricular (LV) remodeling leading to heart failure after acute myocardial infarction (AMI). Being able to identify these patients would represent a step forward towards personalized medicine. The present study aimed to determine the ability of cyclin dependent kinase inhibitor 1C (CDKN1C) to risk stratify AMI patients, in a sex-specific manner.
Methods: CDKN1C expression was measured in blood samples obtained at admission in a test cohort of 447 AMI patients and a validation cohort of 294 patients. The study end-point was LV function assessed by the ejection fraction (EF) at follow-up.
Results: In the test cohort, CDKN1C was lower in patients with a reduced EF (<40%) compared to patients with preserved EF (≥50%). This observation was specific to women. CDKN1C was a significant univariate predictor of LV function in women only. In multivariable analysis including demographic and clinical parameters, CDKN1C predicted LV function in women (odds ratio [95% confidence interval] 0.44 [0.23–0.82]) but not in men (0.90 [0.70–1.16]). Addition of CDKN1C to a multivariable clinical model reduced the Akaike information criterion, attesting for an incremental predictive value, in women ($p = 0.006$) but not in men ($p = 0.41$). Bootstrap internal validation confirmed the added value of CDKN1C in women. The female-specific predictive value of CDKN1C was validated in the independent cohort.
Conclusion: CDKN1C is a novel female-specific biomarker of LV function after AMI.

1. Introduction

Cardiovascular disease is the first cause of death in men and women worldwide, among which acute myocardial infarction (AMI) plays a major role [1,2]. Despite known sex disparities towards the risk of AMI and of developing an adverse outcome after AMI [3–5], diagnosis, treatment and management of AMI are still lacking sex specificity.

Left ventricular (LV) remodeling leading to heart failure (HF) occurs in 28% of patients with AMI [6]. Infarct size, history of MI, age, female sex, LV ejection fraction (EF), diabetes and renal failure are mostly associated with HF after AMI [7,8]. HF can be potentially prevented through changing life style habits, control of risk factors and pharmacological treatment [9]. Inhibition of the renin-angiotensin and β-adrenergic systems is the standard treatment to reduce the risk of LV remodeling after MI. Simultaneous inhibition of angiotensin and the natriuretic peptides degradation emerged as a novel mechanism to prevent HF [10]. Being able to identify patients at risk of HF after AMI would allow tailoring healthcare, in a sex-specific manner, to reduce the socio-economic burden of HF and to improve the quality of life of AMI patients. However, to date, risk stratification after AMI remains a challenge which would benefit from novel biomarkers. Since women and men are not equal towards the risk of developing HF after AMI [4], these novel biomarkers should allow a sex-specific risk stratification.

In our search for novel prognostic biomarkers after AMI, we showed that transcriptomic profiles of blood cells represent a reservoir of novel

Abbreviations: AMI, acute myocardial infarction; CDKN1C, cyclin dependent kinase inhibitor 1C; CPK, creatine phosphokinase; cTn, cardiac troponins; EF, ejection fraction; HF, heart failure; KCNQ1OT1, KQT-like subfamily, member 1 opposite strand/antisense transcript 1; LV, left ventricular; Nt-proBNP, N-terminal pro brain natriuretic peptide; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction.

☆ This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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biomarkers [11]. In a past study, we identified the long noncoding RNA KQT-like subfamily, member 1 opposite strand/antisense transcript 1 (KCNQ1OT1) whose expression levels in blood cells predicted LV function after AMI [12]. KCNQ1OT1 belongs to an imprinted cluster of 9 genes located on the human chromosome 11p15.5 [13]. While KCNQ1OT1 is exclusively expressed from the paternal allele, the other genes are preferentially expressed from the maternal allele. KCNQ1OT1 plays a prominent role in the imprinting of the cluster by recruiting repressive histone and DNA modifications [13]. The most extensively studied genes of the cluster are KCNQ1 and CDKN1C. Defects in KCNQ1, which encodes for a potassium channel, is responsible for the congenital QT syndrome, a cardiac disorder that can result in potentially lethal arrhythmias [14]. CDKN1C encodes for the inhibitor of cell proliferation $p57^{KIP2}$ [15]. CDKN1C was found to be involved in the development of the atherosclerotic plaque through modulation of vascular smooth muscle cells proliferation [16,17]. Interestingly, polymorphisms in functional regions of CDKN1C are associated with the risk of AMI in patients with atherosclerotic vessels [18]. Furthermore, CDKN1C is associated with female-specific malignancies [19–22] and is downregulated by estrogens through an epigenetic mechanism involving KCNQ1OT1 [23]. As KCNQ1OT1 was found to be associated with LV function after an AMI [12], we hypothesized that CDKN1C would as well be associated with LV function, specifically in women.

