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CLINICAL RESEARCH

Long-term reliability of the phospholamban (PLN) p.(Arg14del) risk model in predicting major ventricular arrhythmia: a landmark study

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Aims

Recently, a genetic variant-specific prediction model for phospholamban (PLN) p.(Arg14del)-positive individuals was developed to predict individual major ventricular arrhythmia (VA) risk to support decision-making for primary prevention implantable cardioverter defibrillator (ICD) implantation. This model predicts major VA risk from baseline data, but iterative evaluation of major VA risk may be warranted considering that the risk factors for major VA are progressive. Our aim is to evaluate the diagnostic performance of the PLN p.(Arg14del) risk model at 3-year follow-up.

Methods and results

We performed a landmark analysis 3 years after presentation and selected only patients with no prior major VA. Data were collected of 268 PLN p.(Arg14del)-positive subjects, aged 43.5 ± 16.3 years, 38.9% male. After the 3 years landmark, subjects had a mean follow-up of 4.0 years (± 3.5 years) and 28 (10%) subjects experienced major VA with an annual event rate of 2.6% [95% confidence interval (CI) 1.6–3.6], defined as sustained VA, appropriate ICD intervention, or (aborted) sudden cardiac death. The PLN p.(Arg14del) risk score yielded good discrimination in the 3 years landmark cohort with a C-statistic of 0.83 (95% CI 0.79–0.87) and calibration slope of 0.97.

Conclusion

The PLN p.(Arg14del) risk model has sustained good model performance up to 3 years follow-up in PLN p.(Arg14del)-positive subjects with no history of major VA. It may therefore be used to support decision-making for primary prevention ICD implantation not merely at presentation but also up to at least 3 years of follow-up.

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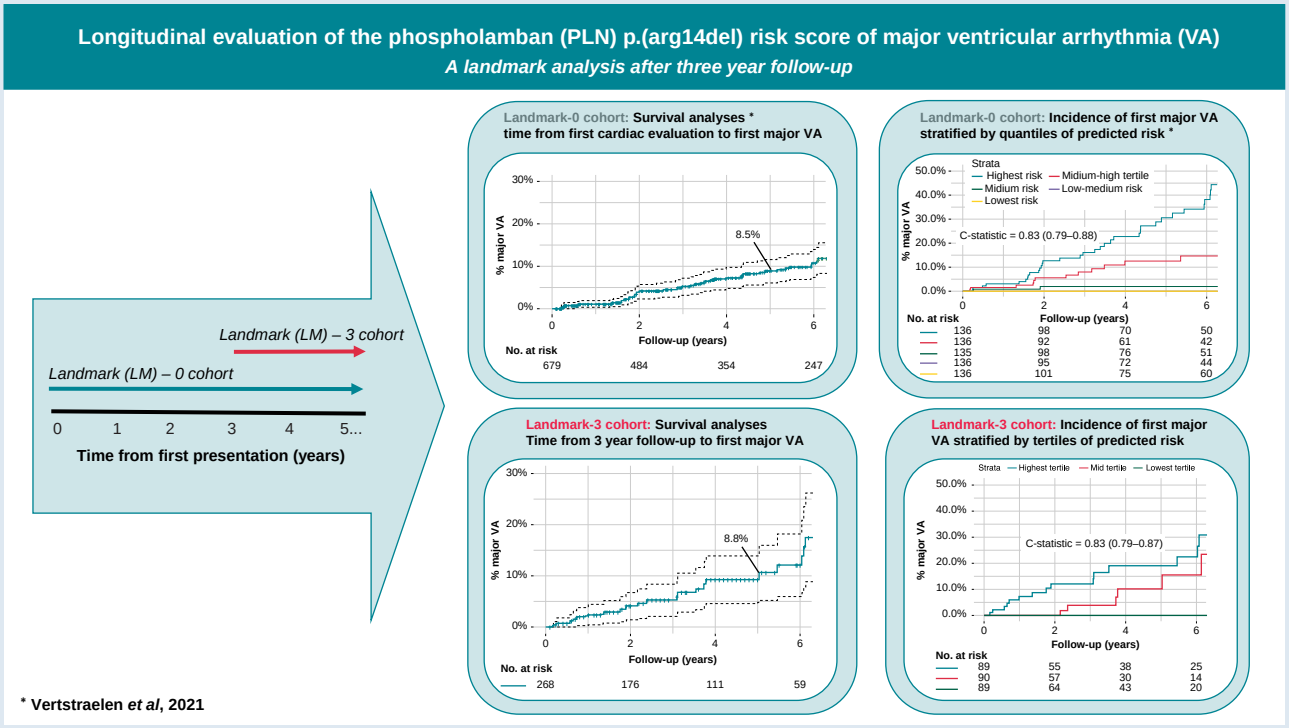
Graphical Abstract



Keywords Phospholamban • PLN p.(Arg14del) risk model • Landmark analysis • Cardiomyopathy • ICD implantation

What's new?

- This study evaluates the phospholamban (PLN) p.(Arg14del) risk score longitudinally using a landmark analysis.
- The PLN p.(Arg14del) risk score yielded good discrimination in the 3 years landmark cohort with a C-statistic of 0.83 (95% confidence interval 0.79–0.87) and calibration slope of 0.97.
- The PLN p.(Arg14del) risk score may be used to support decision-making for primary prevention implantable cardioverter defibrillator not merely at presentation but also up to at least 3 years of follow-up.

Introduction

Phospholamban (PLN) cardiomyopathy is an inherited cardiomyopathy, which is associated with major ventricular arrhythmia (VA) and sudden cardiac death (SCD).[1] Several disease causing variants in PLN, a gene encoding a protein essential for calcium homeostasis, have been reported.[1–3] The pathogenic founder variant PLN p.(Arg14del) leads to the deletion of arginine 14 of the PLN protein resulting in a high risk of both dilated and arrhythmogenic cardiomyopathy.[4] This PLN p.(Arg14del) variant is was first identified in a Greek family in 2006[2] and has since been found in the Germany,[5] Spain,[6] the USA,[7] Canada,[8] China,[9] and Japan.[10] The majority of the PLN p.(Arg14del)-positive individuals were identified in the northern region of the Netherlands. Affected individuals are characterized by low-voltage electrocardiograms (ECGs), abnormal repolarization, development of end-stage heart failure, high arrhythmic burden with premature ventricular contractions (PVC) and VA, and premature death.[4,11,12] To date, there are no specific treatment options for PLN p.(Arg14del)-positive individuals.[13] The primary goal of management is to prevent SCD, for which an implantable cardioverter defibrillator (ICD) is recommended.[14]

Recently, a variant-specific prediction model for PLN p.(Arg14del)-positive individuals was developed to predict individual major VA risk to inform decision-making for primary preventive ICD implantation.[11] This model predicted a 5-year major VA risk at the time of first presentation. However, the baseline risk model has not yet been evaluated for use during follow-up. In follow-up of these individuals, risk factors can progress and iterative evaluation of major VA risk may be warranted. The objective of this study is therefore a longitudinal evaluation of the prediction of VA in PLN p.(Arg14del)-positive individuals using the PLN p.(Arg14del) risk model during follow-up to assess the performance and applicability of the established model in a longitudinal context. Modifying and refining the existing prediction model falls outside the scope of this study.

