Atrial Fibrillation Risk Assessment after Embolic Stroke of Undetermined Source

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Objective: Approximately 20% of strokes are embolic strokes of undetermined source (ESUS). Undetected atrial fibrillation (AF) remains an important cause. Yet, oral anticoagulation in unselected ESUS patients failed in secondary stroke prevention. Guidance on effective AF detection is lacking. Here, we introduce a novel, non-invasive AF risk assessment after ESUS.

Methods: Catch-Up ESUS is an investigator-initiated, observational cohort study conducted between 2018 and 2019 at the Munich University Hospital. Besides clinical characteristics, patients received ≥72 h digital electrocardiogram recordings to generate the rhythm irregularity burden. Uni- and multivariable regression models predicted the primary endpoint of incident AF, ascertained by standardized follow-up including implantable cardiac monitors. Predictors included the novel rhythm irregularity burden constructed from digital electrocardiogram recordings. We independently validated our model in ESUS patients from the University Hospital Tübingen, Germany.

Results: A total of 297 ESUS patients were followed for 15.6 ± 7.6 months. Incident AF (46 patients, 15.4%) occurred after a median of 105 days (25th to 75th percentile 31–33 days). Secondary outcomes were recurrent stroke in 7.7% and death in 6.1%. Multivariable-adjusted analyses identified the rhythm irregularity burden as the strongest AF-predictor (hazard ratio 3.12, 95% confidence interval 1.62–5.80, $p < 0001$) while accounting for the known risk factors age, CHA_2DS_2-VASc-Score, and NT-proBNP. Independent validation confirmed the rhythm irregularity burden as the most significant AF-predictor (hazard ratio 2.20, 95% confidence interval 1.45–3.33, $p < 0001$).

Interpretation: The novel, non-invasive, electrocardiogram-based rhythm irregularity burden may help adjudicating AF risk after ESUS, and subsequently guide AF-detection after ESUS. Clinical trials need to clarify if high-AF risk patients benefit from tailored secondary stroke prevention.

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Introduction

Embolic stroke of undetermined source (ESUS) accounts for approximately 17% (9–25%) of all ischemic strokes worldwide, affecting approximately 1.3 million people per year.[1, 2] After ESUS, patients are facing an annual recurrence rate of 4–5% under acetylsalicylic acid therapy.[3, 4] However, a substantial proportion of ESUS patients suffer from silent atrial fibrillation (AF) as the presumed cause of stroke.[5–8] Although AF is associated with a fivefold increase in stroke risk, this risk can be dramatically reduced by the use of oral anticoagulation (OAC).[9, 10] However, an individual risk assessment of silent, paroxysmal AF as the driving condition underlying ESUS has not been introduced into clinical practice.

Prior studies identified risk factors for AF after ESUS. A previously published high-risk cohort, characterized based on clinical, electrocardiographic, and echocardiographic parameters, reported the detection of AF by continuous rhythm monitoring with implantable cardiac monitors (ICM) in up to 33.3% within 1 year.[6] Another analysis of three registries presented a 7-point score to stratify patients with ESUS into risk groups for underlying AF based on clinical, electrocardiographic, echocardiographic, and both brain and vessel imaging.[11] These previous risk assessments also included the determination of supraventricular extrasystoles and atrial ectopy as relevant predictors of ESUS.[12–14] Randomized clinical trials are currently underway to test the effect of OAC in ESUS patients at adjudicated high risk of underlying AF.[15, 16]

In our prospective Catch-Up-ESUS study, we therefore aimed to examine and independently validate a simple, electrocardiogram (ECG)-based, non-invasive risk stratification approach for the occurrence of AF in ESUS patients that could easily be applied in clinical practice.

Methods

Derivation Cohort
The Catch-Up-ESUS cohort is an investigator-initiated, prospective, single-center, observational trial that enrolled ESUS patients from January 2018 through December 2019. Details on aims and methodology have been reported previously.[17]

Inclusion and Exclusion Criteria
Consecutive ESUS patients aged ≥18 years who were admitted to the neurological stroke unit of the University Hospital of the Ludwig-Maximilians-Universität Munich and who provided written informed consent were enrolled. ESUS was diagnosed based on current diagnostic guidelines as a non-lacunar stroke without evidence of extra- and intracranial arterial stenoses ≥50%, and no
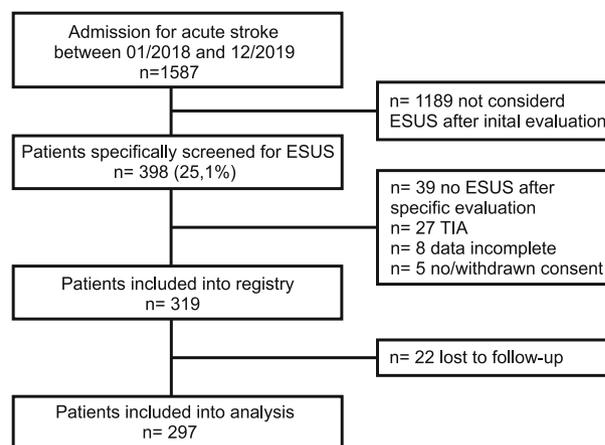
Figure 1: Illustration of the study flow. ESUS = embolic stroke of undetermined source.

overt major indicators for cardiac embolism or other specific causes of stroke.[18] We excluded patients who experienced transient ischemic attacks (Fig 1).