2. Methods

2.1. Test cohort

We enrolled 447 patients from the Luxembourg AMI registry in the present study. Patients were recruited at the Institut National de Chirurgie Cardiaque et de Cardiologie Interventionnelle and the Department of Cardiology of the Centre Hospitalier de Luxembourg between February 2006 and December 2012. All patients had an AMI and were treated with percutaneous coronary intervention. Demographic and clinical data of the patients were collected at admission. 351 patients had a STEMI and 96 had a NSTEMI. STEMI and NSTEMI were defined as previously [24].Arterial blood samples were recovered at the time of reperfusion in PAXgene™ blood RNA tubes (BD Biosciences, Erembodegem, Belgium). In order to assess the LV function at 4-month follow-up, the LV ejection fraction (EF) was determined by echocardiography. The protocol has been approved by the ethics committee and the national committee for data protection of Luxembourg and complies with Helsinki declaration. All patients signed a written informed consent.

2.2. Measurement of CDKN1C expression in the test cohort

Expression levels of CDKN1C in blood samples of the test cohort were assessed using quantitative PCR as described in details elsewhere [12] and as summarized in the Online Supplement.

2.3. Independent validation cohort

Two hundred and ninety four AMI patients from the German Leipzig LIFE- Heart study were enrolled in the validation cohort. The LIFE-Heart study was designed to analyze genetic and non-genetic risk factors of atherosclerosis and related vascular and metabolic phenotypes [25]. All patients were admitted for an AMI as the first manifestation of the coronary heart disease. 75% of the patients were classified as STEMI. Peripheral venous blood samples were collected in PAXgene™ blood RNA tubes (BD Biosciences) at median balloon-to-blood time 0.87 days (IQR 0.65–1.24) after angiographic reperfusion. Gene expression of CDKN1C was analyzed in available microarray data from Illumina HT-12 v4 Expression BeadChips (Illumina, San Diego, CA, USA) [26] and quality control was performed as described elsewhere [27]. Illumina-ID 1718565 was used for that purpose. The follow-up echocardiography was performed at median of 99 days after the AMI. The recruitment phase was conducted at a single tertiary care centre between July 2008 and October 2012. All patients signed a written informed consent and the study was approved by the Ethics Committee of the Medical Faculty of the University of Leipzig, Germany (Reg. No 276–2005) and is registered at ClinicalTrials.gov (NCT00497887).

2.4. Statistical analysis

t-Test or Mann-Whitney test were used to compare two groups of continuous variables following normal or non-normal distribution, respectively. Three-group comparisons were performed using one-way ANOVA for normally distributed data or Kruskal-Wallis one-way ANOVA on ranks for non-normally distributed data. Fisher's exact test was used for qualitative data. All tests were two-tailed and a p value <0.05 was considered significant. The SigmaPlot v12.0 software was used for these analyses.

The association between CDKN1C and LV function was assessed using logistic regression. First, missing data were replaced using 100-fold multiple imputation. Right skewed continuous variables were log2-transformed, and then all continuous variables were scaled to mean = 0 and standard deviation = 1. Both univariate and multivariable analyses were conducted. Ordinal logistic regression was used in the test cohort in which patients were divided into 3 groups of EF (rEF: reduced EF; mrEF: mid-range EF; pEF: preserved EF), and logistic regression was used in the validation cohort in which patients were divided into 2 groups of EF (rEF and pEF). Odds ratios (OR) with 95% confidence intervals (CI) were calculated. The Akaike information criterion (AIC) was used to evaluate models fit. The Wald chi-square test was used to assess the overall significance of each model and the likelihood ratio test was used to compare two models. Bootstrap internal validation was used to identify markers providing the best improvement of prediction. For each of 150 bootstrap samples, the best model was selected based on the AIC. All prediction analyses were performed on the R version 3.3.1 statistical platform using the packages Hmisc, PredictABEL, lmtest, rms and bootStepAIC.

3. Results

3.1. Characteristics of the test cohort

Supplementary Table 1 displays the clinical and demographic characteristics of the test cohort of 447 AMI patients. Median age was 61 and 75% of patients were men. According to the latest guidelines of the European Society of Cardiology for the classification of HF, the test cohort comprised 59% of patients with a preserved EF at 4-month (pEF, EF ≥ 50%), 31% with a mid-range EF (mrEF, EF = 40–49%), and 10% with a reduced EF (rEF, EF < 40%). Patients with rEF were older than patients with mrEF or pEF. The proportion of men was similar in the EF groups. When patients were divided according to their gender (Table 1), we observed that women were older than men (70 vs. 58 years-old, respectively, $p < 0.001$), although age was not associated with the EF group in women, as it was the case in men for whom the elderly were at higher risk of rEF.