Methods

Study design

The longitudinal evaluation of the PLN p.(Arg14del) risk model was performed with a landmark analysis in a cohort obtained from the PLN registry, a retrospective multi-centre cohort study.[15] This study was approved by the Institutional Committee on Human Research at the authors' institution. This study is reported in accordance with the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis statement and conformed to the principles of the Helsinki declaration.[16]

Study population

The study cohort consisted of patients and relatives identified with the pathogenic p.(Arg14del) PLN variant in three Dutch and one Spanish

University Hospital, between 2009 and 2022. The cohort previously used for the development of the PLN p.(Arg14del) risk model at first presentation is regarded as landmark at time point 0 (LM-0) and the new cohort used for the longitudinal evaluation of the PLN p.(Arg14del) risk model is regarded as landmark at time point 3 (LM-3).[11] The LM-3 cohort consisted of 268 (96%) patients from the LM-0 cohort and they were patients already in follow-up from the initial registry. Twelve (4%) new patients were added to the PLN registry since. Both cohorts consist of adult PLN p.(Arg14del)-positive subjects (≥16 years of age) with no history of major VA and 3 years after first presentation. Patient selection and visualization of the landmark analysis is shown in *Figure 1*.

Data collection

Data were collected through electronic case report forms in an online secured data platform. All data from first to last clinical contact with a cardiologist or clinical geneticist were collected both in university and peripheral hospitals. Data of the LM-3 cohort were collected between 2 and 4 years after first clinical contact with a cardiologist or clinical geneticist. The complete design of the registry and definitions of the collected variables has been previously described.[15]

Study outcomes

According to the PLN p.(Arg14del) risk model the primary outcome was a composite endpoint of major VA, including sustained VA, ventricular fibrillation (VF), appropriate ICD intervention, and (aborted) SCD. Sustained VA was defined as ventricular tachycardia (VT) lasting ≥30 s, or <30 s when terminated electrically of pharmacologically. Implantable cardioverter defibrillator intervention was considered appropriate when VT or VF were treated with antitachycardia pacing (ATP) or shock. Sudden cardiac death was defined as witnessed sudden death (with or without documented VF) or death within 1 h of new symptoms or nocturnal deaths in the absence of disease. Sudden cardiac death was considered aborted when a stable circulation returned after a cardiac arrest due to basic life support. Phospholamban p.(Arg14del)-positive subjects were censored if they did not develop one of the endpoints until end of their follow-up.

Predictor variables and prediction model

The predictors selected previously for the PLN p.(Arg14del) risk model were derived from those identified for major VA in patients diagnosed with arrhythmogenic right ventricular cardiomyopathy (ARVC) and dilated cardiomyopathy,[17,18] both phenocopies of PLN associated disease. Additionally, the choice of predictors was informed by insights from a prior cohort study examining PLN p.(Arg14del)-positive individuals.[1] Our patient population did not have sufficient magnetic resonance imaging and biomarker data available to identify possible predictors; however, late gadolinium enhancement (LGE) was analysed in a sub-analysis. Late gadolinium enhancement was a significant predictor for major VA in univariable analysis, yet exclusion of LGE from the multivariable model did not result in a decrease in model performance.[11,19] This is likely attributable to an overlap in predictive ability with other predictors, such as low-voltage ECG. The specific criteria for predictor selection has been previously described.[11] The predictor variables of the PLN risk model, which we evaluate in the LM-3 cohort, include left ventricular ejection fraction (LVEF), low-voltage ECG, 24 h PVC count and the amount of negative T waves inferior or precordial. The prediction model for the individual calculation of the 5-year major VA risk, derived from the Cox proportional hazards model, was published as the following equation (11):

$$P(\text{VA at 5 year}) = 1 - 0.965^{\exp(LP)}$$

LP = 0.466(centring constant) − 0.0364*LVEF + 0.0906 × amount of negative T waves + 0.615 × low-voltage ECG (Yes) + 0.309 × Log(amount of PVC/24 h)

P(VA at 5 years) is the prognostic index (PI) which represents the sum of the regression coefficients multiplied by the product of their predictors, 0.965 is the exponent of the baseline cumulative hazard at 5 years.

Statistical analysis

All statistical analyses were carried out using SPSS version 26 and RStudio version 4.0.3 with the use of packages rms, survival, riskRegression, and mice. Continuous variables with normal distribution were presented as mean ± standard deviation and non-normal variables as median [interquartile range (IQR)]. Categorical variables were presented as frequencies (%). The follow-up time of each subject was calculated from 3 years after the date of first presentation to medical attention for cardiomyopathy related complaint or family screening to the date of reaching the study endpoint or censoring. Patients were censored after heart transplantation, if they died of any other cause than major VA, or at most recent contact with a cardiologist or clinical geneticist. The cumulative incidence was estimated using the Kaplan–Meier method.

Missing data

Nowadays, there is a protocol available for diagnostics and follow-up of PLN p.(Arg14del)-positive individuals;[20] however, this has not always been the case resulting in missing values of some predictor variables in some patients. To account for this problem, all predictors with missing data, together with the outcome, were included in a multiple imputation model which generated a total of 25 imputed datasets. Left and right ventricular ejection fraction values were imputed using the qualitative assessment of ejection fraction as described in echocardiography reports. Our calibration study was performed with all imputed datasets. Rubin's rules were used to combine the results of the Cox regression analyses.

Model evaluation

The approach for the evaluation of the PLN p.(Arg14del) risk model in the LM-3 cohort followed the method described by Royston and Altman for the external validation of a Cox prognostic model.[21] We studied three layers of model performance, namely discrimination, calibration, and a more general fit of the model.