Diagnostic Evaluation of ESUS Patients
All patients received a standardized neurological and cardiac evaluation. Diagnostic measures included a detailed clinical assessment, brain imaging by computed tomography or magnetic resonance imaging, Doppler sonography, and color-coded duplex sonography of extracranial and intracranial vessels, echocardiographic assessment, electrocardiographic monitoring, and blood laboratory tests including N-terminal pro B-type natriuretic peptide (NT-proBNP, normal value <125 pg/ml according to current guidelines[19]). Further details have been reported previously.[17]

Definition of the Rhythm Irregularity Burden
To assess a simple, non-invasive, ECG-based risk stratification, all stroke patients of the derivation cohort received automated, digital ECG monitoring for ≥72 hours using the Draeger infinity delta monitoring system. As part of clinical routine, ECGs were analyzed using the proprietary software application SRAclinic (Apoplex Medical Technologies, Pirmasens, Germany), which relies on a principle component analysis of R-R-intervals, analysis of R-R intervals, and the number of atrial premature contractions.[20, 21] Recordings were interrupted after 24 hours or due to clinical need. Each recording period resulted in an algorithm-based adjudication of AF risk ranging from 0 (lowest risk) to 2 (highest risk). All reports were validated by experienced cardiologists, and patients with documented AF were excluded from further analysis. All remaining reporting results were then averaged, weighting for the respective hours of monitoring, to generate the rhythm irregularity burden ranging from 0 to 2 (Fig 2),
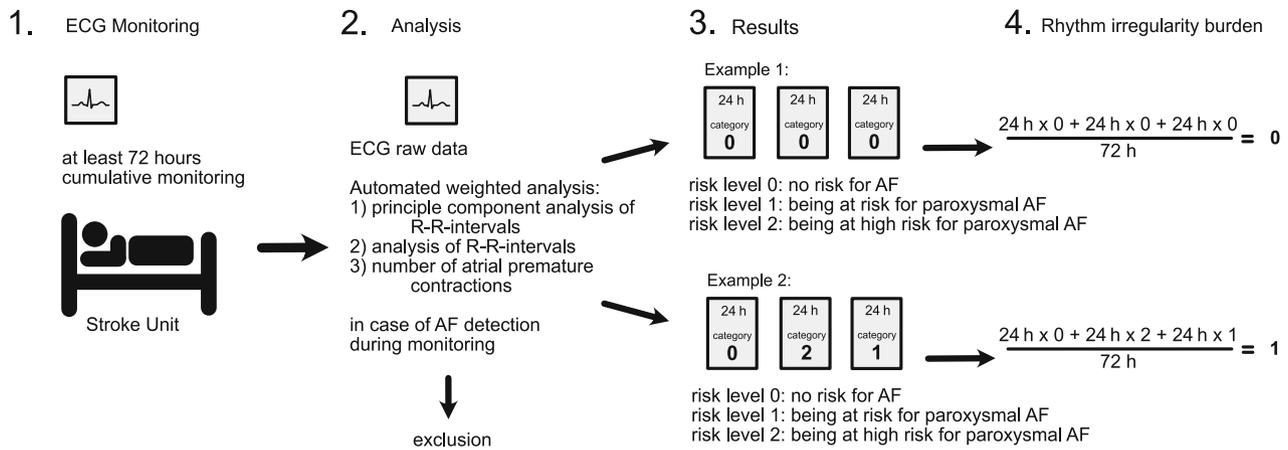
Figure 2: Derivation of the rhythm irregularity burden. Electrocardiogram (ECG) recording lasted ≥72 h during the initial hospitalization for embolic stroke of undetermined source (ESUS) with automated ECG analysis using the proprietary SRAclinic algorithm. This algorithm assigned a risk category of ECG irregularity to each monitoring period. The rhythm irregularity burden by averaging all reporting periods, weighting for the duration of each reporting period. AF = atrial fibrillation.

Follow-Up

We aimed for implantation of an ICM in all enrolled patients. However, ICM implantation was waived for the following reasons: the patient declined ICM implantation; the patient was deemed unable to participate in remote monitoring; and the patient was deemed to be experiencing poor neurological outcome following ESUS. In the case of missing ICM information, we performed 24-hour Holter monitoring at each follow-up. Standardized remote or on-site follow-up was scheduled at 3, 6, and 12 months after enrollment, and the full ICM interrogation or the Holter report was made available to the study team. The adjudication of outcomes was recorded continuously until the end of follow-up. The follow-up included an assessment of neurological status by the modified Rankin Scale. Good neurological outcome was defined as modified Rankin Scale 0–2. Occurrence of AF was diagnosed by an experienced cardiologist using remote or on-site ICM interrogation, ECG, and Holter ECG analysis. In the case of incident AF, medical secondary prevention of ESUS was changed from acetylsalicylic acid to OAC, unless contraindications were identified. Recurrent strokes were diagnosed clinically or by cerebral imaging at follow-up.