3.2. Expression of CDKN1C according to sex and EF group

Expression levels of CDKN1C were measured by quantitative PCR in blood samples obtained at reperfusion in the 447 AMI patients of the test cohort. As shown in Fig. 1A for all patients, CDKN1C expression was lower in rEF patients compared to pEF patients. Patients with mrEF had comparable levels of CDKN1C to patients with rEF and pEF. CDKN1C levels were comparable between men and women (Fig. 1B). Interestingly, while CDKN1C expression was similar between the 3 EF groups in men, women with rEF had reduced CDKN1C expression compared to the mrEF and pEF groups (Fig. 1C).

3.3. Association of CDKN1C with LV function after AMI

Both univariate and multivariable analyses were conducted to assess the association between CDKN1C and LV function measured 4 months after AMI. The 3-group classification (rEF, mrEF, pEF) was used as endpoint in these analyses. We first observed that CDKN1C was a significant univariate predictor of LV function in all patients (OR [95% CI] 0.82 [0.68–0.98]) (Supplementary Fig. 1A). When this association was assessed separately in men and women, we found that it failed to reach significance in men (OR [95% CI], 0.91 [0.73–1.12]) while it was stronger in women (OR [95% CI] 0.56 [0.37–0.83]) (Supplementary Fig. 2 B, C).

The following parameters were included in multivariable analyses: age, body mass index (BMI), sex, diabetes, hypertension, hypercholesterolemia, prior MI, smoking, type of MI (STEMI vs NSTEMI), white blood cells count, ischemic time (i.e. delay between chest pain onset and reperfusion), Nt-proBNP, CPK and cTnT levels. In all patients, prior AMI, CPK and Nt-proBNP were significantly associated with LV function (Fig. 2A). The association between CDKN1C and LV function was of borderline significance ($p = 0.054$). In men, CPK and Nt-proBNP, not CDKN1C, were significantly associated with LV function (Fig. 2B). In women, CDKN1C showed the strongest association with LV function (OR [95% CI] 0.44 [0.23–0.82] (Fig. 2C). Importantly, adding the interaction between CDKN1C and sex in the multivariable model was

Table 1
Demographic and clinical features of MI male and female patients of the test cohort.

