

Serial measurement of interleukin-6 and risk of mortality in anticoagulated patients with atrial fibrillation: Insights from ARISTOTLE and RE-LY trials

Julia Aulin^{1,2} | Ziad Hijazi^{1,2} | Agneta Siegbahn^{2,3} | Ulrika Andersson² | John H. Alexander⁴ | Stuart J. Connolly⁵ | Michael D. Ezekowitz⁶ | Bernard J. Gersh⁷ | Christopher B. Granger⁴ | John Horowitz⁸ | Elaine M. Hylek⁹ | Renato D. Lopes⁴ | Salim Yusuf⁵ | Lars Wallentin^{1,2} | Jonas Oldgren^{1,2}

¹Department of Medical Sciences, Cardiology, Uppsala University, Uppsala, Sweden

²Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden

³Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden

⁴Duke Clinical Research Institute, Duke University Medical Center, Duke Health, Durham, NC, USA

⁵Population Health Research Institute, McMaster U and Hamilton Health Sciences, Hamilton, ON, Canada

⁶Thomas Jefferson Medical College and the Heart Center, Wynnewood, PA, USA

⁷Mayo Clinic College of Medicine, Rochester, MN, USA

⁸University of Adelaide, Adelaide, SA, Australia

⁹Boston University Medical Center, Boston, MA, USA

Correspondence

Julia Aulin, Uppsala Clinical Research Center, Dag Hammarskjölds väg 38, S-751 85 Uppsala, Sweden.
Email: julia.aulin@ucr.uu.se

Funding information

The ARISTOTLE trial was funded by Bristol-Myers Squibb, Princeton, NJ, and

Abstract

Background: The inflammatory biomarker interleukin-6 (IL-6) is associated with mortality in atrial fibrillation (AF).

Objective: To investigate if repeated IL-6 measurements improve the prognostication for stroke or systemic embolism, major bleeding, and mortality in anticoagulated patients with AF.

Methods: IL-6 levels by ELISA were measured at study entry and at 2 months in 4830 patients in the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial with 1.8 years median follow-up. In the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial, IL-6 was measured at study entry, 3, 6, and 12 months in 2559 patients with 2.0 years median follow-up. Associations between a second IL-6 measurement and outcomes, adjusted for baseline IL-6, clinical variables, and other cardiovascular biomarkers, were analyzed by Cox regression.

Results: Median IL-6 levels were 2.0 ng/L (interquartile range [IQR] 1.30-3.20) and 2.10 ng/L (IQR 1.40-3.40) at the two time-points in ARISTOTLE, and, in RE-LY, 2.5 ng/L (IQR 1.6-4.3), 2.5 ng/L (IQR 1.6-4.2), 2.4 ng/L (IQR 1.6, 3.9), and 2.4 ng/L (IQR 1.5, 3.9), respectively. IL-6 was associated with mortality; hazard ratios per 50% higher IL-6 at 2 or 3 months, respectively, were 1.32 (95% confidence interval, 1.23-1.41; $P < .0001$) in ARISTOTLE, and 1.11 (1.01-1.22, $P = .0290$) in RE-LY; with improved C index from 0.74 to 0.76 in ARISTOTLE, but not in the smaller RE-LY cohort.

Clinical Trial Registration: www.ClinicalTrials.gov NCT00412984 and NCT00799903.

Manuscript handled by: Jean Connors.

Final decision: Jean Connors, 29 May 2020.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals LLC on behalf of International Society on Thrombosis and Haemostasis

Pfizer Inc, New York, NY, and coordinated by the Duke Clinical Research Institute, Durham, NC, and Uppsala Clinical Research Center, Uppsala, Sweden. The RE-LY trial was funded by Boehringer-Ingelheim, Ingelheim, Germany, and coordinated by the Population Health Research Institute, Hamilton, Canada, and Uppsala Clinical Research Center, Uppsala, Sweden.

There were no consistent associations with second IL-6 and stroke or systemic embolism, or major bleeding.