Definition of Outcomes

The primary endpoint was the detection of at least 30 seconds of AF. Secondary endpoints were recurrent stroke or death, or a combination of these. For each outcome, time to event was determined from enrolment. In the case of uncertainty, we adjudicated the time of the event to have occurred at the midpoint between the last known status without the event and the indexing follow-up. We administratively terminated follow-up at 6 months after the last patient in, on July 31, 2020.

Validation Cohort

For external validation, we used an independent, well-characterized cohort of prospectively enrolled ESUS patients at the Department of Neurology of the Tübingen University Hospital, Germany, between September 2015 and July 2017 (NCT04352790).[6] Participants systematically received an ICM for AF detection. The rhythm irregularity burden was determined from standard Holter ECGs using the SRAclinic software application.

Statistical Analysis

Discrete data are presented as absolute and relative frequencies, and were compared using the Fisher's exact tests. Continuous variables are presented as the mean ± standard deviation or median [25th to 75th percentile] and were compared by Student t-tests or Wilcoxon tests, as appropriate. We present data for the unstratified cohort and stratified by age ≤60 years or >60 years, by sex, by occurrence of AF during follow-up, by presence of an ICM, and by presentation with a rhythm irregularity burden of 0 or >0.

To predict the primary outcome of AF during follow-up, we calculated time to event analyses fitting univariable Cox regression models to identify suitable predictors for incident AF. All predictors significant by univariable adjusted analysis were tested in a multivariable adjusted Cox regression model using AF as the outcome. Model fit was adjudicated by plotting Martingale residuals. The multivariable adjusted model derived in our derivation cohort was subsequently replicated in the validation cohort. A meta-analysis of the primary outcome in both cohorts was calculated and visualized by a forest plot. For all outcomes, we drew cumulative incidence plots; for the outcome of AF, we stratified the plots by age

and sex, respectively, and calculated log-rank tests. We assessed the predictive value of our new rhythm irregularity burden by means of a receiver operating characteristics analysis. Given that quantifiable echocardiographic data was not available in the full cohort, we conducted a sensitivity analysis including only participants that would have had quantifiable echocardiographic data.

We performed analyses using Stata v12.0 (StataCorp, College Station, TX, USA) and R-Studio (Integrated Development for R, RStudio, Boston, MA). We considered a two-sided $p < 0.05$ significant.

Ethical Considerations
The study was approved by the Ethics Committee at the Ludwig-Maximilians-Universität, Munich, Germany (project number 17-685). The validation cohort was similarly approved by the ethics committee at the Medical Faculty of Eberhard-Karls-University and University Hospital Tübingen (project number 522/2012BO2). Both studies were registered at www.clinicaltrials.gov, all patients provided written informed consent to study participation.

Results
From 1,587 patients with acute stroke screened between January 2018 and December 2019, the final derivation cohort comprised 297 (19%) patients with ESUS (Fig 1).

Derivation Cohort Characteristics
Patients were aged 67.9 ± 12.7 years on average and predominantly men (63.6%). The majority was functionally independent before the stroke (median modified Rankin Scale 0 [25th–75th, 0–1]) and most patients had mild strokes (median National Institutes of Health Stroke Scale at admission 2 [1–4]). Strokes were diagnosed by computed tomography ($n = 277$; 93.3%) or magnetic resonance imaging ($n = 235$; 79.1%), with $n = 215$ patients (72.4%) receiving both imaging modalities. Table 1 summarizes baseline characteristics of the overall cohort. AF patients were older (73.3 ± 9.2 vs 66.9 ± 13.1 years, $p = 0.002$) and had higher prevalence of hypercholesterolemia (47.8% vs 31.5%, $p = 0.042$). AF patients also had a higher median CHA_2DS_2-VASc score (5 [4–6] vs 4 [3–6], $p = 0.003$) and a higher rhythm irregularity burden (0.5 [0.0;1.0] vs, 0.0 [0.0–0.4], $p < 0.001$). By laboratory testing, their median NT-proBNP levels were higher (321 [126–731] vs. 172 [80–400] pg/ml, $p = 0.004$).

Primary and Secondary Outcomes
During a mean follow-up duration of 15.6 ± 7.6 months, the primary outcome of AF occurred in 46 patients (15.4%; Fig 3A). In those equipped with an ICM, the AF detection rate was 18.4% ($n = 33$), whereas it was 11.0%

($n = 13$) in those with intermittent Holter monitoring. The median time to AF detection was 105 (31–338) days. As secondary outcome recurrent stroke occurred in 7.7% ($n = 23$) and death in 6.1% ($n = 18$). The combined outcome of all three distinct events was documented in 77 patients (25.9%; Fig 3A). AF was detected at similar rates in men and women (Fig 3B), but more frequently in patients aged >60 years (Fig 3C).