	Men	pEF	mrEF	rEF	*p* Value	Women	pEF	mrEF	rEF	*p* Value
	(*N* = 339, 75%)	(*N* = 197, 58%)	(*N* = 113, 33%)	(*N* = 28, 9%)	(pEF vs mrEF vs rEF)	(*N* = 109, 25%)	(*N* = 69, 63%)	(N = 28, 26%)	(*N* = 12, 11%)	(pEF vs mrEF vs rEF)
Age, median (range), y	58 (30–88)	56 (30–86)	58 (30–88)	66 (47–86)	**0.002**	70 (35–91)	69 (35–91)	62 (37–89)	73 (37–83)	0.795
Body mass index, median (range)	27 (18–51)	28 (19–47)	27 (18–51)	28 (21–35)	0.124	26 (19–47)	25 (19–47)	26 (20–43)	28 (19–34)	0.93
Blood cell counts at admission, median (range)										
White blood cells, ×109/L	10.76 (1.1–29)	10.5 (1.1–29)	11.32 (5.8–29)	11.6 (4.8–21.7)	0.087	10.4 (3.1–94.7)	9.2 (3.1–22.8)	11.3 (4.9–19.48)	14.1 (8.12–19.91)	**0.004**
Neutrophils, %	74 (23.9–93.7)	72.4 (23.9–89.2)	75.1 (53.8–93.7)	79.7 (56.8–93.1)	<**0.001**	75 (45.6–93.5)	75 (56.7–93.5)	74.4 (50.9–91)	91.6 (45.6–91.8)	0.463
Lymphocytes, %	16 (3.1–29.5)	17.9 (5.4–69.5)	15.05 (3.1–34.9)	12.9 (3.7–27)	<**0.001**	15 (3.6–42)	16 (3.6–37.6)	14.8 (5.2–34.4)	11.2 (61–42)	0.255
Monocytes, %	6.3 (1.1–22)	6.45 (2.1–16.2)	6.25 (2.2–22)	5.89 (1.1–13.1)	0.673	5.6 (0.8–12.7)	5.5 (1.1–11.5)	5.7 (3.4–12.7)	5.7 (0.8–7.4)	0.458
Platelets, ×109/L	230 (117–724)	228 (12–724)	239.5 (117–377)	195 (157–404)	0.35	245 (76–502)	246 (76–481)	234.5 (156–502)	274 (147–399)	0.809
Biomarkersmedian (range)										
CK, U/L	1631 (34–13,038)	1248 (76–9574)	2254.5 (34–13,038)	3796 (105–9363)	<**0.001**	1368 (82–6799)	1111.5 (82–4078)	3122.5 (201–6799)	3129 (981–5627)	<**0.001**
cTnT, μg/L	3.74 (0.02–25.66)	2.76 (0.02–25.6)	5.36 (0.03–24.7)	7.81 (0.19–24.3)	<**0.001**	4.09 (0.01–26.9)	2.91 (0.01–24.7)	5.84 (0.83–26.9)	6.4 (1.04–24)	<**0.001**
High-sensitivity CRP, mg/L	8.5 (0.3–333.4)	5 (0.3–251.2)	15.6 (0.69–333.4)	17.2 (2.26–301)	<**0.001**	9.1 (0.91–292)	7.7 (0.91–292)	9.8 (2.87–128)	28 (2.7–71)	**0.03**
Nt-proBNP, pg/mL	156 (5–35,000)	113.9 (10.55–12,746)	220.5 (5–35,000)	745.4 (41.8–12,894)	<**0.001**	526 (35.7–15,679)	502.2 (60.3–15,679)	289 (45.2–5014)	774.7 (35.7–13,977)	0.157
4-month EF, median (range), %	50 (15–89)	55 (50–89)	45 (40–49)	34.5 (15–38)	<**0.001**	50 (20–81)	55 (50–81)	45 (40–49)	32.5 (20–39)	<**0.001**
Medical history, n (%)										
Previous MI	43 (13)	21 (11)	16 (14)	6 (21)	0.24	6 (6)	2 (3)	3 (11)	1 (8)	0.164
Diabetes mellitus	70 (21)	39 (20)	22 (19)	9 (32)	0.31	27 (25)	11 (16)	10 (36)	6 (50)	**0.011**
Hypertension	159 (47)	98 (50)	45 (40)	16 (57)	0.13	56 (51)	32 (46)	15 (54)	9 (75)	0.41
Hypercholesterolemia	141 (42)	88 (45)	40 (35)	13 (46)	0.24	56 (51)	37 (54)	11 (39)	8 (67)	0.25
Smoking	159 (47)	73 (37)	60 (53)	7 (25)	**0.027**	45 (41)	27 (39)	14 (50)	4 (33)	0.56

Left ventricular function was determined by the 4-month ejection fraction (EF). pEF indicates preserved EF (EF ≥ 50%), mrEF indicates mid-range EF (40% ≤ EF ≤ 49%), rEF indicates reduced EF (EF < 40%). White blood cell count and N-terminal pro-brain natriuretic peptide (Nt-proBNP) were determined at reperfusion. Creatine phosphokinase (CPK), cardiac troponin T (cTnT) and high-sensitivity C-reactive protein (CRP) are peak values.
P values < 0.05 are indicated in bold.

