

Bi-atrial high-density mapping reveals inhibition of wavefront turning and reduction of complex propagation patterns as main antiarrhythmic mechanisms of vernakalant

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Received 8 June 2020; editorial decision 16 January 2021; accepted after revision 22 January 2021; online publish-ahead-of-print 26 February 2021

Aims

Complex propagation patterns are observed in patients and models with stable atrial fibrillation (AF). The degree of this complexity is associated with AF stability. Experimental work suggests reduced wavefront turning as an important mechanism for widening of the excitable gap. The aim of this study was to investigate how sodium channel inhibition by vernakalant affects turning behaviour and propagation patterns during AF.

Methods and results

Two groups of 8 goats were instrumented with electrodes on the left atrium, and AF was maintained by burst pacing for 3 or 22 weeks. Measurements were performed at baseline and two dosages of vernakalant. Unipolar electrograms were mapped (249 electrodes/array) on the left and right atrium in an open-chest experiment. Local activation times and conduction vectors, flow lines, the number of fibrillation waves, and local re-entries were determined. At baseline, fibrillation patterns contained numerous individual fibrillation waves conducting in random directions. Vernakalant induced conduction slowing and cycle length prolongation and terminated AF in 13/15 goats. Local re-entries were strongly reduced. Local conduction vectors showed increased preferential directions and less beat-to-beat variability. Breakthroughs and waves were significantly reduced in number. Flow line curvature reduced and waves conducted more homogeneously in one direction. Overall, complex propagation patterns were strongly reduced. No substantial differences in drug effects between right and left atria or between goats with different AF durations were observed.

Conclusions

Destabilization of AF by vernakalant is associated with a lowering of fibrillation frequency and inhibition of complex propagation patterns, wave turning, local re-entries, and breakthroughs.

Keywords

Atrial fibrillation • Mapping • Re-entry • Sodium channel block • Vernakalant • Antiarrhythmic mechanism

Introduction

Atrial fibrillation (AF) is characterized by fast and irregular atrial activity. On the longer term, AF leads to electrophysiological and structural changes that contribute to prolongation of AF episodes and loss

of efficacy of antiarrhythmic drugs.^{1,2} Moreover, AF stabilization is associated with increased fibrillation frequency, a higher number of waves propagating through the atria and fibrosis content.^{2,3} AF perpetuation has also been suggested to be dependent on stable and/or transient local rotors that act as sources of fibrillatory conduction in

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What's new?

- Vernakalant reduces complex propagation, breakthroughs, and re-entrant patterns.
- Sodium current inhibition by vernakalant leads into fewer wave turning events forcing waves to propagate uniformly in one direction.
- Effects of vernakalant, on cycle length and the complexity of propagation, were comparable between both atria and preserved up to 22 weeks of atrial fibrillation.

the cardiac tissue around this source.⁴ Conversely, mapping of fibrillation patterns during drug-induced termination of AF showed that Class I and III antiarrhythmic drugs reduced the complexity of propagation patterns.⁵ The relatively novel antiarrhythmic drug vernakalant inhibits both the fast sodium current and several potassium currents.⁶ We previously demonstrated that the antiarrhythmic effect of vernakalant is mainly explained by sodium current inhibition.⁷ This Class I effect resulted in slowing of conduction velocity (CV), and a prolongation of the effective refractory period (ERP) prolongation due to post-repolarization refractoriness.⁷

In theory, the ERP prolongation could lead to a prolongation of the wavelength and closure of the excitable gap. However, the ERP prolongation by Class I antiarrhythmic drugs (e.g. vernakalant) goes along with conduction slowing that might counteract wavelength prolongation. As a matter of fact, Wijffels *et al.* demonstrated that both Class I and III drugs cause a prolongation of the excitable gap during AF.⁸ It has been proposed that the antiarrhythmic effect of Class I drugs is due to the decrease of wavefront turning.⁹ When sodium channel conductance is reduced a shift in the source-sink relation occurs. This leads to a lower maximal curvature of the wavefront and wider wave turns in general.¹⁰ Computer modelling and experimental work has shown that sufficient reduction of sodium channel conductance will also lead to detachment of wavefronts from an obstacle leading to a wider core around which a wavefront pivots.^{9,10} This mechanism implies that wavefronts will occupy a larger space (hence reducing the maximal number of wavefronts) and a longer cycle length as wavefronts have to conduct over longer trajectories to re-excite the atrial tissue. Moreover, reduced wave turning has been suggested to widen the excitable gap leading to less wavefront wavetail interaction additionally reducing the number of newly generated wavefronts ultimately leading to termination of AF. As wave turning also occurs during transmural conduction, Class I compounds may also affect breakthrough patterns during AF.

We hypothesized that administration vernakalant of reduces complex propagation patterns during AF leading to less spatial variability of conduction vectors, reduction of wavefront turning, fewer breakthrough patterns, and less re-entrant activity. To address this hypothesis, we performed high-density bi-atrial mapping of persistent AF in goats with a substrate of electrical remodelling only (3 weeks of AF) and in goats with a combined substrate of both electrical and structural remodelling (22 weeks of AF).^{2,3} The effects of vernakalant were tested at two clinically relevant plasma levels of vernakalant and compared to baseline values within the same animal.

Methods

Animal model

This protocol was approved by the local ethics committee and complied with the Dutch and European directives. Goats with a weight of 46–86 kg were used. Goats were anesthetized (see [Supplementary material online](#)) and electrodes were implanted on the pericardium above the left atrium (LA) and connected to an implanted neurostimulator (Itrel 3/4, Medtronic, MI, USA). In the first week, AF was induced by burst-pacing (1 s, 50 Hz bursts) every other second and every 10 seconds thereafter. AF was maintained for either 3 (3wAF, $n=8$) or 22 weeks (22wAF, $n=8$).