First, calibration was evaluated by analysing the calibration slope to assess the degree of agreement between the observed and predicted hazards of major VA. Accordingly, the calibration slope was plotted for all imputed datasets. Regarding the calibration slope a value of 1 indicates a perfect calibration (i.e. the PI from the LM-3 cohort is identical to the linear predictor of the risk model), a slope <1 indicates poorer discrimination and conversely if the slope is >1 this indicates better discrimination. Next, the predictors were evaluated for linearity with the martingale residuals (plots illustrated in Supplementary material online, *Figure S1*) and for misspecification by comparing the hazard ratios in univariable analysis. In addition, the discriminative ability of the prediction model was evaluated with Harrell's concordance (*C*)-statistic and graphically analysed with a cumulative incidence graph stratified by predicted risk tertiles during follow-up. A *C*-index of >0.7 suggest a good discrimination and >0.8 strong discrimination. The number of predicted risk groups was chosen based on the spread of the risk values. More groups would harm the discriminative power between neighbouring groups, and it would make the cumulative incidence curves unstable. For the second layer of model performance, calibration was performed with the predicted risk of each subject in 25 imputed datasets presented graphically with the 5-year predicted risk of major VA probabilities plotted against the observed risk predictions. Lastly, in the third layer a more strict calibration and model fit assessment was performed by comparing the baseline survival function (i.e. covariate-adjusted survival) of the LM-3 dataset with the LM-0 dataset as shown in Supplementary material online, *Table S1*.

Results

The final LM-3 cohort included 268 PLN p.(Arg14del)-positive subjects with no history of major arrhythmias. The baseline characteristics are shown in *Table 1*. A noticeable difference between baseline characteristics is disease progression of the LM-3 cohort compared to the LM-0 cohort. This resulted in a higher frequency of ICD implants, low-voltage ECG, T-wave inversion, non-sustained ventricular tachycardia, and right ventricular dysfunction.

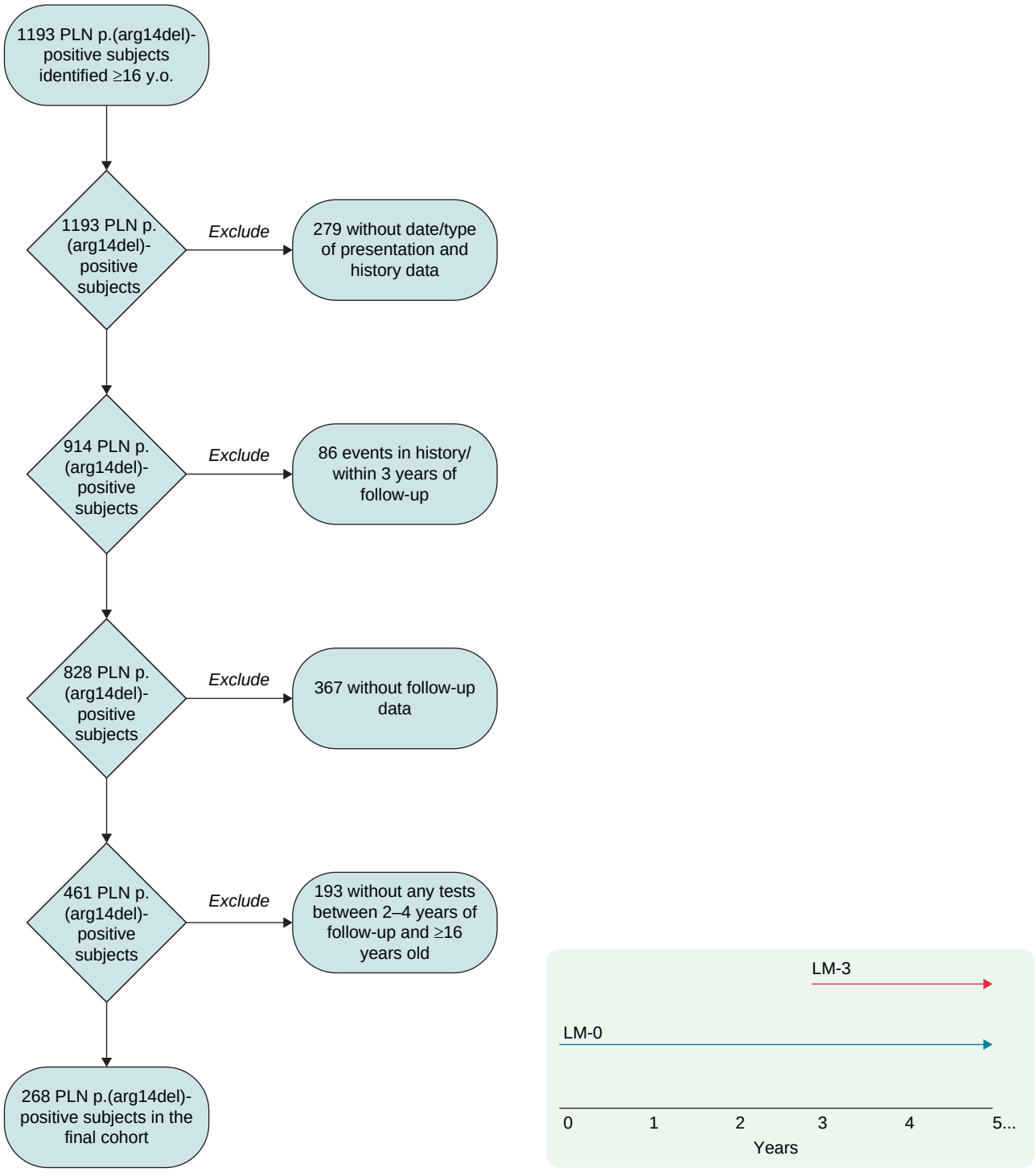


Figure 1 Inclusion of study patients. (*A*) Flowchart summarizing the selection of the study population with the PLN p.(Arg14del) pathogenic variant. Type of presentation and history data should be available for exclusion of PLN p.(Arg14del)-positive subjects with major VA at baseline or in history. Most subjects with no baseline or follow-up data were only genetically counselled in the participating university hospitals with no cardiologic data available and treated by their local cardiologists. Events in history and during a 3-year follow-up period consisted spontaneous VT/VF ($n = 51$), aborted SCD ($n = 19$), appropriate ICD intervention ($n = 9$), death ($n = 7$), heart transplantation ($n = 7$), VT-storm ($n = 1$) and undefined arrhythmic events ($n = 10$). Tests between 2 and 4 years of follow-up included electrocardiogram, transthoracic echocardiogram, or magnetic resonance imaging and Holter monitor. (*B*) Landmark at time point 0 (LM-0) represent the cohort for the development of the PLN p.(Arg14del) risk score at time of first presentation, landmark at time point 3 (LM-3) represent the new cohort for the longitudinal evaluation. ICD, implantable cardioverter defibrillator; PLN, phospholamban; SCD, sudden cardiac death; VA, ventricular arrhythmia; VF, ventricular fibrillation; VT, ventricular tachycardia.