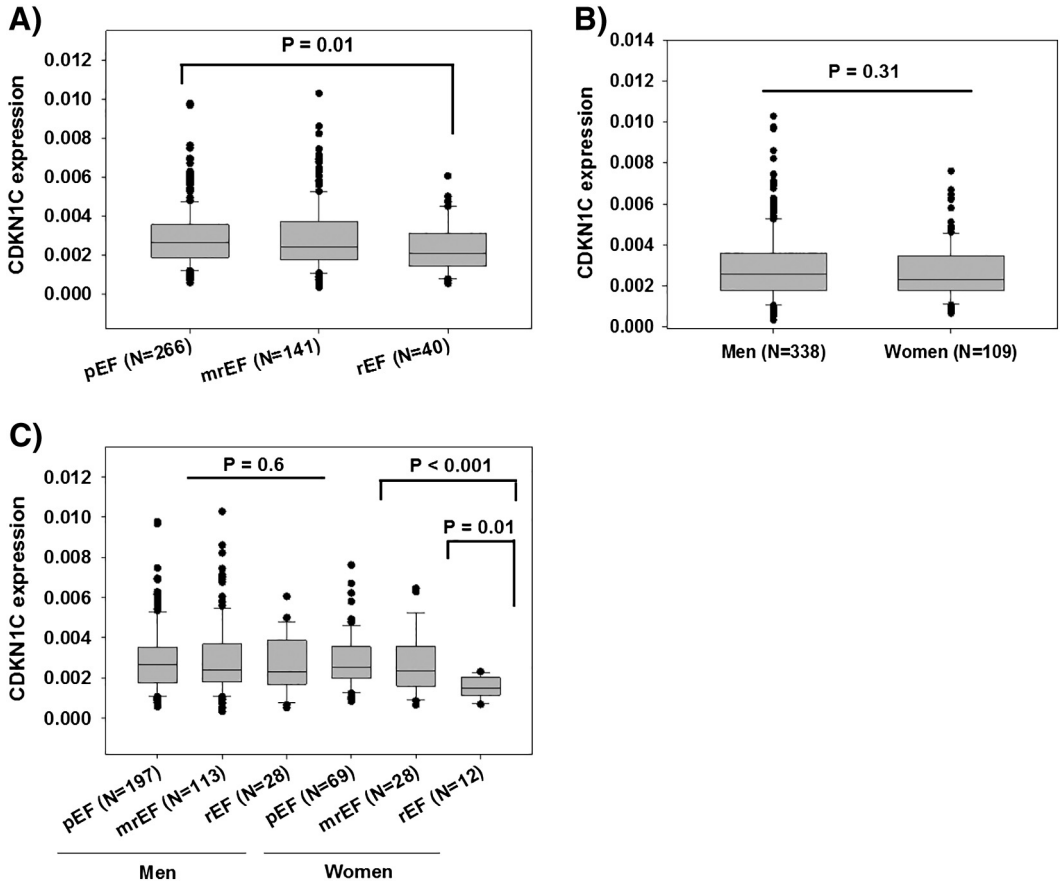

Fig. 1. Expression of CDKN1C in AMI patients. CDKN1C was measured by quantitative PCR in blood samples recovered at reperfusion in 447 AMI patients. (**A**) Boxplots show the expression of CDKN1C in all patients according to their 4-month EF. Patients with EF ≥ 50% were considered as having a pEF, 40% ≤ EF < 49% was considered as mrEF, and EF < 40% was considered as rEF. (**B**) Expression levels of CDKN1C in men and women. (**C**) Expression levels of CDKN1C in the three EF groups separately in men and women. mrEF: mid-range ejection fraction; pEF: preserved ejection fraction; rEF: reduced ejection fraction.

significant ($p = 0.005$, likelihood ratio test), thus confirming that the predictive value of CDKN1C is dependent on sex.

To determine whether CDKN1C could increase the prediction of LV function by a clinical model including all the variables listed above, we calculated the AIC of the clinical model alone and after addition of CDKN1C. A lower AIC reflects a better model fit and therefore an improvement of prediction. As shown in Supplementary Table 2, adding CDKN1C resulted in a significant reduction of AIC in women ($p = 0.006$), but not in all patients ($p = 0.053$) and men ($p = 0.41$). Thus, CDKN1C improves the prediction of LV function in women only.

Then we used bootstrap internal validation to determine which biomarker(s) among Nt-proBNP, CPK, cTnT and CDKN1C were necessary to reach the best improvement of prediction by a clinical model including age, BMI, sex, diabetes, hypertension, hypercholesterolemia, prior MI, smoking, type of MI (STEMI vs NSTEMI), leucocytes level, and ischemic time. In all patients, 53 models (35% of 150 bootstrap iterations) containing CPK and CDKN1C were selected to generate the best improvement of prediction (Supplementary Fig. 2A). In men, 63 models (42% of 150 bootstrap iterations) containing Nt-proBNP and CPK provided the best improvement of prediction (Supplementary Fig. 2B). In women, 63 models (42% of 150 bootstrap iterations) providing the best improvement of prediction contained CDKN1C with either CPK or cTnT (Supplementary Fig. 2C). Most importantly, in women, CDKN1C was selected 126 times (84%) over the 150 bootstrap iterations as generating the best model improvement, while Nt-proBNP was selected only 56 times (37%) (Supplementary Fig. 2C). These results show that, while Nt-proBNP and CPK are useful predictors of LV function in men, CDKN1C is the best predictor in women.