Conclusions: Persistent systemic inflammatory activity, assessed by repeated IL-6 measurements, is associated with mortality independent of established clinical risk factors and other strong cardiovascular biomarkers in anticoagulated patients with AF.

KEYWORDS

anticoagulants, atrial fibrillation, inflammation, interleukin-6, mortality, stroke

1 | INTRODUCTION

Atrial fibrillation (AF) is a well-known risk factor for stroke and mortality.^{1,2} Anticoagulation treatment markedly reduces these events in patients with AF.^{3,4} Biomarkers of inflammatory activity, such as interleukin-6 (IL-6) and C-reactive protein, are associated with cardiovascular events in healthy individuals and in patients suffering from various cardiac diseases other than AF.⁵⁻⁸ In patients with AF, a single measurement showing higher levels of the inflammatory biomarkers C-reactive protein and, even more consistently, IL-6 has been associated with increased risk of mortality, independently of clinical risk factors and other cardiovascular biomarkers.^{9,10} However, the variability of inflammatory biomarkers over time, and the associations between serial measurements of inflammatory biomarkers and cardiovascular events have previously not been described in larger cohorts of patients with AF.

In this study, we aimed to investigate changes in the IL-6 concentration over both short- and long-term follow-up, up to 12 months. Further, we aimed to assess the associations between a second IL-6 measurement obtained after short-term follow-up (2-3 months) and stroke or systemic embolism, bleeding, and mortality, and evaluate if the additional information gained from a second IL-6 measurement improved the prognostication for these outcomes, on top of baseline measurements, in patients with AF on anticoagulation treatment within the serial biomarker substudies of the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial¹¹ and the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial.¹²

2 | METHODS

2.1 | Study design and participants

In the ARISTOTLE trial, 18 201 patients with AF and at least one additional risk factor for stroke were randomized to apixaban or warfarin for stroke prevention; details have been published previously.^{11,13} Biomarker samples of IL-6 were available from 4830 patients at study entry and at 2 months, with a median follow-up

Essentials

- Interleukin-6 (IL-6) is associated with mortality in patients with atrial fibrillation (AF).
- Repeated IL-6 measurements were conducted in ARISTOTLE and RE-LY trial participants.
- Higher IL-6 levels 2-3 months after study entry were associated with increased mortality.
- In patients with AF, IL-6 assessment at any time adds relevant information on risk of mortality.

time of 1.8 years. In the RE-LY trial, 18 113 patients with AF and at least one additional risk factor for stroke were randomized to dabigatran or warfarin for stroke prevention; details have been published previously.^{12,14} Biomarker samples of IL-6 were available from 2559 patients at study entry and at any postbaseline time-point at 3, 6, and 12 months, with a median follow-up time of 2.0 years. Both cohorts consisted of consecutive patients at participating sites who met all inclusion criteria of the main trial and accepted to participate in the serial biomarker substudy with additional sample collections. Approval by the appropriate ethics committees was obtained at all sites. All participants provided written informed consent.

2.2 | Outcomes

In both the ARISTOTLE and RE-LY trials, the primary outcome was stroke or systemic embolism and the primary safety outcome was major bleeding events, adapted from the International Society on Thrombosis and Haemostasis criteria. Clinical events committees blinded for study treatment adjudicated all outcomes using pre-specified criteria.^{13,14} The present study investigated the association of a second IL-6 measurement at 2 months in the ARISTOTLE and at 3 months in the RE-LY trials, with the subsequent outcomes stroke or systemic embolism, major bleeding events, and all-cause mortality.