Table 1 Baseline characteristics

Characteristics	LM-0 cohort	LM-3 cohort	*P*-value
Total, *n*	679	268	
Demographics			
Age (years)	42 (27–55)	46 (30–63)	0.42
Sex, male	294 (43)	103 (38)	0.19
Caucasian ethnicity	679 (100)	268 (100)	
Proband	113 (17)	47 (18)	0.70
History			
Cardiac syncope <6 months before inclusion	4 (0.6)	6 (2.2)	*0.037*
NYHA class ≥ II	62 (9)	20 (7)	0.44
First degree family member with major VA	91 (13)	49 (18)	0.068
Presentation reason: family screening vs. symptomatic or abnormal test result	574 (85)	201 (75)	*<0.001*
ICD implantation before inclusion	10 (1)	42 (16)	*<0.001*
ECG/continuous rhythm monitoring			
Atrial fibrillation/flutter	56 (8)	26 (10)	0.44
Low-voltage ECG[a]	95 (15) (*n = 618*)	55 (27) (*n = 202*)	*<0.001*
TWI in ≥ 3 precordial leads[b]	77 (14) (*n = 559*)	44 (23) (*n = 191*)	*0.003*
TWI in ≥ 2 inferior leads[b]	49 (9) (*n = 559*)	30 (16) (*n = 191*)	*0.006*
NSVT[c]	67 (10)	39 (15)	*0.034*
24 h PVC count >500	125 (31) (*n = 406*)	47 (36) (*n = 132*)	0.28
Imaging			
LVEF <50%	105 (17) (*n = 627*)	35 (18) (*n = 196*)	0.66
Median LVEF (%)	54 (48–58) (*n = 532*)	55 (47–59) (*n = 196*)	0.45
RV dysfunction	62 (10) (*n = 254*)	34 (17) (*n = 217*)	*0.022*
LGE on MRI	77 (29) (*n = 262*)	28 (38) (*n = 74*)	*0.036*

Categorical data are presented as percentage and continuous data as mean ± standard deviation. Significant *P*-values are shown in italic.
ECG, electrocardiogram; ICD, implantable cardioverter defibrillator; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NSVT, non-sustained ventricular tachycardia; NYHA, New York heart Association; RV, right ventricular; TWI, T-wave inversion; VA, ventricular arrhythmia.
[a]Low-voltage ECG is defined as peak-to-peak QRS amplitude of <0.5 mV in the limb leads and/or <1 mV in the precordial leads.
[b]TWI, T-wave inversion, T-wave is only considered inverted if the voltage shift is ≥ 0.1 mV
[c]NSVT is defined as three or more consecutive premature ventricular contractions with a rate >100/min, lasting <30 s.

Outcomes

During a median follow-up time of 3.1 years (IQR 1.4–5.5), 28 (10%) PLN p.(Arg14del)-positive subjects experienced the composite endpoint of major VA, corresponding to a crude annual event rate of 2.6% and 5-year cumulative incidence of 10.4%. *Figure 2* shows the cumulative incidence of major VA, which increases quite consistently throughout the follow-up period. Most common event was appropriate ICD intervention ($n = 20$), followed by spontaneous VT/VF ($n = 4$), VT-storm ($n = 3$), and aborted SCD ($n = 1$). Appropriate ICD intervention included ATP (30%) and shock (70%). Of the subjects with data available on the ventricular rate and/or ICD settings 83% had an event with >200 beats per minute. During follow-up, 53 (20%) of the subjects received an ICD for primary or secondary prevention, 6 (2%) subjects underwent heart transplantation, and 4 (1%) received a left ventricular assist device. At last follow-up, 24 (9%) PLN p.(Arg14del)-positive subjects had died caused by: witnessed sudden cardiac death ($n = 6$), unwitnessed sudden cardiac death ($n = 1$), heart failure/cardiogenic shock ($n = 11$), other cardiovascular cause of death ($n = 2$), non-cardiac cause of death ($n = 1$), and unknown cause of death ($n = 3$).

Longitudinal evaluation

In order to evaluate the PLN p.(Arg14del) risk model longitudinally in the LM-3 cohort, we first assessed the discrimination of the risk model. The mean calibration slope in the LM-3 cohort is 0.97. The individual predictors were evaluated in univariable analysis between the LM-0 and LM-3 cohort and showed similar hazard ratios for LVEF, 24 h PVC count, and low-voltage ECG, but T-wave inversion was significantly different between cohorts, as shown in *Table 2*. This did not affect the model performance, as the model yielded a *C*-statistic of 0.83 [95% confidence interval (CI) 0.79–0.87]. Discrimination is graphically shown in *Figure 3* and presents good discrimination between predicted risk tertiles. *Figure 4* shows the calibration of the agreement between predicted and actual probabilities over a 5-year time period which is excellent. Finally, a stricter calibration and model fit assessment are shown in Supplementary material online, *Table S1* with similar baseline survival hazards.