3.4. Independent validation

The association between CDKN1C and LV function was independently assessed in patients from the German LIFE-Leipzig Heart study. 294 AMI patients having blood samples at admission and follow-up echocardiographic data within 6 months (median 99 days, IQR 85–141 days) were enrolled. Characteristics of patients are shown in Tables 3–5 in the Online Supplement. Median age of these patients was 60 years, 72% were men and 40% had LV dysfunction assessed by an EF ≤ 50%. An EF ≤ 50% was considered as a criterion for LV dysfunction in this cohort in order to compensate the low number of rEF patients (EF < 40%, $n = 29$), especially in women ($n = 5$). Univariate and multivariable analyses were conducted with logistic regression to address the association between CDKN1C and LV dysfunction, in all patients and in men and women separately (Table 2). CDKN1C was a significant univariate predictor in all patients but not in men. In women, the association had a borderline significance ($p = 0.068$). In multivariable analyses including age, sex, white blood cells count and Nt-proBNP, the association between CDKN1C and LV dysfunction was significant in all patients as well as in women, but not in men (Table 2). These data independently validate the association between CDKN1C and LV function after AMI, especially in women.

4. Discussion

We report a sex-specific association between the CDKN1C gene and LV function after AMI. The blood expression pattern of this gene may

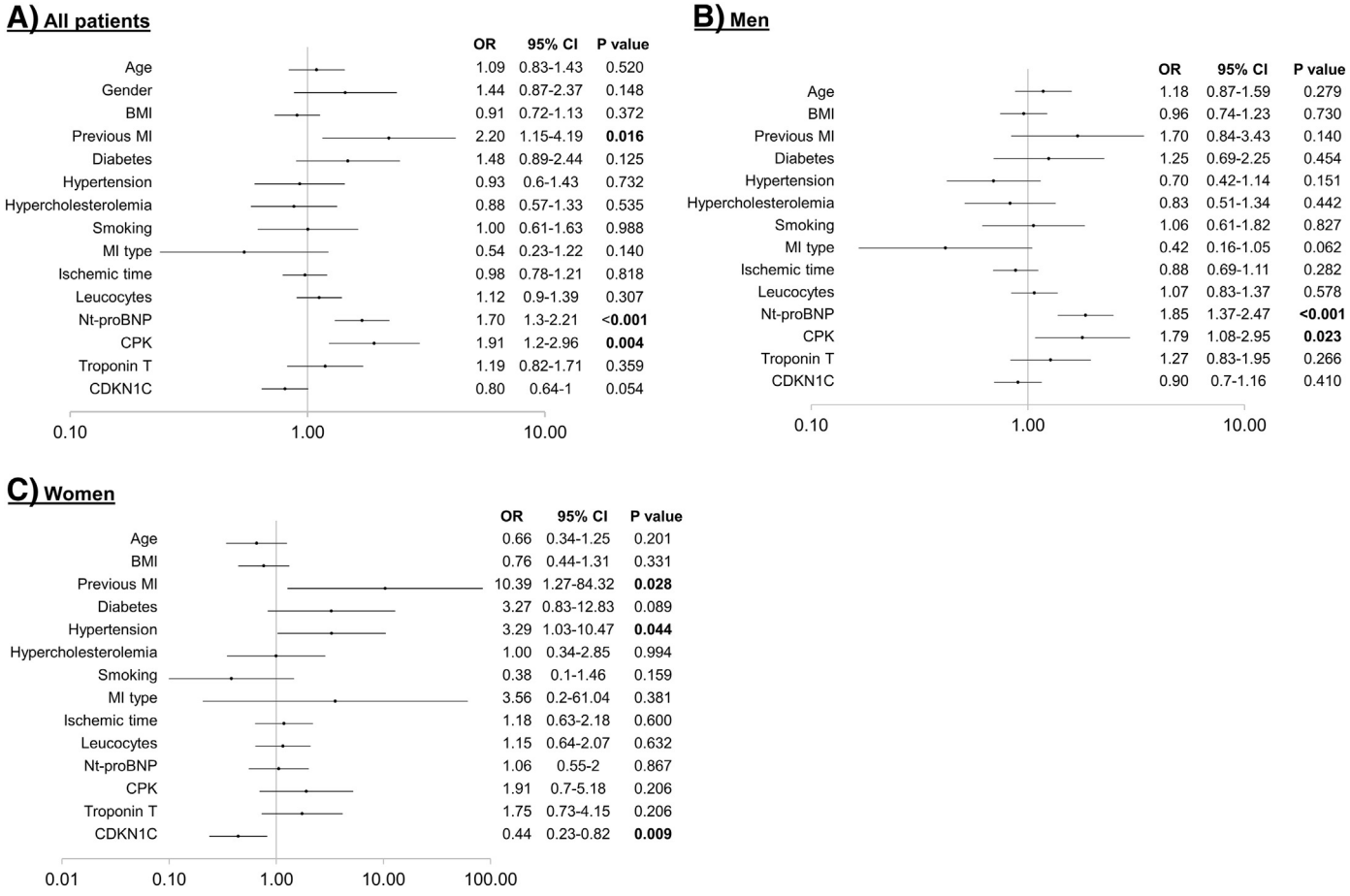

Fig. 2. Multivariable analyses. Ordinal regression was used to evaluate the association of CDKN1C and the demographic and clinical parameters of patients with LV dysfunction at 4 months, in all patients (**A**), and in men (**B**) and women separately (**C**). Odds ratios (OR) with 95% confidence intervals (CI) are shown. X axis is in log scale. BMI: body mass index; CI: confidence intervals; CPK: creatine phosphokinase; MI: myocardial infarction; Nt-proBNP: N-terminal pro-brain natriuretic peptide; OR: adds ratios.

help to risk stratify AMI patients for a more personalized healthcare approach.

Risk stratification at an early stage after AMI is necessary to start aggressive treatments as soon as possible to prevent HF development. Neurohumoral blockade through inhibition of the renin-angiotensin system and beta-blockers represent the standard treatment approach to prevent HF development after AMI [9]. However, predicting outcome shortly after AMI is challenging, notably due to fluctuating levels of current biomarkers such as Nt-proBNP [28]. Blood samples of the test cohort were collected at the time of reperfusion (i.e. within 12 h of chest pain onset) and at median balloon-to-blood time 0.87 days in the validation cohort. Hence, CDKN1C, combined with clinical and demographic parameters of patients, provides an early (<1 day) prediction of outcome which would allow adapting healthcare depending on risk category.