Open chest experiment

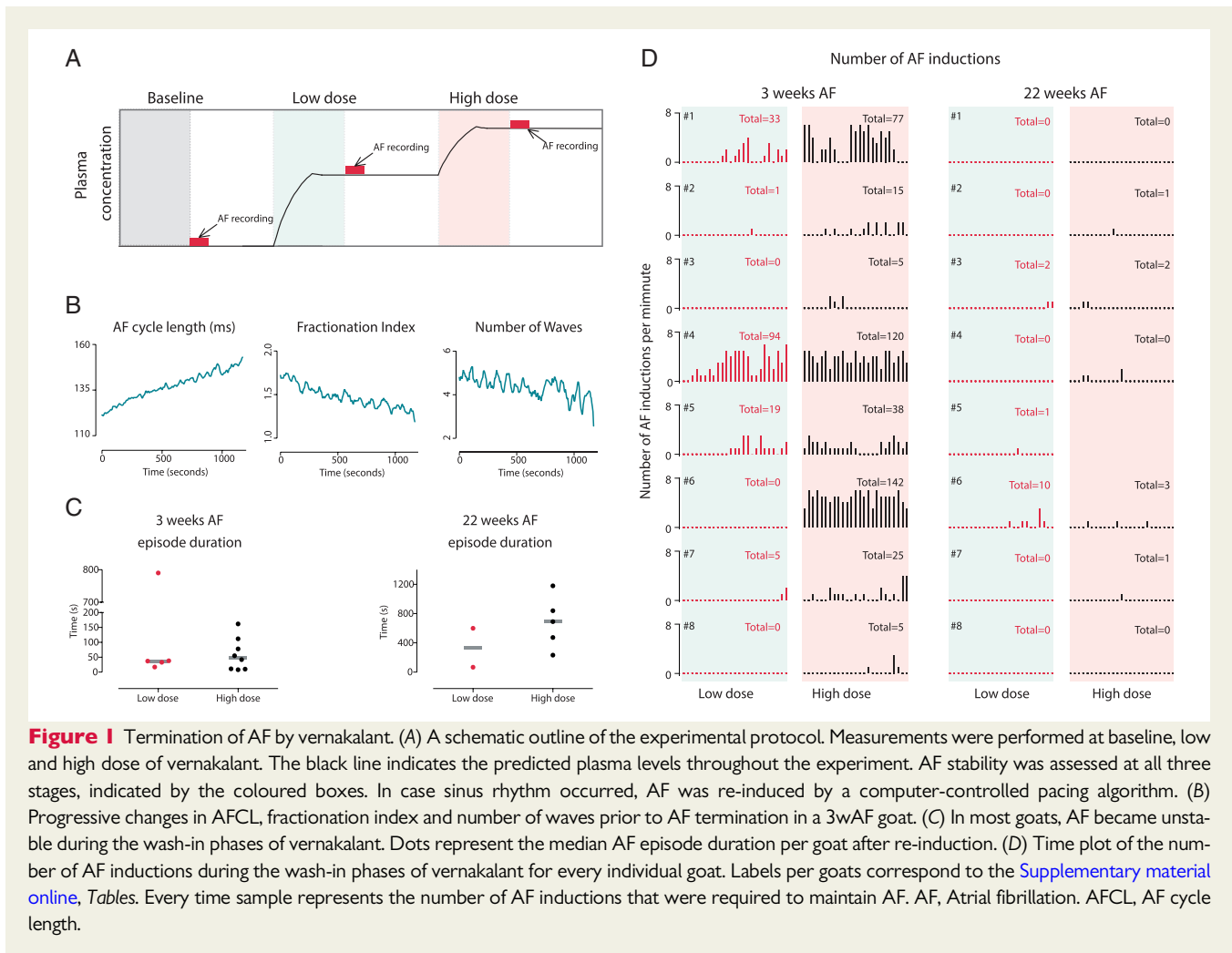
Mapping arrays containing 249 electrodes (2.4 mm inter-electrode distance, 14.3 cm²) were placed on the right atrial (RA) and the LA epicardial free wall, covering about 50% of the epicardial surface. AF was recorded in 60-second files, 1.039 kHz sampling rate and 16 bit AD-conversion. ERP and CV measurements are described in detail in the [Supplementary material online](#). All measurements were performed at baseline and during vernakalant infusion, targeting stable plasma levels of $\sim 3\mu\text{g/ml}$ (low dose, LD) and $\sim 5\mu\text{g/ml}$ (high dose, HD). *Figure 1A* depicts the predicted plasma levels during the experiment and see [Supplementary material online](#) for a detailed description. To analyse AF recordings in the presence of vernakalant at clinically relevant plasma levels,⁷ a 60-second AF recording was selected between 15 and 18 minutes after start of infusion. In the presence of vernakalant AF could become unstable in some goats; in this case the recording with the longest AF duration (≥ 8 second long and > 2 second after the pacing manoeuvre) was selected. A computer-controlled algorithm for AF maintenance continuously monitored the rhythm. In case AF terminated and no pacing measurements were performed, AF was re-initiated after 1 second of sinus rhythm by a 1 second burst of 50 Hz to avoid reverse remodelling.