2.3 | Biochemical methods

Venous blood samples were obtained in citrate tubes before start of study treatment and at 2 months in the ARISTOTLE substudy cohort and at 3, 6, and 12 months in the RE-LY substudy cohort. In both trials, all tubes were centrifuged immediately and plasma samples frozen in aliquots and stored at -70°C until analyzed centrally at the Uppsala Clinical Research Center Laboratory, Uppsala, Sweden. Plasma concentrations of high-sensitivity IL-6 were analyzed using an ELISA technique, R&D Systems Inc, Minneapolis, MN. The local coefficient of variation for this method is 12% at 0.43 ng/L and 8% at 4.9 ng/L. The methodology of the other cardiovascular biomarkers adjusted for in the statistical models have been described in detail previously and were analyzed as follows: cardiac troponin I with the high-sensitivity assay by ARCHITECT i1000SR (Abbott Diagnostics) and N-terminal B-type natriuretic peptide (NT-proBNP) with the Cobas Analytics e601 Immunoanalyzer (Roche Diagnostics) using high-sensitivity assays; cystatin C with the ARCHITECT ci8200; growth-differentiation factor-15 (GDF-15) with the Elecsys precommercial assay kit P03 from Roche Diagnostics with the same standardization as the recently introduced routine reagent.^{10,15-18}

2.4 | Statistical analyses

Summary of demographics and baseline characteristics by randomized treatment and in total for the participants in the ARISTOTLE serial biomarker substudy, and in the RE-LY serial biomarker substudy, were summarized using frequencies for categorical variables and median and 25th and 75th percentiles for continuous variables for the 4830 ARISTOTLE patients and 2517 RE-LY patients with available measurements at both study entry and at 2 or 3 months, respectively. IL-6 measurements were natural log-transformed before analysis. IL-6 over time by randomized treatment groups was presented as geometric means and treatment group differences as ratios of geometric means. The intraclass correlation coefficient was calculated using variance components from linear mixed models with patient as random effect. The intraclass correlation coefficient is the proportion of the total variance in log(IL-6) that is accounted for by the between-patient variance. The associations between the second IL-6 measurements and outcomes were investigated using Cox proportional hazards regression, excluding all events before the second measurement of IL-6 in both cohorts. Analyses of major bleeding events only included patients on study treatment. Four different multivariable models (O, A, B, and C) were used. Model O included month 2 IL-6 (ARISTOTLE) or month 3 IL-6 (RE-LY), baseline IL-6, and randomized treatment. Model A included for the outcome of stroke/systemic embolism in addition to the covariates in model O heart failure, diabetes, previous stroke/systemic embolism/transient ischemic attack, hypertension, history of vascular disease, gender, and age. For the outcome of all-cause mortality, model A also included systolic blood pressure, cystatin C (renal function), and smoking status. For major bleeding events, in addition to the covariates in the model for all-cause mortality, the model included hemoglobin

and use of nonsteroidal anti-inflammatory drugs/antiplatelets. Model B included the same covariates as in model A with addition of the cardiac biomarkers troponin I and NT-proBNP. Model C included the same covariates as in model B with addition of the biomarker GDF-15. Relative hazard with 95% confidence interval (CI) bounds was plotted according to continuous IL-6 level at month 2 in ARISTOTLE and month 3 in RE-LY, with the median as reference point. The model was fitted using restricted cubic splines, adjusted for baseline IL-6.

Complete case analysis was implemented for both studies. In the ARISTOTLE trial, there were <1% missing data on covariates. In the RE-LY trial, 74% had complete data on all covariates, including other biomarkers, available and the analyses of models A-C were thus carried out on this analysis set. Because the number of events in the RE-LY trial were few, shrinkage was applied to the models by penalized maximum likelihood estimation. The shrinkage parameter was for each model based on approximate degrees of freedom chosen to obtain 1 degree of freedom per 10 events.

The increased discriminative value of a second IL-6 measurement was investigated by estimating the C index for survival data¹⁹ for models with established clinical risk factors, including baseline IL-6, and the other biomarkers troponin I, NT-proBNP, and GDF-15, with and without a second IL-6 at 2 months (ARISTOTLE) or 3 months (RE-LY).

The effects of treatment assignment on outcome in relation to inflammation marker level were investigated by using a Cox model including randomized treatment, inflammation marker level, and the interaction between treatment and inflammation marker level. All statistical tests were two-tailed and performed at the 0.05 significance level. Because the analyses were exploratory, no adjustments for multiple comparisons were performed. All analyses were performed using SAS version 9.4 for Windows (SAS Institute Inc) and R version 3.5 (The R Foundation).