Table 2
Independent validation.

Group	Univariate analysis		Multivariable analysis	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
All patients ($N = 294$)	0.50 (0.27–0.92)	**0.027**	0.46 (0.23–0.97)	**0.040**
Men ($N = 212$)	0.58 (0.28–1.19)	0.140	0.67 (0.29–1.58)	0.360
Women ($N = 82$)	0.31 (0.090–1.09)	0.068	0.18 (0.038–0.90)	**0.037**

Odds ratio are for the association of CDKN1C with LV dysfunction as assessed by an EF ≤ 50% at follow-up of 99 days in median. The univariate analysis assessed the prediction value of CDKN1C alone. Variables included in multivariable analysis were age, sex, leucocytes level, and Nt-proBNP. CI: confidence interval; OR: odds ratio.
P values < 0.05 are indicated in bold.

Few biomarkers have been shown to be women-specific. Plasma levels of proneurotensin are higher in women than men and are associated with the risk of diabetes, cardiovascular disease, breast cancer, and all-cause mortality in women, but not in men [29]. In women with HFpEF, thymosin beta 4 is upregulated and predicts mortality independently of Nt-proBNP and other risk factors [30]. To our knowledge, no sex-specific biomarker may serve as outcome predictor after AMI. We observed a lower expression of CDKN1C in blood samples from AMI women with rEF at 4-month follow-up as compared to women with mrEF and pEF. This difference was absent in men. Accordingly, CDKN1C displayed a significant association with LV function only in female patients. Therefore, CDKN1C may be useful to aid in risk stratification of women suffering an AMI.

The end-point used in the test cohort was LV function at 4 months as classified into 3 groups of EF according to European Society of Cardiology guidelines for the management of HF patients [31]. Although this classification is not dedicated to the post-AMI setting but to acute and chronic HF, it was selected for the present study to refine the analyses of the prediction value of CDKN1C. In past studies [12, 32, 33], LV function after AMI was determined using a dichotomized EF with a cut-off value of 40%. In the validation cohort, we opted for this dichotomized classification and a cut-off EF of 50% was chosen to compensate the low number of women in the low to moderate EF groups. This strategy has been adopted previously [33].

For prediction analyses, we calculated the AIC instead of the more widely used area under the curve to take into account the multiplication of variables entered into prediction models. This approach, used in past studies [34, 35], allows to avoid model overfitting. In addition, bootstrap

internal validation was conducted to confirm the association between CDKN1C and LV function. This approach was chosen for its suitability to small sample size [36], as it was the case for women in the rEF group of the test cohort.