Analysis of conduction direction

Unipolar atrial electrograms were analysed offline using custom-made analysis software (MATLAB 8.1, The Mathworks, Inc., Natick, MA, USA). Local activation times in the electrograms were identified with a previously validated probabilistic annotation algorithm, see [Supplementary material online](#) for a detailed description. AF cycle length (AFCL), unipolar fractionation index (ratio of far-field to local deflections, FI) and conduction vectors were determined based on local activation times. Conduction vectors were determined by a plane fit through the central activation time and its direct spatial neighbours (min. 3 and max 8). Using the fitted plane, the direction of conduction (V_{θ}) and CV were assessed. Circular statistics was applied to determine circular variance and preferentiality of direction of conduction (Pref) was calculated as Pref = 1 - circular variance. Hence, Pref = 0 means an equally distribution of directions and Pref = 1 means that only 1 direction occurred. The absolute beat-to-beat differences in V_{θ} were determined for each activation at each electrode. Local temporal organization of conduction (> 3 beats with angle difference of < 30 degrees) was considered to be linked activity. All vector-based parameters took the periodic angular continuity into account. Flow lines were computed based on the local vector fields, and are described in detail in the [Supplementary material online](#).

Wave and re-entry analysis

Next, we analysed the propagating fibrillation waves on the epicardial surface. Waves were defined by clusters of activation times that are connected in space and time by an apparent CV of $\geq 20\text{cm/s}$. See the [Supplementary material online](#) for a summary of the wave reconstruction



steps and threshold motivation. Intra-wave preferentiality of conduction direction was determined based on the alignment of conduction vectors within a wave, $\text{intra-wave}_n = 1 - (\text{variance of } V_{\theta} \text{ wave}_n)$. Intra-wave preferentiality was determined in waves covering an area of ≥ 10 electrodes.

Re-entrant activity was identified based on conduction paths. Conduction paths were determined as the shortest contiguous trajectory between a starting and Endpoint of a wave, considering a CV ≥ 20 cm/s. If the trajectory had ≥ 1 self-intersection after $\geq 75\%$ of the mean AFCL it was considered to be a local re-entry. Re-entrant activity was characterized for the number of rotations, lifespan and percentage of time being present in the recording. To explore the effects of local re-entries on AF dynamics, we investigated how much local re-entries disrupted the AFCL and number of waves/cycle. For this analysis, the AFCL and number of waves/cycle were determined in the entire field-of-view of the mapping electrode where the local re-entry occurred. To identify changes in AF dynamics the period where a re-entry occurred was compared to a window of 500 ms before the re-entry initiated and to 500 ms after re-entry terminated.

Statistics

Data were analysed using IBM SPSS statistics version 19.0.0. Data were tested with linear mixed-effects model using a diagonal covariance structure with dose and duration of AF as fixed variables and animal identity as

random variable. In case of the analysis of local re-entries, all re-entries were treated as independent phenomena. To avoid the normality assumption, a Friedman test was used to explore the association between local re-entries and AF dynamics. Conditions with < 4 observations were not included for analysis. Results are reported as mean \pm SD. Statistical significance was taken as $P < 0.05$.

Results

Basic electrophysiological effects of vernakalant

Vernakalant reduced CV and prolonged ERP at different cycle lengths ([Supplementary material online, Tables S1 and S2](#)). The median AF inducibility by S1S2 pacing was not affected. In 3wAF goats, the wavelength during pacing increased in the LA somewhat. In contrast, the wavelength in the right atrium in 22wAF goats did not show a significant prolongation.

AF termination and wave complexity

Towards AF termination, vernakalant induced a progressive prolongation of AFCL, the 5th percentile of the AFCL distribution

(AFCLp5, a surrogate parameter of refractory period during AF), and a reduction in irregularity of AFCL ((p50-p5)/p50) and of FI (Supplementary material online Tables S1 and S2). Despite, large fluctuations in the number of waves/cycle a strong decrease in number of waves was observed just before cardioversion (Figure 1B).

Pilot data evaluation confirmed that the goats were in persistent AF (>24 h) on the day before the experiment. Persistent AF was confirmed for 15 goats during a 30-minute stabilization period after surgery but one animal in the 3wAF group cardioverted after induction of anaesthesia. Thirteen out of the remaining 15 goats cardioverted after vernakalant administration. The other two goats belonged to the 22wAF group. In the animals where AF became unstable, AF episode duration had a trend towards shorter durations in the 3wAF goats compared to 22wAF goats (Figure 1C) and required many more AF inductions to maintain AF (Figure 1C). AF segment lengths used for AF analysis were 60 seconds at baseline, 55 ± 12 seconds at LD and 43 ± 20 seconds at HD.

An example of the complexity of propagation patterns can be appreciated in Figure 2. Overall, 3wAF goats had a lower total number (RA + LA) of waves/cycle compared to 22wAF, 15 ± 2.1 vs. 20 ± 6.0 , respectively. Vernakalant reduced both waves and breakthroughs at a comparable extent in the RA and LA irrespective of the animal group (Figure 3). The threshold for conduction block affects the quantification of the number of breakthroughs. A threshold sensitivity analysis on the number of breakthroughs revealed that the reduction in number of breakthroughs waves is independent on the applied threshold (Supplementary material online, Figure S1).

Local re-entries

In the 3wAF goats at baseline, a total of 1,667 local re-entries in 66,318 waves, were identified in 429 seconds. In 22wAF goats at baseline, a total of 1,438 local re-entries in 84,033 waves were identified in 480 seconds (Supplementary material online, Table S3). At baseline, only transient re-entries appeared within the field-of-view with an average number of rotations of 1.27 ± 0.08 (3wAF) and 1.19 ± 0.09 (22wAF). Local re-entries generally occupied a small portion of surface of the mapping area, exhibited the by multiple waves being present during a re-entry (Supplementary material online, Table S3). Vernakalant strongly, and dose-dependently, reduced the occurrence of local re-entries (Figure 4A). In two cases, vernakalant led to a highly stable and reoccurring re-entrant circuit that almost filled the mapped area. In most other cases, the maximum number of rotations, re-entry lifespan or the re-entry lifespan corrected for cycle length exhibited a trend towards somewhat less stable re-entrant circuits but these data did not reach significance in the current sample size (Supplementary material online, Table S3).