Prediction of AF
Age, NT-proBNP, the CHA_2DS_2-VASc score, and the rhythm irregularity burden were significant univariate predictors of AF (Table 2). The rhythm irregularity burden was the only significant predictor of AF after multivariable adjustment (hazard ratio [HR] 3.12, 95% confidence interval [CI] 1.62–5.80, $p < 0.001$; Table 2). This result remained unchanged in our sensitivity analysis excluding participants without quantifiable echocardiographic data (Table S1).

We stratified patients at a rhythm irregularity burden of 0 or >0. Patients with a burden >0 were older (75.4 ± 8.8 vs 63.1 ± 12.6 years, $p < 0.001$), were more likely hypertensive (80.9% vs 64.8%, $p = 0.004$), had a higher National Institutes of Health Stroke Scale at admission (3 [1–5] vs 2 [1–4], $p = 0.014$), presented with a higher CHA_2DS_2-VASc score (6 [5–6] vs 4 [3–5], $p < 0.001$), and had a higher NT-proBNP (328.5 [167–684.5] vs 121 [59–302] pg/ml, $p < 0.001$). The risk of developing AF during follow-up was significantly higher in those with a rhythm irregularity burden >0 compared with those with a burden of 0 (25.5% vs 8.6%, $p < 0.001$; Fig 4).

Independent Validation
For independent validation in an external cohort from the University of Tübingen, we derived the rhythm irregularity burden in patients with ischemic stroke of undetermined etiology,[22] prospectively recruited between September 2015 and July 2017. Details have been reported previously.[6] Overall, 118 patients were included, of whom 33.3% were diagnosed with AF within 1 year and 56 (47.5%) within the total duration of follow-up to 5 years. Patients from the validation cohort were older (71.4 ± 10.5 years vs 67.9 ± 12.7 years), and had a more distinctive cardiovascular risk profile with higher incidence of hypertension (84.7% vs 71.3%) and higher NT-proBNP (median 258 pg/ml vs 191 pg/ml; Table 1).

Results of the univariable Cox regression for the predictors age, NT-proBNP, CHA_2DS_2-VASc score, and rhythm irregularity burden are presented in Table 2. By multivariable Cox regression using these predictors, the rhythm irregularity burden remained the

Table 1. Baseline characteristics

Parameter	Munich Overall (n = 297)	Munich Stratified by AF incidence AF during FU (n = 46)	Munich Stratified by AF incidence no AF during FU (n = 251)	p	Tübingen Overall (n = 118)	Tübingen Stratified by AF incidence AF during FU (n = 56)	Tübingen Stratified by AF incidence no AF during FU (n = 62)	p
Age [years]	67.9 ± 12.7	73.3 ± 9.2	66.9 ± 13.1	0.002	71.4 ± 10.5	74.8 ± 8.3	68.4 ± 11.4	<0.001
Male sex	189 (63.6%)	25 (54.4%)	164 (65.3%)	0.18	69 (58.5%)	35 (62.5%)	34 (54.8%)	0.46
BMI [kg/m^2]	26.6 ± 4.3	27.7 ± 4.6	26.4 ± 4.2	0.07	N/A	N/A	N/A	N/A
Hypertension	213 (71.3%)	33 (71.7%)	180 (71.7%)	1.00	100 (84.7%)	51 (91.1%)	49 (79.0%)	0.079
Hypercholesterolemia	101 (34.0%)	22 (47.8%)	79 (31.5%)	0.042	40 (33.9%)	23 (41.1%)	17 (27.4%)	0.125
Diabetes mellitus	63 (21.2%)	11 (23.9%)	52 (20.7%)	0.70	17 (14.4%)	11 (19.6%)	6 (9.7%)	0.189
Ever smoker	98 (33.0%)	10 (21.7%)	88 (35.1%)	0.09	20 (16.9%)	4 (7.1%)	16 (25.8%)	0.007
NIHSS admission	2 (1;4)	2 (1;4)	2 (1;4)	0.22	2 (1;5)	2 (1;6)	2 (0;5)	0.428
NIHSS discharge	1 (0;3)	0 (0;1)	1 (0;3)	0.006	N/A	N/A	N/A	N/A
mRS discharge	1 (1;2)	1 (0;2)	1 (1;2)	0.14	1 (0;2)	1 (0;2)	1 (0;2)	0.584
Prior stroke	74 (24.9%)	16 (34.8%)	58 (23.1%)	0.10	N/A	N/A	N/A	N/A
Antiplatelet therapy at discharge	274 (92.3%)	44 (95.7%)	230 (91.6%)	0.55	92 (78.0%)	39 (69.6%)	53 (85.5%)	0.047
Anticoagulation at discharge	25 (8.4%)	1 (2.2%)	24 (9.6%)	0.15	26 (22.0%)	17 (30.4%)	9 (14.5%)	0.047
CHA$_2$DS$_2$-VASc	5 (3;6)	5 (4;6)	4 (3;6)	0.003	5 (4;6)	6 (5;6)	5 (4;6)	<0.001
Rhythm irregularity burden	0.0 (0.0;0.6)	0.5 (0.0;1.0)	0.0 (0.0;0.4)	<0.001	0.0 (0.0;1.0)	1.0 (0.0;1,0)	0.0 (0.0;0.0)	<0.001
P-wave terminal force V1 (μV*ms)	3,168± 2,277	3,165± 2068	3,169 ± 2,317	0.98	N/A	N/A	N/A	N/A
P-wave terminal force V1 ≥ 4,000 μV ms	87 (29.3%)	15 (32.6%)	72 (28.7%)	0.60	N/A	N/A	N/A	N/A
NT-proBNP [pg/ml]	191 (83;448)	321 (126;731)	172 (80;400)	0.004	258 (94;578)	355 (152;975)	209 (62;336)	0.005