Clinical implications

As we demonstrate that the PLN p.(Arg14del) risk model has good model performance in the LM-3 cohort, these results can be used,

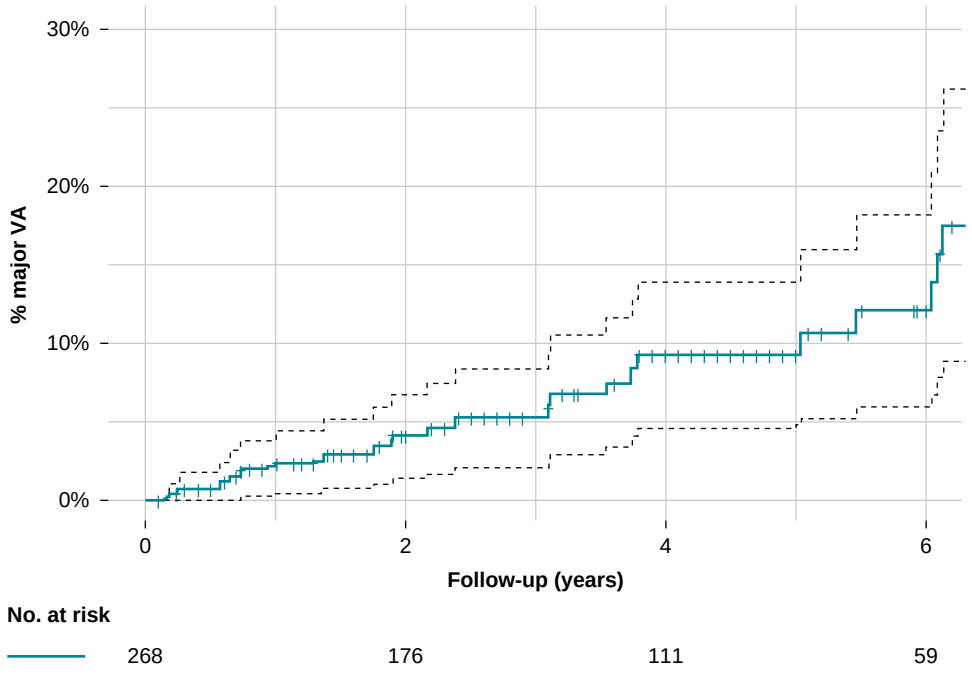


Figure 2 Survival analysis. Kaplan–Meier analysis with time to event from 3-year follow-up to first major VA. The cumulative event rate is plotted with a 95% confidence interval. VA, ventricular arrhythmia.

Table 2 Comparison of individual predictor hazard ratios between the LM-0 and LM-3 cohort

Predictors	LM-0 cohort Hazard ratio (95% CI)	LM-3 cohort Hazard ratio (95% CI)	*P*-value
LVEF, per 1% decrease	1.08 (1.05–1.09)	1.09 (1.06–0.12)	0.59
Leads with negative T waves inferior or precordial (per 1 increase)	1.2 (1–1.3)	0.90 (0.74–1.1)	*0.007*
Low-voltage ECG	3.9 (2.3–6.6)	14.1 (5.2–37.9)	0.15
Logarithm of PVC count/24 h (per 1 log increase)	1.5 (1.3–1.7)	1.4 (1.1–2)	0.85

Significant *P*-values are shown in italic.
CI, confidence interval; ECG, electrocardiogram; LVEF, left ventricular ejection fraction; PVC, premature ventricular contractions.

for example, when a 5% risk major VA risk threshold is chosen to justify an ICD implantation. This resulted in 107 (40%) PLN p.(Arg14del)-positive subjects in the LM-3 cohort that are recommended for an ICD implantation, with 25 of the subjects experiencing major VA. More notably, 161 ICD implantations were avoided with three of these subjects experiencing major VA without ICD implantation. These results are comparable to the LM-0 cohort, see *Figure 5*.

Discussion

Main findings

In this study, we sought to evaluate the PLN p.(Arg14del) risk model for repeated use during follow-up, using a cohort selected 3 years after first presentation, without prior major VA. In this cohort, the model accurately distinguished PLN p.(Arg14del)-positive subjects who were at risk of major VA. Furthermore, calibration was good as the predictions of survival reflected the observed data. Therefore, the PLN p.(Arg14del) risk model can also be used during follow-up.

Model performance

The evaluation of the PLN p.(Arg14del) risk model started with the assessment of the discriminative ability. The mean calibration slope was 0.97, and comparison of the hazard ratios showed no significant difference for LVEF, low-voltage ECG, and 24 h PVC count in univariable analysis. On the contrary, T-wave inversion did show a significant difference in hazard ratios with a hazard ratio beneath 1 suggesting that a higher amount of T-wave inversion would result in a lower major VA risk. A plausible explanation for the misfit of T-wave inversion is the fact that T-wave inversion can be a non-pathognomonic finding on the ECG, for example in lead V1. This is also shown in the non-linearity of T-wave inversion in Supplementary material online, *Figure S1*.

The discrimination was also assessed by evaluating the *C*-statistic, which yielded a *C*-index of 0.83 (95% CI 0.79–0.87) in the LM-3 cohort

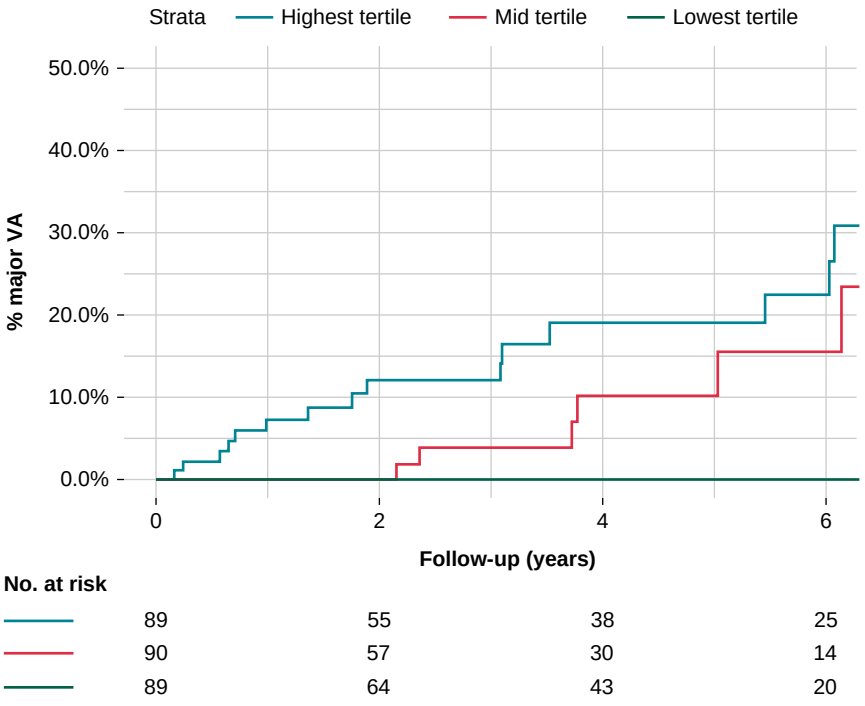


Figure 3 Kaplan–Meier plot. Incidence of first major ventricular arrhythmia stratified by tertiles of predicted risk.