In bootstrap internal validation analyses, we observed that significant markers of LV function differed between men and women. In men, Nt-proBNP and CPK appeared to be the best predictors while in women, CDKN1C, along with cTnT or CPK, showed the highest predictive value. In women, Nt-proBNP had a poor if no incremental predictive value, consistent with an insignificant OR in multivariable analyses (OR 95% CI 1.06 [0.55–2]). Accordingly to previous studies [37–39], levels of Nt-proBNP were higher in women compared to men (Supplementary Table 1, $p < 0.001$). This difference was observed only in the pEF group. Furthermore, NT-proBNP was correlated with EF in men ($r = -0.26$, $p = 1.7 \times 10 - 6$) but not in women, which is consistent with the weakness of this biomarker to predict LV function in women. The absence of predictive value of Nt-proBNP in women in this cohort, while significant in men (OR 95% CI 1.85 [1.37–2.47]), firstly speaks against a general (i.e. equally in men and women) use of this biomarker for risk stratification after AMI and secondly illustrates the need for novel women-specific biomarkers. As biomarkers reflect the underlying pathogenesis of the LV remodeling process which is significantly affected by sex [40, 41], using sex-specific biomarkers would allow a more accurate risk-stratification of the patients. From our data, it can be suggested that CDKN1C might be one of these novel biomarkers.

From a clinical perspective, being able to accurately identify high risk patients after AMI in a sex-specific manner would certainly represent a step forward towards personalized medicine and would allow reducing the economic burden of HF which is still expected to raise continuously over the next decade [42].

From a technological perspective, utilization of CDKN1C in clinical practice will require the development of in vitro diagnostic kits based on the measurement of gene expression in blood samples. This is feasible, as shown for the Corus® CAD test which measures gene expression levels in blood samples to evaluate the likelihood of obstructive coronary artery disease in symptomatic non-diabetic patients [43]. Further developments are required to achieve robust, accurate, fast and cost-effective in vitro diagnostic kits. However, before engaging into in vitro diagnostics development, the predictive value of CDKN1C will have to be thoroughly validated in independent and adequately-sized patient cohorts.

From a mechanistic perspective, sudden death of cardiomyocytes after AMI triggers an inflammatory response which is essential for healing [44]. If dysregulated, this inflammatory response contributes to the development of LV dysfunction and HF. The lower expression of CDKN1C – a cell cycle inhibitor [15] – in blood cells from patients with rEF observed in the present study is consistent with blood cell proliferation and activation of inflammation, which may be excessive and may induce LV remodeling leading to a rEF. Lower levels of CDKN1C in rEF patients are also consistent with the higher levels of KCNQ1OT1 levels observed in our previous study [12], since KCNQ1OT1 silences CDKN1C [45]. It could be argued that CDKN1C expression in blood samples may be a mere indication of the extent of inflammation and would not provide any additional predictive information than blood cells count. The absence of predictive value of leukocytes in multivariable analyses and the unmodified association between CDKN1C and outcome in females after adjustment with leukocytes count (multivariate OR 0.31 [0.13–0.74]) compared to the unadjusted OR of 0.44 [0.23–0.82] (Fig. 2C) argue against this possibility. The exact contribution of CDKN1C in the impairment of LV function after AMI remains to be fully characterized.

The main strength of our study relies on the confirmation of the sex-specific predictive value of CDKN1C in two independent cohorts. Most of presumed novel biomarkers did not reach clinical application due to lack of proper validation. Nevertheless, further large-scale testing of the predictive value of CDKN1C is needed. As far as limitations are concerned, the low number of women, especially women with impaired EF, certainly affected the statistical power of our study. The use of bootstrap internal validation and validation in an independent cohort allowed, to some extent, to compensate this hurdle. Furthermore, changing the EF cut-off for patient classification in the validation cohort is another drawback of the study. The selection of CDKN1C as a candidate sex-specific marker for this study was based on previous findings of an association with the clustered gene KCNQ1OT1 and LV function after AMI [12], as well as its known association with gynecological cancers [19–22] and AMI risk [18]. The other maternally-expressed genes of the cluster (e.g. KCNQ1, SLC22A18 or PHLDA2) represent additional potential sex-specific markers that deserve to be investigated. It is conceivable that other genes than CDKN1C may possess a predictive value as well. It will be interesting in future studies to evaluate the incremental value of panels of genes and not only single genes. Finally, serial blood samples would be desirable to determine the evolution of the expression levels of CDKN1C in the few hours and days following AMI.

5. Conclusion

We identified CDKN1C as a female-specific biomarker of LV function after AMI. Further validation in independent patient cohorts is warranted before engaging into the development of an in vitro diagnostic test for risk stratification after AMI.

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Competing interests

The authors have declared that no competing interest exists.

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