3 | RESULTS

3.1 | Baseline characteristics and demographics

Baseline characteristics and demographics are presented in Table S1 A,B. In ARISTOTLE, the median age of the patients was 70 years (interquartile range [IQR] 63, 76) and approximately 34% were women. In RE-LY, the median age was 72 years (IQR 66, 77) and approximately 35% were women. In both cohorts, the study treatment groups were well balanced regarding physical measurements and comorbidities.

3.2 | Distribution and changes of IL-6 levels over time in AF

In ARISTOTLE, the median IL-6 level was 2.0 ng/L (IQR 1.30, 3.20) at entry. At 2 months follow-up, the median level was higher at 2.10 ng/L (IQR 1.40, 3.40; $n = 4830$). The ratio of geometric means (95% CI) for

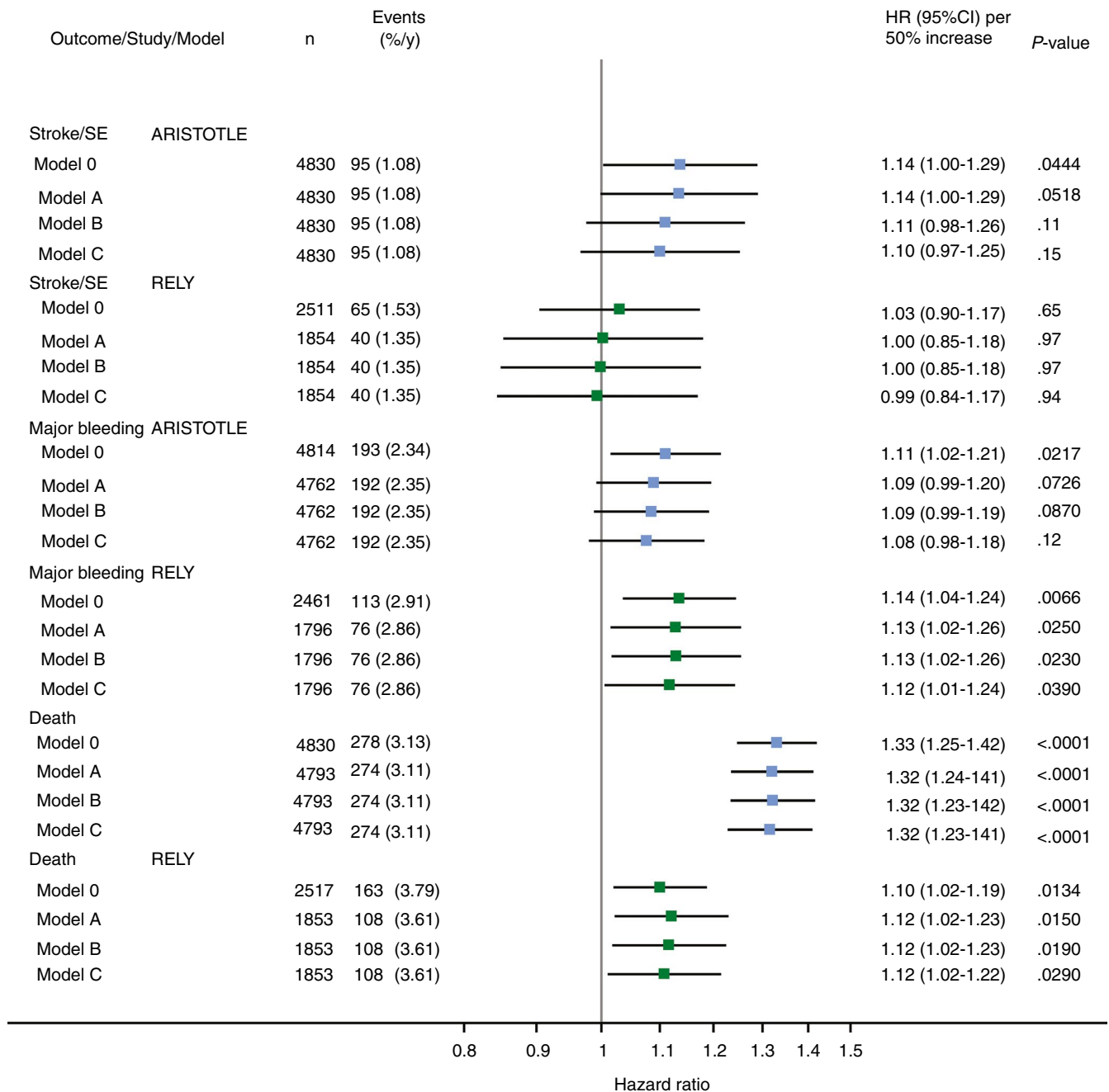


FIGURE 1 The impact of second measurement of IL-6 level on outcomes in both the ARISTOTLE and RE-LY serial biomarker substudies. Cox proportional hazards model adjusted for baseline IL-6 level, randomized treatment, baseline characteristics, and other biomarkers at baseline according to model 0, A, B, and C, respectively. Model 0 included month 2 IL-6 (ARISTOTLE) or month 3 IL-6 (RE-LY), baseline IL-6, and randomized treatment. Model A included for the outcome of stroke/systemic embolism in addition to the covariates in model 0 heart failure, diabetes, previous stroke/systemic embolism/transient ischemic attack, hypertension, history of vascular disease, gender, and age. For the outcome of all-cause mortality, model A also included systolic blood pressure, cystatin C (renal function), and smoking status. For major bleeding events, in addition to the covariates in the model for all-cause mortality, the model included hemoglobin and use of nonsteroidal anti-inflammatory drugs/antiplatelets. Model B included the same covariates as in model A with addition of the cardiac biomarkers troponin I and NT-proBNP. Model C included the same covariates as in model B with addition of the biomarker GDF-15.