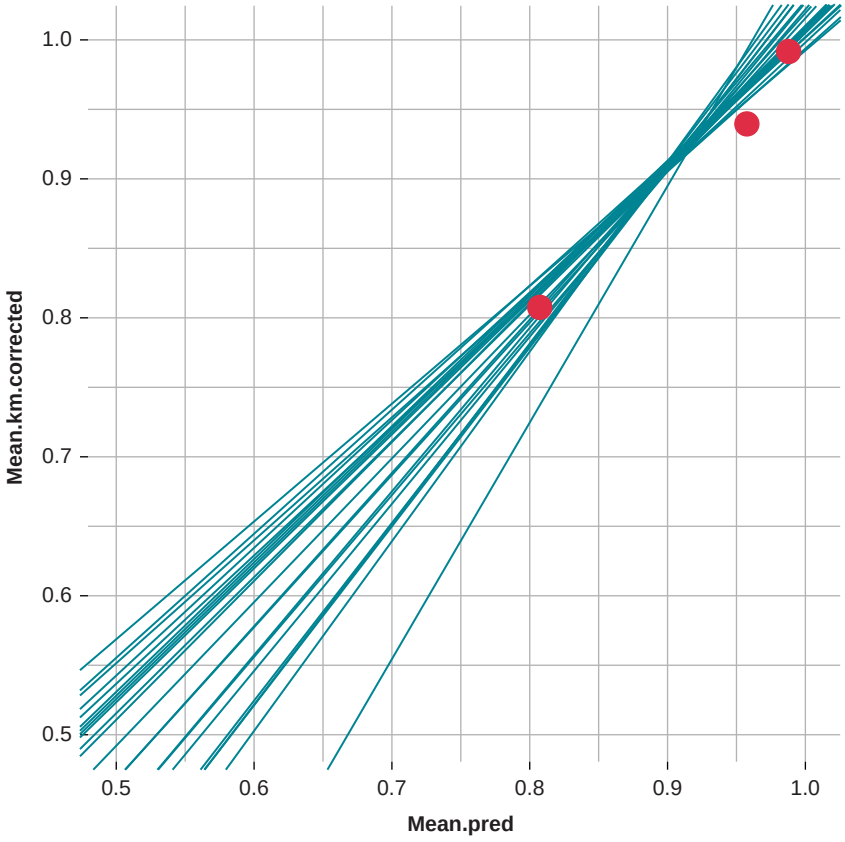


Figure 4 Calibration plot presenting the agreement between predicted (x-axis) and observed 5-year risk probabilities of major VA of 25 imputed datasets. The black lines represent the continuous calibration hazards of all imputed datasets ($n = 25$). The circles represent the tertiles of mean predicted risk. VA, ventricular arrhythmia.

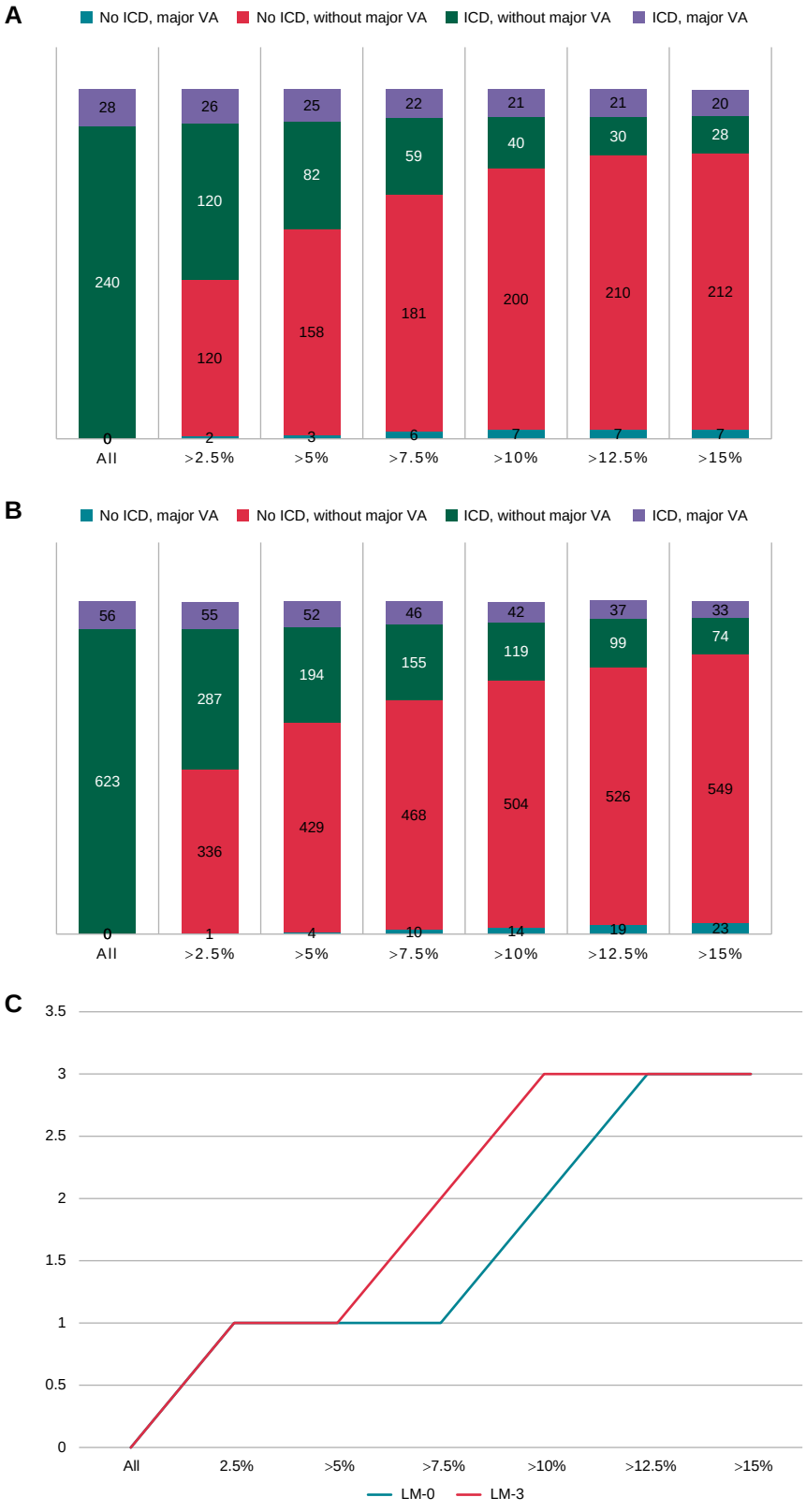


Figure 5 Comparison risk threshold of the PLN p.(Arg14del) risk model in the LM-0 and LM-3 cohort. The y-axis represents the clinical implication of the chosen risk threshold on the x-axis with the PLN p.(Arg14del) risk model in the LM-3 cohort ($n = 268$ patients) (*A*) and the LM-0 cohort ($n = 679$ patients) (*B*). The cohorts are divided by proportions of subjects experiencing major VA as well as implantable cardioverter defibrillator (ICD) implantation. (*C*) Represents the comparison between the LM-0 and LM-3 cohort (blue and orange line, respectively) of 'missed' subjects with major VA and without ICD implantation. PLN, phospholamban; VA, ventricular arrhythmia.