Considering that local re-entries did not occupy the entire mapping area, the effects of local re-entries on its immediate surrounding was investigated for AFCL and number of waves. In Figure 4B, and zoom ins in Figure 4C, depicts the temporal changes in AFCL and waves/cycle when a re-entry appears and terminates. This particular example illustrates a baseline recording in a goat of the 22wAF group. In these graphs, no consistent change in AFCL or waves/cycle is apparent when a local re-entry is initiated or terminated. To quantify whether the presence of local re-entries goes along with changes in AFCL or waves/cycle, we determined the change in AFCL or waves/cycle before, during, and after an occurrence. Specifically, we

compared a 500 ms window directly before initiation (indicated by black lines in Figure 4B) of the local re-entry and a 500 ms window directly after the end of the local re-entry (indicated by red lines in Figure 4B). The averages for Δ AFCL or Δ waves/cycle, during and after the re-entry, were centred on zero (Supplementary material online, Table S3). Vernakalant did not affect this behaviour, indicating that the presence of a local re-entry did not associate with detectable changes of AFCL or number of waves. We further explored the histogram morphology of the Δ AFCL and Δ wave/cycle. To test whether re-entries went along with changes in AF dynamics, we compared the entire population of re-entries to a sample of waves that was randomly selected outside the re-entry occurrences. A two-way Kolmogorov–Smirnov test indicated that both populations (random sampling vs. local re-entries) indicated similar distributions in AFCL and waves/cycle.

Conduction directions and wavefront turning

When wave maps (Figure 2) of baseline recordings are inspected in more detail, it can be appreciated that wave trajectories conducted in all directions and often underwent sharp pivots. After administration of vernakalant wave trajectories became more uniformly aligned. To quantify the stability of conduction directions in the temporal domain we analysed the circular distribution of conduction vectors at each individual electrode, an example is presented in Figure 5A. An almost uniform distribution of conduction directions can be appreciated at baseline but vernakalant led to a strong redistribution to a strong preferential direction. Vernakalant increased preferentiality of conduction direction in almost all goats (Figure 5B). Beat-to-beat changes in direction were reduced (Figure 5C) and linking of fibrillation waves became more prevalent (Figure 5D). The effects were similar for both atria and in both groups of animals.

Next, we analysed turning at the larger spatial domain. Figure 6A illustrates flow lines at baseline and after HD vernakalant infusion. It becomes clear that flow line curvature strongly reduced. Reduced wavefront turning is further illustrated by an increased alignment of conduction vectors within waves, Figure 6B.

Discussion

In this study, we found that vernakalant reduces complex propagation patterns during AF and inhibits wavefront turning considerably. In addition, vernakalant strongly reduced AF stability, even after 3 or 22 weeks of AF. The reduction in pattern complexity was reflected by fewer waves, breakthroughs, and local re-entries and went along with AFCL prolongation and reduction of wavefront turning. We conclude that AF termination induced by vernakalant is associated with: 1 AFCL prolongation, 2 inhibition of wave turning, 3 reduction of the number of waves, 4 inhibition of local re-entry formation, and 5 inhibition of breakthrough waves. Wavelength did not substantially increase, except for a minor increase the LA at 3 weeks of AF. This finding is consistent with earlier findings of our group suggesting that the widening of the excitable gap and reduction of wavefront–wave-tail interactions is a pivotal mechanism in termination of AF.⁸