Note: Table reports baseline characteristics of both cohorts and stratified by occurrence of atrial fibrillation during follow-up. Parameters are expressed as mean ± standard deviation for normally distributed continuous measures and as median (25th;75th) for non-normally distributed measures, and as absolute and relative frequencies for discrete measures.

Abbreviations: AF = atrial fibrillation; FU = follow-up; mRS = modified Rankin scale; NIHSS = National Institutes of Health Stroke scale.

strongest and most significant predictor of AF (HR 2.20, 95% CI 1.45–3.33, $p <$ 0001; Table 2). AF occurred significantly more frequently in patients with a rhythm irregularity burden of >0 compared with those with a burden of 0 (78.4% vs 32.9%, $p <$ 0.001; Fig 4). By receiver operating characteristics analysis incorporating the rhythm irregularity burden, we obtained a c-statistic of 0.66.

A meta-analysis of the derivation and validation cohorts revealed a combined hazard ratio of 2.43 (95% CI 1.72–3.44) for the rhythm irregularity burden, with no indication of cross-cohort heterogeneity (Fig 5).
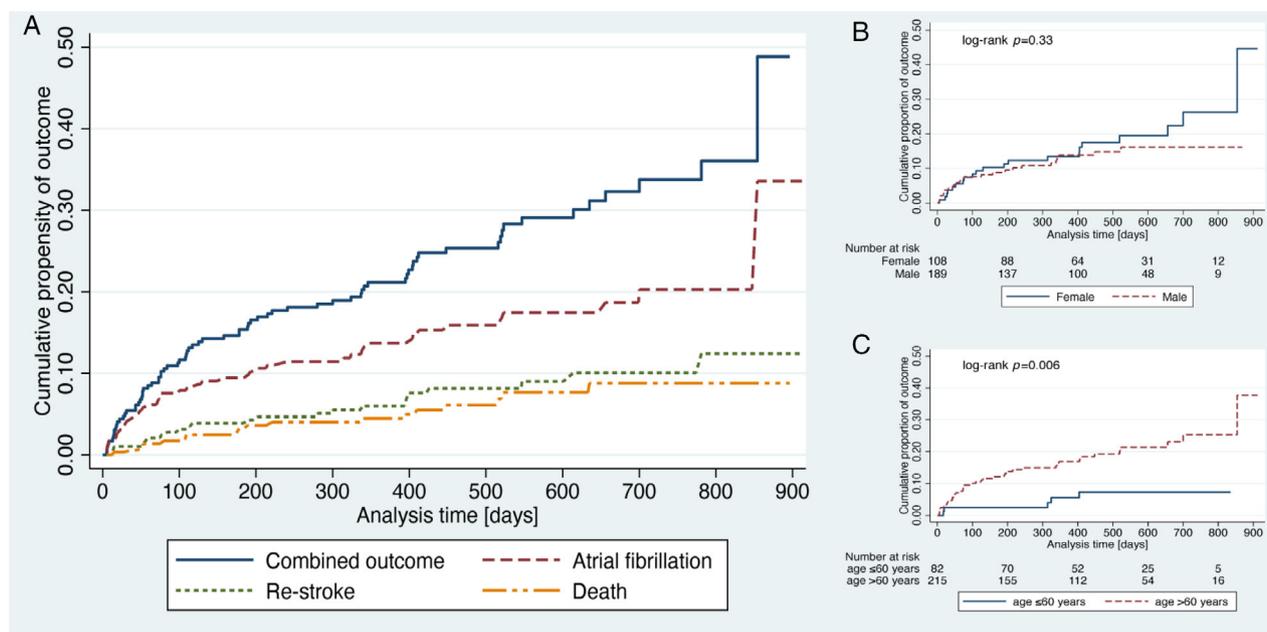
Figure 3: Cumulative incidence plots of the respective outcomes. (A) Unstratified cumulative incidence curves for the outcomes of atrial fibrillation (primary outcome), recurrent stroke, death, and the combination of these three outcomes (secondary outcomes). Importantly, all four curves are independent of each other, drawn to the same scale and overlaid for comparative visualization. (B) Cumulative incidence curves for the primary outcome of atrial fibrillation, stratified by sex. (C) Cumulative incidence curves for the primary outcome of atrial fibrillation, stratified by age ≤60 years or >60 years. Comparisons in B and C by log-rank tests. [Color figure can be viewed at www.annalsofneurology.org]

Discussion

In Catch-Up-ESUS, a prospective cohort study reflecting clinical reality, we derived a simple, ECG-based approach to predict AF as the most likely adjudicated cause of ESUS. We identified the rhythm irregularity burden as the strongest predictor of AF, detecting up to 25.5% AF during follow-up. This finding was validated in an external cohort. Most importantly, the rhythm irregularity burden can be determined non-invasively by rhythm monitoring during hospitalization for ESUS, and does not require elaborate and time-consuming evaluation.