which equals the *C*-index of 0.83 (95% CI 0.78–0.88) in the LM-0 cohort. The second step in evaluating the PLN p.(Arg14del) risk model was to assess the calibration, which we believe is acceptable judging the calibration plot in *Figure 4*. Also a more stricter type of evaluating the model performance is comparing the baseline survival hazard, as shown in Supplementary material online, *Table S1*, and this confirmed our believes.

Clinical implications

The evaluation of the PLN p.(Arg14del) risk model is clinically relevant in daily practice, as clinicians use the risk model during follow-up to guide them on implantation of an ICD. Implantation of an ICD is still the only intervention to prevent SCD in PLN p.(Arg14del)-positive individuals. However, over 90% of the subjects remain event free after 5 years of follow-up and thus, better risk stratification is needed to avoid unnecessary ICD implantations, while protecting those at risk for SCD. Herein lies the greatest clinical utility of this PLN p.(Arg14del) risk model: the individual estimation of the 5-year major VA risk with a nomogram or the online calculator,[11,22] not only at first clinical presentation, but based upon the presented data also during follow-up. After a 3-year follow-up, the event rates in the LM-3 cohort were similar to the LM-0 cohort, 10.4% vs. 10.6%, respectively. This suggest that PLN p.(Arg14del)-positive subjects remain at risk even though they did not experience major VA at baseline and in years of follow-up. The evenly spread of events during follow-up in *Figure 2* also substantiates this idea and highlights the importance of the use of this risk model during follow-up.

The previously suggested 5-year major VA risk of >5% seems still reasonable in the LM-3 cohort to justify implantation of an ICD while at all times considering other individual factors than major VA. In the published PLN p.(Arg14del) LM-0 cohort, a major VA risk >5% would lead to the treatment of 246 subjects with an ICD. Among them, 52 experienced major VA, resulting in 433 avoided ICD implantations compared to treating all patients with four subjects with major VA and without ICD implantation. With this threshold in the LM-3 cohort, 107 subjects were recommended for an ICD implantation. Twenty-five of these experienced a major VA and two of these inappropriate shock, while 161 ICD implantations were avoided with three subjects experiencing major VA later on. A risk threshold of 7.5% in the LM-3 cohort will barely increase the number of subjects experiencing major VA without ICD implantation ($n = 1$) and thus a higher threshold can be debated. A lower risk threshold will increase the number of ICD implantations without experiencing major VA and will probably cause more harm from the ICD implantation (ICD-related complications or inappropriate shocks) than benefits.[23]

The PLN p.(Arg14del) risk model calculates a major VA risk and does not recommend a specific type of ICD. Prior to inclusion in the LM-3 cohort, 42 subjects had an ICD implantation. During follow-up, 53 subjects were treated with an ICD implantation. Most of the ICD implantations were transvenous (TV-) ICDs, only five subcutaneous (S-)ICDs and four cardiac resynchronization therapy with defibrillator devices in total. Phospholamban p.(Arg14del)-positive individuals can be ineligible for an S-ICD due to low-voltages that are not accurately detected by the S-ICD.

The choice of the ICD type is made collaboratively between the physician and the patient through shared decision-making.

The generalizability of the PLN p.(Arg14del) risk model to other genetic variants remains uncertain; however, it appears unlikely as that the predictive efficacy of the model is tied to specific characteristics to the PLN p.(Arg14del) variant.

Prior studies

In 2022, the ARVC risk calculator was also evaluated in a longitudinal design.[24] In this study, the 5-year risk of VA was predicted longitudinally using the baseline ARVC risk calculator prediction, with updated risk factors and time-varying Cox regression. This study demonstrated that using the updated risk factors and time-varying Cox regression resulted in a model with strong discrimination. The predictions of the ARVC risk calculator at baseline worsened during follow-up. This underlines the value of iterative risk predictions with updated risk factors.

The power of the ARVC calculator was previously assessed in PLN p.(Arg14del)-positive individuals and yielded a C-statistic of 0.74 which was significantly lower than the PLN p.(Arg14del) risk model with a C-statistic of 0.83. This was not a surprising result as the ARVC risk calculator was used for all patients while only a fraction of patients had Task Force-compatible ARVC diagnosis.[11]

Limitations

The preferred strategy for the evaluation and of a risk prediction model is external validation, which means assessing the performance of a risk prediction model in an independent dataset. 'Independent' is defined as collected separate from the derived dataset of the original model, for example data collected by different investigators in a different geographical location.[21] This however, is currently not feasible as there are not yet other large enough PLN p.(Arg14del) cohorts available, but this might change in the future as more PLN p.(Arg14del)-positive individuals are found in an increasing number of countries and continents. To be able to evaluate the PLN risk score and check for its model performance during follow-up, we used a new dataset of predictors collected after 3 years of follow-up within the same cohort (96% of the subjects overlapping).

Another limitation of this study is inherent to its retrospective design, because not all subjects underwent full evaluation as, for the initial years, there was no standardized protocol. This has resulted in missing data. Currently, new identified PLN p.(Arg14del)-positive individuals will undergo extensive evaluations. The final limitation is the true risk for SCD, which is masked by ICD implantation in subjects at high risk for major VA. If ICD recipients were removed from the cohort the power would be insufficient and this would also lead to selection bias and an underestimated true risk for SCD. Other observational datasets such as in hypertrophic cardiomyopathy or ARVC, also concern similar issues and included ICD recipients as well.