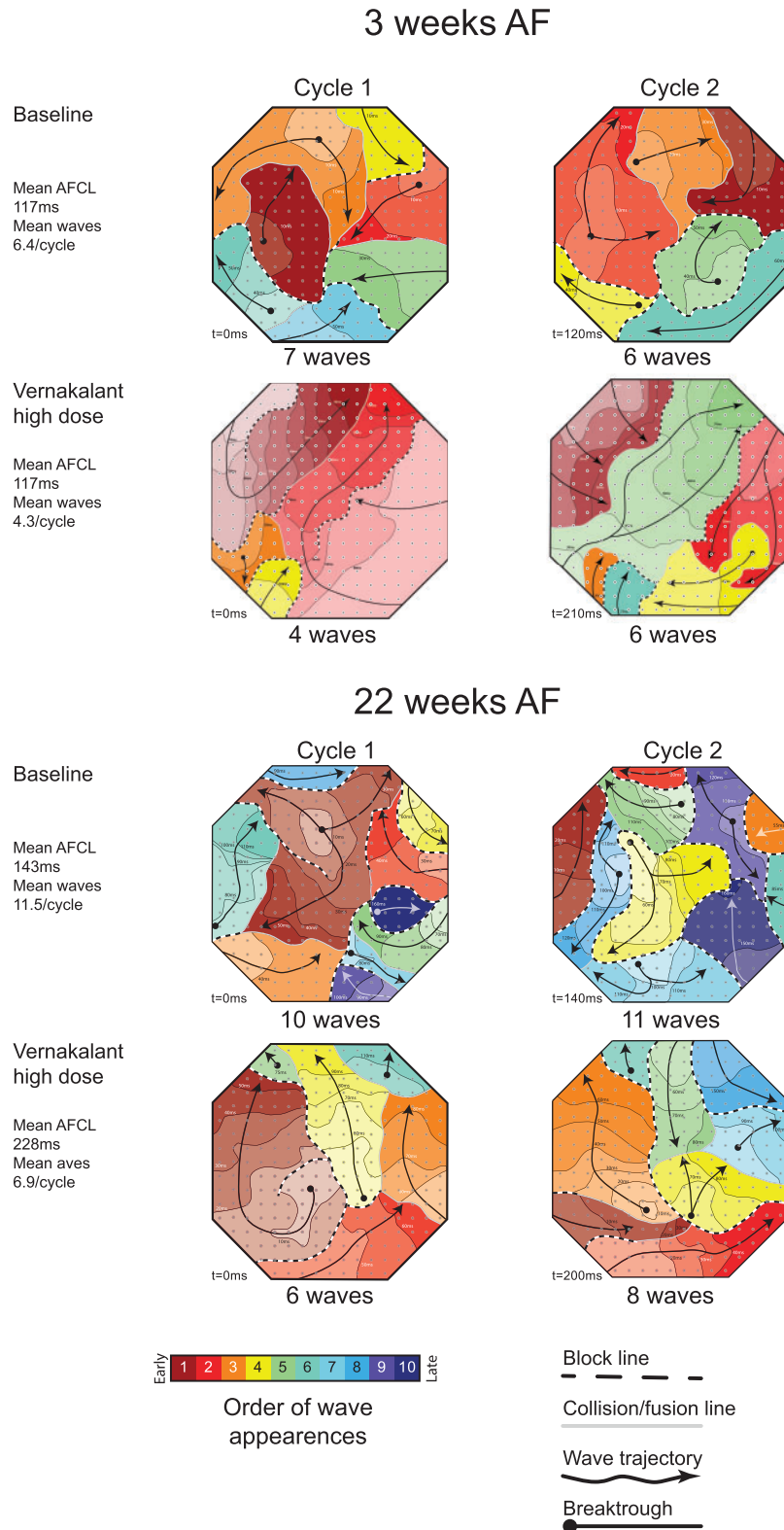


Figure 2 Effect of vernakalant on fibrillation patterns. Wave maps depict the territory of individual fibrillation waves within the mapped area during 1 AF cycle. Two consecutive cycles at baseline and after high-dose administration of vernakalant are presented. The colour code describes the order of appearance within 1 AF cycle. Light grey dots represent the positions of the recording electrodes. Both groups of goats had highly variable beat-to-beat changes in conduction directions. After vernakalant administration, the number of waves decreased and conduction directions were more organized and repetitive. AF, Atrial fibrillation.

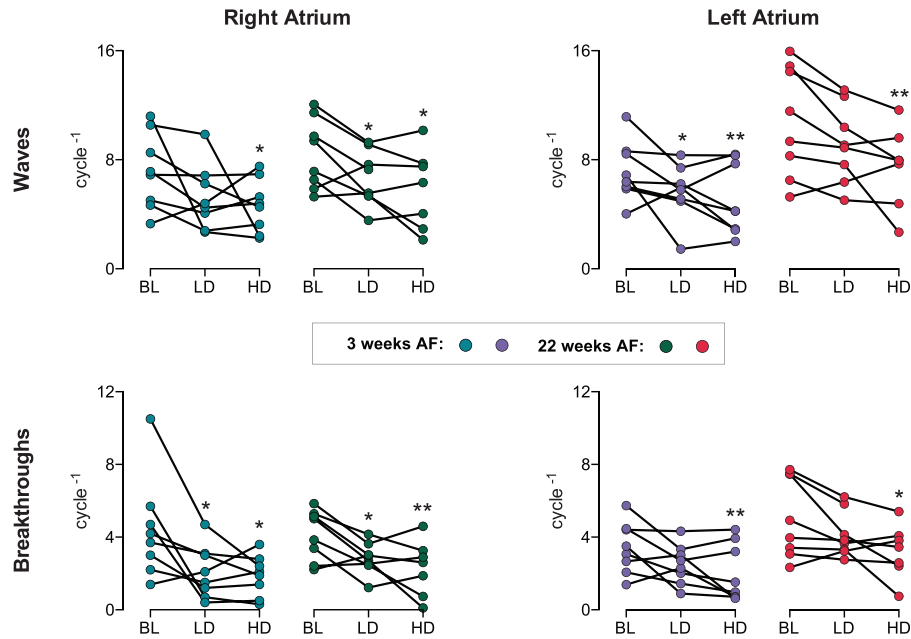


Figure 3 The number of waves and breakthroughs per AF cycle recorded on the RA and LA epicardial surface. Vernakalant decreased the number of waves and breakthroughs. LD or HD vs. baseline * $P < 0.05$, ** $P < 0.01$. AF, Atrial fibrillation; LA, left atrium.