Since the definition of ESUS,[18] the largest trials enrolling ESUS patients were NAVIGATE ESUS and RE-SPECT ESUS.[3, 4] Both studies encompassed over 12,600 participants and hence set the benchmark for clinically characterizing those patients. Our cohort was highly comparable with these benchmark studies, both regarding clinical characteristics and stroke severity. Yet, in ESUS patients, the critical clinical challenge remains to identify the undetermined source. Particularly the identification of AF, as such an underlying cause of stroke is of importance, because AF detection changes the antithrombotic regimen from antiplatelet therapy to OAC in most patients.[7] Prior efforts to provide OAC to all ESUS patients, as attempted in NAVIGATE ESUS and RE-SPECT ESUS, have not been successful due to an excess of adverse events and a too heterogenous ESUS population.[3, 4, 23] This is

despite the fact that several investigations were able to show that a relevant proportion of ESUS patients actually present with incident AF. Particularly in patients poststroke of undetermined source based on the TOAST criteria, the CRYSTAL AF study identified 12.4% AF at 12 months in patients randomized to receive an ICM.[5] In contrast, patients aged younger than 50 years with ESUS had a significantly lower incidence of AF, even when equipped with ICM.[24] More recently, the FIND-AF-randomized trial used three times 10-day Holter recordings in patients after an ischemic stroke and detected AF in 13.5% after 6 months.[25] In our current investigation, the overall cumulative incidence of AF was 9.1% after 6 months and 12.5% after 12 months follow-up, and it was 10.6% (6 months) and 14.5% (12 months) when restricted to those patients equipped with an ICM. To further parallel prior efforts, we used 30 seconds duration of the arrhythmia to assign the diagnosis of AF. This is particularly in line with the CRYSTAL AF study and a more recent expert-based white paper on screening for AF post-stroke.[5, 7]

Various efforts have been made to identify ESUS patients at increased AF risk. Measures of left atrial dysfunction, demographic and anthropometric measures, and clinical preconditions were used to predict AF during long-term follow-up.[26–28] The AF-ESUS score identified age, hypertension, echocardiographic hypertrophy and ejection fraction, ECG-derived supraventricular

Table 2. Uni- and multivariable Cox regression

	Munich				Tübingen			
	Univariable		Multivariable		Univariable		Multivariable	
Parameter	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age (yr)	1.04 (1.02–1.07)	0.001	1.02 (0.98–1.06)	0.268	1.06 (1.03–1.09)	<0.001	0.99 (0.95–1.04)	0.815
Male sex	0.75 (0.42–1.34)	0.335	N/A	-	N/A	-	N/A	-
BMI (kg/m^2)	1.05 (0.99–1.12)	0.095	N/A	-	N/A	-	N/A	-
Hypertension	0.88 (0.46–1.68)	0.704	N/A	-	N/A	-	N/A	-
Hypercholesterolemia	1.81 (1.01–3.24)	0.048	N/A	-	N/A	-	N/A	-
Diabetes mellitus	1.29 (0.65–2.54)	0.466	N/A	-	N/A	-	N/A	-
Ever smoker	0.55 (0.27–1.10)	0.092	N/A	-	N/A	-	N/A	-
NIHSS admission	0.93 (0.85–1.02)	0.109	N/A	-	N/A	-	N/A	-
Antiplatelet therapy at discharge	2.45 (0.59–10.13)	0.217	N/A	-	N/A	-	N/A	-
Anticoagulation at discharge	0.18 (0.02–1.30)	0.089	N/A	-	N/A	-	N/A	-
CHA$_2$DS$_2$-VASc	1.31 (1.09–1.57)	0.004	1.08 (0.82–1.42)	0.586	1.57 (1.27–1.94)	<0.001	1.64 (1.19–2.25)	0.002
Rhythm irregularity burden	3.90 (2.25–6,73)	0.000	3.07 (1.62–5.80)	<0.001	2.66 (1.88–3.76)	<0.001	2.20 (1.45–3.33)	<0.001
P-wave terminal force V1 (μV*ms)	1.00 (1.00–1.00)	0.95	N/A	-	N/A	-	N/A	-
NT-proBNP (pg/ml)	1.00 (1.00–1.00)	0.022	1.00 (1.00–1.00)	0.402	1.00 (1.00–1.00)	<0.001	1.00 (1.00–1.00)	0.188