Conclusion

Our findings show that the PLN p.(Arg14del) risk model has a good model performance in a longitudinal design and can be used for PLN p.(Arg14del)-positive individuals in their follow-up to inform decision-making for primary prevention ICD implantation.

Supplementary material

Supplementary material is available at *Europace* online.

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Data availability

The data underlying this article are available in the article and in its online Supplementary material.

References

1. van Rijsingen IAW, van der Zwaag PA, Groeneweg JA, Nannenberg EA, Jongbloed JDH, Zwinderman AH, *et al.* Outcome in phospholamban R14del carriers results of a large multicentre cohort study. *Circ-Cardiovasc Gene* 2014;**7**:455–U191.
2. Haghighi K, Kolokathis F, Gramolini AO, Waggoner JR, Pater L, Lynch RA, *et al.* A mutation in the human phospholamban gene, deleting arginine 14, results in lethal, hereditary cardiomyopathy. *Proc Natl Acad Sci U S A* 2006;**103**:1388–93.
3. Deiman FE, Bomer N, van der Meer P, Grote Beverborg N. Review: precision medicine approaches for genetic cardiomyopathy: targeting phospholamban R14del. *Curr Heart Fail Rep* 2022;**19**:170–9.
4. van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, *et al.* Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. *Eur J Heart Fail* 2012;**14**:1199–207.
5. Posch MG, Perrot A, Geier C, Boldt LH, Schmidt G, Lehmkuhl HB, *et al.* Genetic deletion of arginine 14 in phospholamban causes dilated cardiomyopathy with attenuated electrocardiographic R amplitudes. *Heart Rhythm* 2009;**6**:480–6.
6. Lopez-Ayala JM, Boven L, van den Wijngaard A, Penafiel-Verdu P, van Tintelen JP, Gimeno JR. Phospholamban p.arg14del mutation in a Spanish family with arrhythmogenic cardiomyopathy: evidence for a European founder mutation. *Rev Esp Cardiol (Engl Ed)* 2015;**68**:346–9.
7. DeWitt MM, MacLeod HM, Soliven B, McNally EM. Phospholamban R14 deletion results in late-onset, mild, hereditary dilated cardiomyopathy. *J Am Coll Cardiol* 2006;**48**:1396–8.
8. Cheung CC, Healey JS, Hamilton R, Spears D, Gollob MH, Mellor G, *et al.* Phospholamban cardiomyopathy: a Canadian perspective on a unique population. *Neth Heart J* 2019;**27**:208–13.
9. Zhang XL, Xie J, Lan RF, Kang LN, Wang L, Xu W, *et al.* Genetic basis and genotype-phenotype correlations in Han Chinese patients with idiopathic dilated cardiomyopathy. *Sci Rep* 2020;**10**:2226.
10. Tabata T, Kuramoto Y, Ohtani T, Miyawaki H, Miyashita Y, Sera F, *et al.* Phospholamban p.Arg14del cardiomyopathy: a Japanese case series. *Intern Med* 2022;**61**:1987–93.
11. Verstraelen TE, van Lint FHM, Bosman LP, de Brouwer R, Proost VM, Abeln BGS, *et al.* Prediction of ventricular arrhythmia in phospholamban p.Arg14del mutation carriers-reaching the frontiers of individual risk prediction. *Eur Heart J* 2021;**42**:2842–50.
12. van Drie E, Taal SEL, Schmidt AF, Verstraelen TE, de Brouwer R, Schoormans D, *et al.* Influence of stressful life events and personality traits on PLN cardiomyopathy severity: an exploratory study. *Europace* 2024;**26**:euad368.
13. Eijgenraam TR, Boukens BJ, Boogerd CJ, Schouten EM, van de Kolk CWA, Stege NM, *et al.* The phospholamban p.(Arg14del) pathogenic variant leads to cardiomyopathy with heart failure and is unresponsive to standard heart failure therapy. *Sci Rep* 2020;**10**:9819.
14. Zeppenfeld K, Tfelt-Hansen J, de Riva M, Winkel BG, Behr ER, Blom NA, *et al.* 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death developed by the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC) Endorsed by the Association for European Paediatric and Congenital Cardiology (AEPC). *Eur Heart J* 2022;**43**:3997–4126.
15. Bosman LP, Verstraelen TE, van Lint FHM, Cox M, Groeneweg JA, Mast TP, *et al.* The Netherlands arrhythmogenic cardiomyopathy registry: design and status update. *Neth Heart J* 2019;**27**:480–6.
16. Collins GS, Reitsma JB, Altman DG, Moons KG, Group T. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *Circulation* 2015;**131**:211–9.
17. Sammani A, Kayvanpour E, Bosman LP, Sedaghat-Hamedani F, Proctor T, Gi WT, *et al.* Predicting sustained ventricular arrhythmias in dilated cardiomyopathy: a meta-analysis and systematic review. *Esc Heart Fail* 2020;**7**:1430–41.
18. Cadrin-Tourigny J, Bosman LP, Nozza A, Wang W, Tadros R, Bhonsale A, *et al.* A new prediction model for ventricular arrhythmias in arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J* 2022;**43**:e1–e9.
19. van der Voorn SM, Bourfiss M, te Riele ASJM, Taha K, Vos MA, de Brouwer R, *et al.* Exploring the correlation between fibrosis biomarkers and clinical disease severity in PLN p.Arg14del patients. *Front Cardiovasc Med* 2022;**8**:802998.
20. Taha K, Asselbergs FW, de Boer RA, van der Heijden JF, Wilde AAM. Protocol for diagnostic work-up and follow-up of PLN mutation carriers. 2019.
21. Royston P, Altman DG. External validation of a Cox prognostic model: principles and methods. *BMC Med Res Methodol* 2013;**13**:33.
22. PLN 5 year VA risk calculator in candidates for primary prevention ICD implantation. 2021. https://plnriskcalculator.shinyapps.io/final_shiny/.
23. Olde Nordkamp LR, Postema PG, Knops RE, van Dijk N, Limpens J, Wilde AA, *et al.* Implantable cardioverter-defibrillator harm in young patients with inherited arrhythmia syndromes: a systematic review and meta-analysis of inappropriate shocks and complications. *Heart Rhythm* 2016;**13**:443–54.
24. Carrick RT, Te Riele A, Gasperetti A, Bosman L, Muller SA, Pendleton C, *et al.* Longitudinal prediction of ventricular arrhythmic risk in patients with arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhythm Electrophysiol* 2022;**15**:e011207.