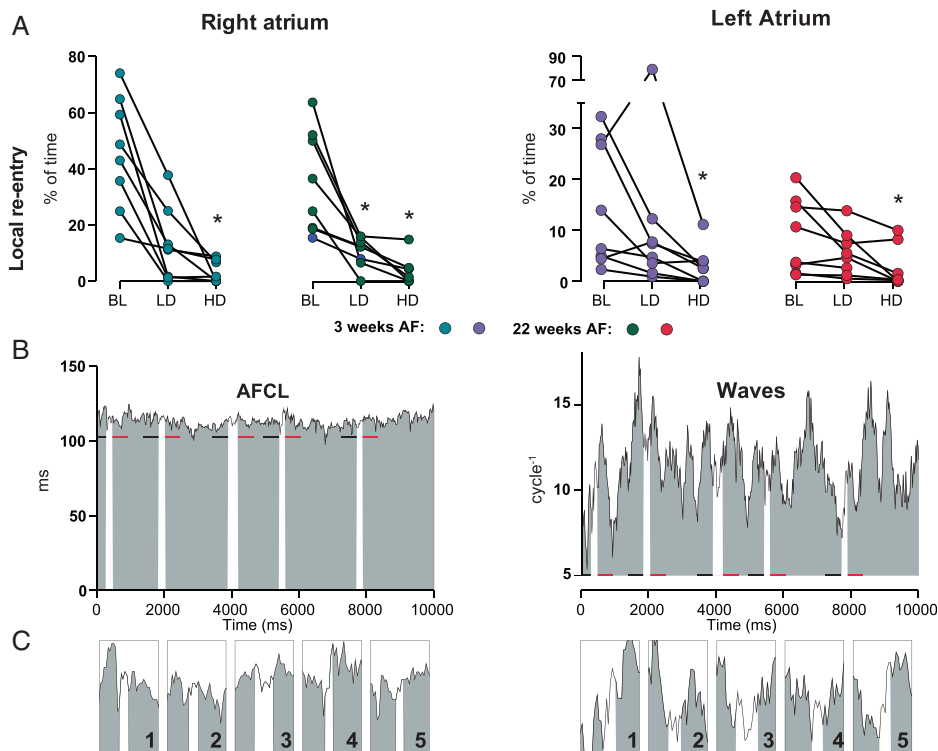
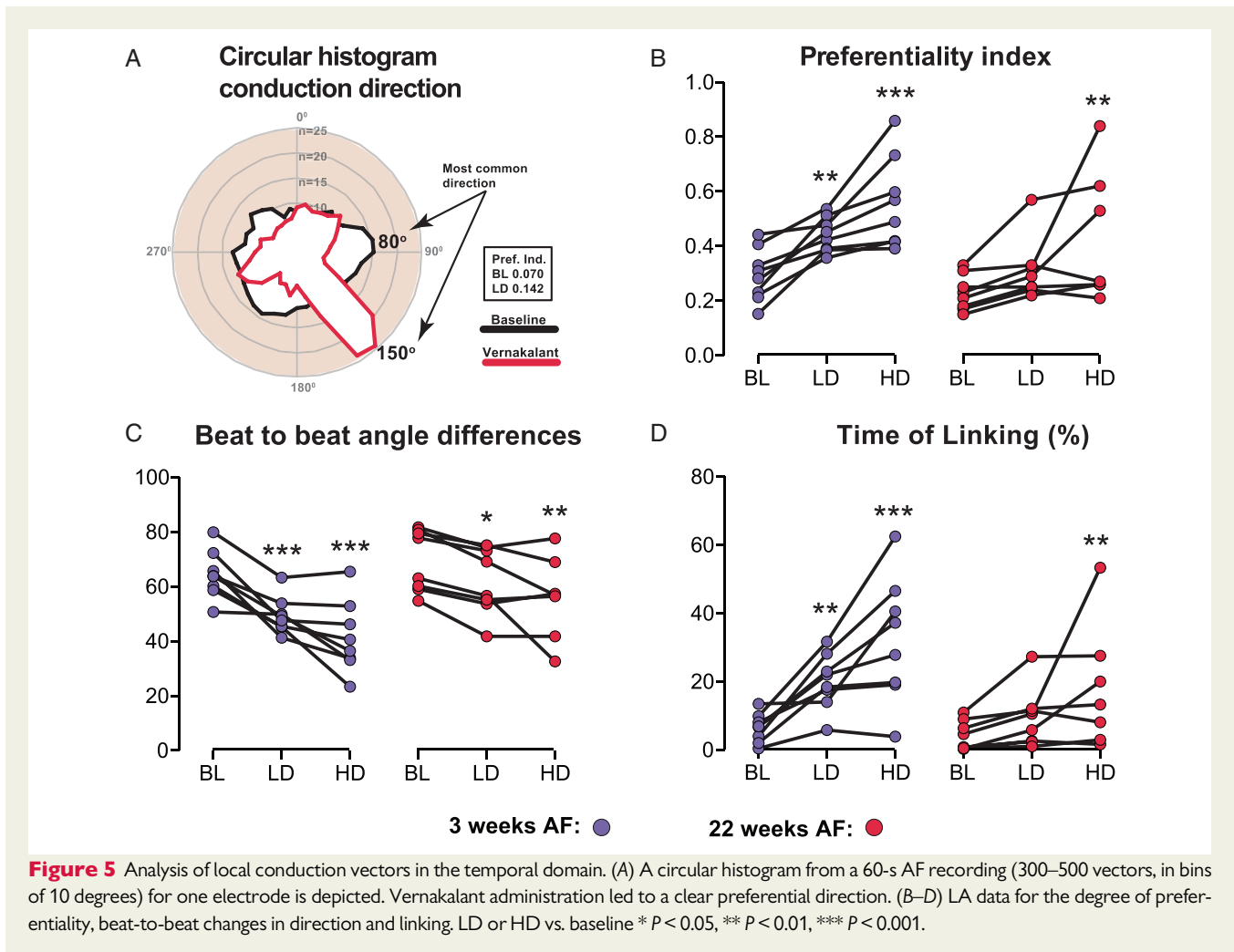


Figure 4 The effect of vernakalant on the presence of local re-entries. (A) Vernakalant strongly decreased the prevalence of local re-entries in both atria and groups. * $P < 0.05$. (B) The dynamics of AFCL and number of waves in a 10 s window of a 22wAF goat at baseline. The white column indicates the period when a re-entry was present. Black lines, before the white column represent the 500 ms window before re-entry. The red lines represent the 500 ms window after the re-entry. (C) A zoom in of the five different occurrences of re-entries. From these graphs it can be appreciated that re-entries did not consistent associate with changes in AFCL or number of waves.