Abbreviations: BMI = body mass index; CI = confidence interval; HR = hazard ratio; NIHSS = National Institutes of Health Stroke scale.

irregularity, and imaging characteristics of the extracranial vasculature and of the brain as relevant risk factors for AF.[11] Importantly, the ascertainment of AF was restricted to non-systematic documentation of symptomatic episodes; asymptomatic AF episodes may thus have been missed.[11] Another study considered a CHA$_2$DS$_2$-VASc score ≥4, atrial runs on Holter ECG, a left atrial size >45 mm on transthoracic echocardiography, a left atrial appendage flow ≤0.2 mm/s, or spontaneous contrast on transesophageal echocardiography to define an increased AF risk.[6] This study used ICM monitoring for AF ascertainment, but restricted monitoring to the small high-risk subset.[6] As a limitation to both studies, their sophisticated, invasive, and time-consuming diagnostics may not be readily available in clinical routine. In the present study, we aimed to systematically ascertain AF in all participants. Reflecting clinical routine outside a clinical trial, we managed to accomplish this goal by continuous ICM monitoring in most patients. More importantly, we identified the rhythm irregularity burden as the single most important predictor of AF during follow-up with a hazard ratio >3, even after multivariable adjustment. In addition, we validated the rhythm irregularity burden in a well-characterized, independent cohort. Unlike in prior studies, our rhythm irregularity burden can be determined non-invasively during hospitalization and does not require additional elaborate diagnostic procedures.

Atrial ectopy has been the focus of recent research. Miyazaki et al. reported a strong association between atrial ectopy and documentation of AF on 7-day ECG recordings. The median time to AF detection was 50 hours,[12] a duration typically covered by monitoring in the stroke unit. The Find-AF study found an excess of recurrent strokes during follow-up in a small subset of 67 patients presenting with atrial ectopy on a 7-day ECG recorded after an index stroke of unknown etiology.[13] Yet, the development of AF as the actual underlying etiology was not systematically examined.[13] Most recently, Ntaios et al. associated atrial ectopy with the occurrence of AF in ESUS patients, but only very few patients were followed by continuous AF monitoring.[14] In the present study, we aimed to overcome several of these prior limitations. Specifically, patients were only enrolled into our study once current paroxysmal AF was ruled out by at least 72 hours of ECG monitoring. All patients received an assessment of the rhythm irregularity burden to maximize statistical power. Also, all patients were systematically evaluated for incident AF during follow-up, the majority using continuous ICM monitoring. Most importantly, the present study was independently validated, yielding the strongest
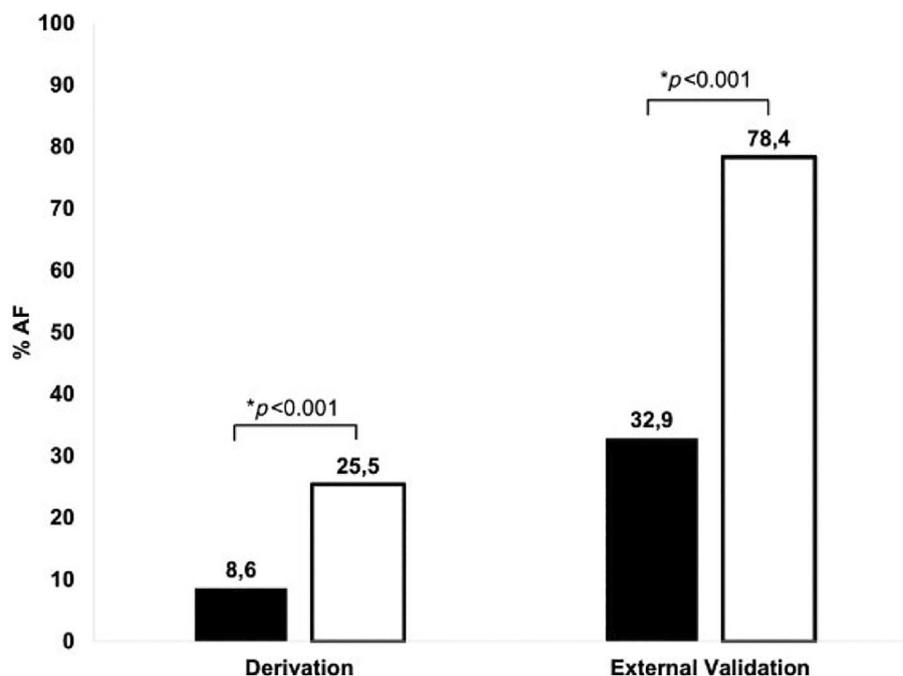
Figure 4: Incidence of atrial fibrillation (AF) in low and high-risk patients. Patients in the derivation and the validation cohorts are stratified by a rhythm irregularity burden of 0 (left columns) or >0 (right columns).

evidence so far for rhythm irregularity as a relevant risk factor with the potential to stratify ESUS patients into clinically relevant subgroups.

To stratify ESUS patients into distinct risk groups amenable to differential therapeutic regimens will be one imminent next task to improve care. Few clinical trials are currently underway aiming to address this task. The ATTICUS and ARCADIA trials both require elaborate technical evaluation for stratification.[15, 16] Based on the present results, a rhythm irregularity burden >0 may simply and non-invasively stratify ESUS patients with an excess AF risk. Conversely, AF risk in ESUS patients with a rhythm irregularity burden of 0 may be considerably low. Such comparably simple stratification may facilitate implementation into clinical practice.[8, 29] Prospective randomized clinical trials are warranted to further address the issue. As such,

the FIND-AF-2 study is currently underway to substantiate atrial ectopy for individual risk stratification after ESUS (http://www.clinicaltrials.gov: NCT04371055).

Some considerations are required interpreting our data. Catch-Up-ESUS is a single center, non-randomized cohort study and, hence, is primarily hypothesis-generating. However, independent validation of the present findings in an external cohort clearly substantiates our findings. We aimed to enroll all consecutive qualifying patients. Yet, our sample size study was limited to investigate a more comprehensive prediction model for AF post-ESUS. Nevertheless, our study facilitates future sample size calculations by providing evidence of expectable effect sizes of several predictors, including the rhythm irregularity burden. A clear strength of the present study was the high percentage of patients equipped with ICMs for
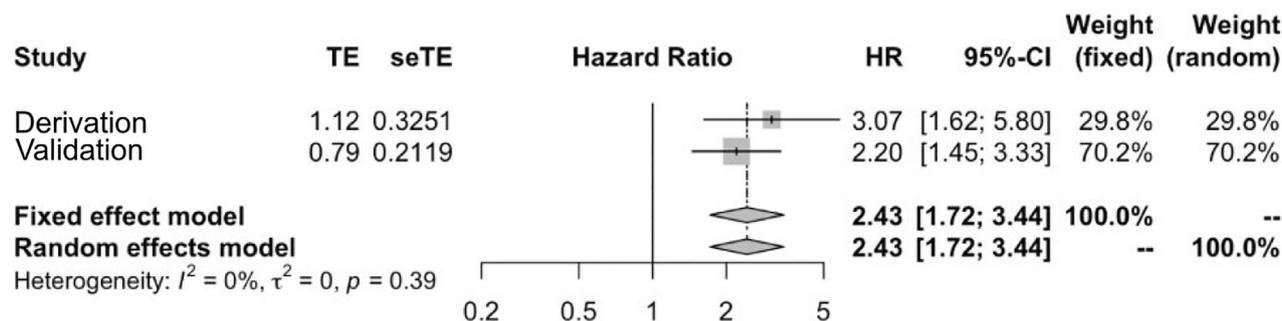
Figure 5: Visualization of rhythm irregularity burden meta-analysis results. Derivation cohort: CATCH-Up ESUS cohort from Munich; validation cohort: embolic stroke of undetermined source cohort from Tübingen. CI = confidence interval; HR = hazard ratio for the rhythm irregularity burden; TE = treatment estimate; seTE = standard error of the treatment estimate.

continuous rhythm monitoring. Compared with prior efforts without ICMs, our AF detection rate may be more reliable. It reflects clinical reality that we were unable to equip all participants with an ICM in our derivation cohort. This may underestimate the incidence of AF, especially when compared with our validation cohort that was designed to include only patients with an ICM. Yet, we submit that our validation cohort replicates well our derivation findings. Similarly, not the full spectrum of available diagnostic procedures was studied. This pertains particularly to quantifiable echocardiographic data in all patients, which would have enabled the analysis of left atrial size, a known predictor of AF, in comparison with the rhythm irregularity burden. Additionally, future research needs to clarify to what extent easily determinable supraventricular ectopy influences the rhythm irregularity burden, and to what extent atrial ectopy and, hence, an increased rhythm irregularity burden may be the consequence of acute stroke rather than the predictor of atrial fibrillation underlying the index event. Importantly, the rhythm irregularity burden can be determined from both surveillance monitor recordings in the stroke unit and Holter ECGs supporting transferability across different ECG recording settings.

In conclusion, we conducted and validated an analysis of AF predictors in two well-characterized cohorts of patients post-ESUS and identified the rhythm irregularity burden as an important predictor. This predictor can be determined easily and non-invasively during the initial hospitalization at the stroke unit and thereafter. Our results may help to identify patients at high risk for AF who benefit from intensified rhythm monitoring. Future randomized clinical trials may inform if differential therapeutic regimens are beneficial in different strata of ESUS patients.

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Author Contributions

A.S.F., K.F., M.F.S., M.D., S.M., S.K., and L.K. were responsible for conception and design of the study. A.S.F., K.F., M.F.S., S.S., J.M., J.W., E.E., S.C., S.P., K.P., C.S.Z., U.Z., S.K., and L.K. were responsible for data acquisition and analysis. A.S.F., K.F., M.F.S., E.E., S.C., S.P., U.Z., J.B., A.K., W.S., M.D., S.M., S.K., and L.K. were responsible for drafting a significant portion of the manuscript or figures.

Potential Conflicts of Interest

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