Complexity of propagation patterns

In patients with longstanding persistent AF, more complex propagation patterns are observed than in patients with acutely induced AF.^{11,12} Similarly, atria in the goat model of persistent AF displayed more complex propagation patterns after longer AF duration, 6 months vs. 3 weeks of AF.³ The increase of complex patterns was associated with increased levels of endomyocardial fibrosis at the thin epicardial layer contributing to (endo-) epicardial dyssynchronous activity.² Also in this study, we found an increase of AF complexity with increasing duration of AF. Moreover, shortening of the action potential due to electrical remodelling increases the atrial activation rate.¹ Already experimental work by Moe *et al.* showed that high-activation frequencies, as present during AF, lead to complex patterns. In this study, a high frequency aconitine-induced focus could induce and drive AF in canine atria as it resulted in rapid activation in the vicinity of the focus but to 1:1 propagation failure, conduction block, and wave splitting further away from the focus.¹³ In line with this observation, it was later shown that high frequency stimulation of pectinate bundles leads to fibrillatory conduction with generation of new wavefronts.¹⁴ Indeed, when the cycle and wavelength is reduced, the more circuits that can be formed and AF becomes sustained. For instance,

experimental work by Scheussler *et al.* showed that acetylcholine-induced ERP shortening increased the number of wavefronts and small re-entrant circuits, ultimately leading to stable AF.¹⁵ In the multiple wavelet model of sustained AF, it was predicted that prolongation of the wavelength will reduce of the number of simultaneous waves and AF stability.¹³ ERP prolongation by Class I and III antiarrhythmic drugs does indeed reduce the number of fibrillation wavefronts, at least briefly before termination of AF⁵ but the mechanism that decreases the number of waves in the presence of antiarrhythmic drugs may not be related to wavelength prolongation. For example, the action of Class I drugs leads to ERP prolongation with a concurrent slowing of CV leaving the wavelength unaffected. Yet, such compounds can effectively terminate AF and this effect was suggested to be related to a widening of the excitable gap.⁸

In this study, vernakalant reduced complexity of conduction patterns without a substantial wavelength prolongation but AFCL prolonged profoundly suggesting that also in the presence of vernakalant the excitable gap during AF is prolonged. In line with the previous study by Wijffels *et al.* widening of the excitable gap resulting from inhibition of wavefront turning may have resulted in fewer wave front wave tail interactions, less generation of new wavefronts and thus

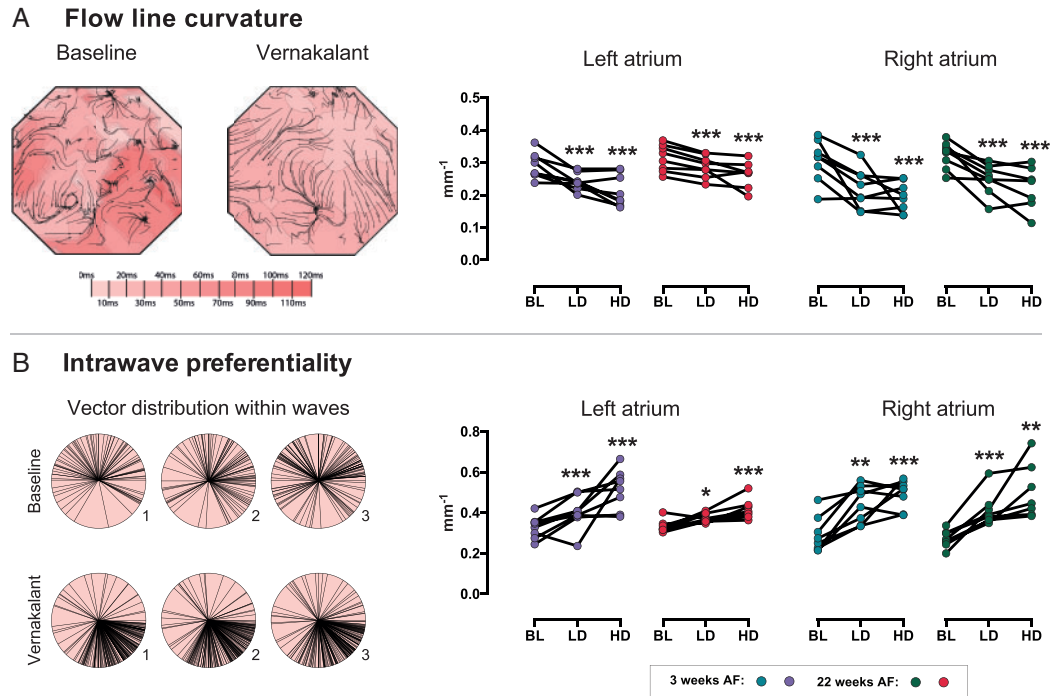


Figure 6 (A) Curvature analysis of flow lines for the quantification of wavefront turning. The two left panels depict an example (LA 3wAF) of flow lines field at a given time point during baseline AF and after high dose vernakalant administration. A clear reduction in wave front turning can be appreciated. Flow line curvature was reduced in all conditions. (B) The two left panels depict the distribution of conduction vectors of 3 waves in the LA in a 3wAF goat, at baseline and high dose vernakalant. Conduction vectors are more aligned to one direction at high dose vernakalant compared to baseline. Vernakalant led to a higher intrawave preferentiality. Individual averages are presented. LD or HD vs. baseline * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

termination of AF.^{8,9} Alternatively, destabilization of local re-entry may have contributed to destabilisation of the arrhythmia.¹⁶ Both concepts will be discussed in the two following paragraphs.

Wavefront turning

Action potential propagation within cardiac tissue is dependent on a sufficient source current generated by the wavefront to depolarize the unexcited tissue downstream (sink) up to the threshold for sodium channel activation.¹⁷ This relation between source and sink is depending on the tissue architecture, degree of coupling between cells, and strength of the depolarizing current. The potent inhibition of sodium current by vernakalant,^{6,7} as also suggested by the results of this study, is expected to affect the source/sink balance and to decrease the safety factor for wavefront propagation.¹⁰ Numerical and *in vitro* investigations have predicted reduced wavefront curvature and pivoting points to become wider when excitability is reduced.¹⁰ Indeed, at the site of a pivot point, the CV is reduced most strongly when excitability is lowered, leading to wider turns.⁹ This implies that during dyssynchronous conduction and AF, excitable atrial tissue oriented perpendicular to the wavefront is particularly prone to conduction block under conditions of Na⁺-channel blockade. Indeed, we observed the instalment of a preferred direction of conduction, a decrease of turning wavefronts (e.g. reduced flow line curvature) and conduction paths were inclined to repeatedly follow the same

trajectory. These data illustrate that wavefronts were potently inhibited from turning.

Moreover, inhibition of turning may have contributed to the observed reduction of breakthrough patterns. In previous work, we found that transmural conduction was the predominant mechanism for breakthroughs in the goat.¹⁸ A sharp turn is likely to occur when the epicardial surface is activated by a wavefront that conducted along a small transmural bundle. Breakthroughs may therefore be prone to conduction block as wavefront turning occurs when a wave emerges at the atrial surface. Preferential block of breakthroughs in the presence of Na⁺-channel block may also be due to the relatively large mass of the epicardial surface as compared to the small source of the transmural bundle. The block of breakthroughs may in turn contribute to the widening of the excitable gap as demonstrated in our earlier work in computational and animal models.^{8,19}

In summary, this study shows that a reduction in complexity of propagation patterns and breakthroughs is associated with an inhibition of turning.

Local re-entries

Local re-entries are obviously dependent on turning. Indeed, congruent to the reduction of turning the number of local re-entries was lower in the presence of vernakalant. 'Rotors' have been proposed to be the perpetuators of AF, being a high frequency source that

results in wave break and complex conduction.⁴ Antiarrhythmic action of (state dependent) sodium channel inhibition in the context of rotor driven AF has been investigated by Nattel *et al.*¹⁶ The researchers concluded that sodium current inhibition led to AF termination by an increase in the size of the core of the rotor (leading to AFCL prolongation), less daughter wavelet formation, and increased rotor drift leading to rotor termination once it hit a boundary. A number of our observations are congruent to this study, such as AFCL prolongation, fewer waves, and reduced presence of local re-entries. However, our data cannot be taken as an argument that AF is driven by local re-entries. First, we could not determine the drift of the local re-entries because they were of very short lifespan, generally less than 2 rotations. Despite the short lifespan, we observed a trend towards a small reduction of the number of rotations, ranging from 0.1 to 0.5 rotations. The limited effect on lifespan suggests that the reduction of local re-entries was driven by the inhibition of re-entry formation, as can be anticipated by reduced wavefront turning. Second, we investigated whether in the presence of local re-entries would modulate the number of waves but we could not identify a relation between the occurrence of local re-entries and AF dynamics, such as AFCL or number of waves. The action of local re-entries as perpetuators of AF is not supported by our observations, but can also not be excluded. It should be noted, however, that in many recently published studies 'rotors' have been identified as phase singularities in filtered AF electrograms which strongly over estimates wavefront turning.²⁰ In this study, analysis methods were only based on local activation times to avoid the low specificity of phase singularities to detect wavefront turning.

Limitations

We used large electrode arrays on both atria but our approach did not allow the electrical activity to be captured in the entire atria. There might have been an AF driver outside the field-of-view that was not captured because the study was not designed to track a stationary source outside the field-of-view. The inter-electrode distance of the mapping arrays was limited to 2.4 mm. A re-entry with a smaller rotating wavefront than the spatial resolution could have remained undetected.

This study was conducted in the goat model of AF because this experimental approach allowed well-controlled conditions. Young and healthy animals were included. Atrial remodelling was therefore considered to be driven by the presence of AF. It is reasonable to assume that the degree of atrial remodelling in the average patient, of older age and having comorbidities, is more advanced which leads to an earlier loss of AF termination efficacy compared to this model of persistent AF. Further validation in human subjects would be required to further assess the proposed mechanisms of action occur in man.

Conclusions

During untreated AF, we observed complex propagation patterns with wavefronts that propagated in varying directions. Administration of vernakalant strongly prolonged ERP and AFCL, reduced the number of fibrillation waves and breakthroughs as well as local re-entries. Vernakalant strongly reduced wave turning and increased preferential of conduction direction.

As vernakalant did not or at best marginally increase wavelength it is likely that the inhibition of wavefront turning and breakthroughs resulted in fewer re-entries, widening of the excitable gap, reduction of wavefront–wavellet interactions, less generation of new wavefronts, and thus lowering of the complexity of propagation patterns and finally in the termination of AF.

Supplementary material

Supplementary material is available at *Europace* online.

Acknowledgements

This study was performed within the framework of CTMM, the Centre for Translational Molecular Medicine (www.ctmm.nl), project COHFAR (grant 01C-203), and supported by the Netherlands Heart Foundation (CVON2014-09, RACE V Reappraisal of Atrial Fibrillation: Interaction between hyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF), the European Union (CATCH ME: Characterizing Atrial fibrillation by Translating its Causes into Health Modifiers in the Elderly, No. 633196, the ITN Network AFibTrainNet, No. 675351 and the ERACoSysMED H2020 ERA-NET Cofund project Systems medicine for diagnosis and stratification of atrial fibrillation), and Merck.

Conflict of interest: M.S.D. supplied vernakalant for this study. U.S. is a cofounder and shareholder of Your Rhythmics BV and received funding from Roche. Diagnostics and EP Solutions. PK received consultancy fees from Acutus Medical. All remaining authors have declared no conflicts of interest.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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