month 2 in relation to baseline was 1.05 (1.03-1.06) and the intraclass correlation coefficient was 0.59. Comparison between randomized treatment groups, adjusted for baseline IL-6 level, showed a slightly lower increase in IL-6 levels in the apixaban treated group compared with the warfarin treated group at 2 months; geometric mean 2.18 ng/L

(95% CI, 2.13-2.23) vs 2.26 ng/L (95% CI, 2.20-2.31), respectively, a ratio of geometric means of 0.96 (95% CI, 0.93-1.00, $P = .0351$).

In RE-LY, the median IL-6 level was 2.5 ng/L (IQR 1.6, 4.3; $n = 2517$) at entry, 2.5 ng/L (IQR 1.6, 4.2; $n = 2517$) at 3 months, 2.4 ng/L (IQR 1.6, 3.9; $n = 1040$) at 6 months, and 2.4 ng/L (IQR 1.5,

3.9; $n = 1039$) at 12 months. The ratio of geometric means (95% CI) was for month 3 in relation to baseline 1.00 (0.97-1.03), month 6/baseline 0.93 (0.89-0.97), and month 12/baseline 0.90 (0.87-0.94). The intraclass correlation coefficient for baseline versus the month 3 measurement was 0.47. Comparison between randomized treatment groups, adjusted for baseline IL-6 level, showed no significant difference between treatment groups at 3 months ($P = .26$).

3.3 | Second IL-6 measurement and association with outcomes

In the ARISTOTLE substudy cohort, during follow-up, after 2 months, there were a total of 95 strokes or systemic embolic events with a yearly event rate of 1.08%, 193 major bleeding events with a yearly event rate of 2.34%, and 278 deaths with a yearly event rate of 3.13%. In Cox proportional hazards model adjusted for baseline IL-6 level, randomized treatment, and baseline characteristics, there were no significant associations between increasing IL-6 levels and the outcomes of stroke or systemic embolism and major bleeding events. For all-cause mortality, a significant association was seen with increasing IL-6 level at 2 months with a hazard ratio (HR) of 1.32 (95% CI, 1.24-1.41; $P < .0001$) per 50% higher IL-6 level at 2 months. The association remained unchanged after extending the multivariable adjustment by adding the cardiovascular biomarkers troponin I, NT-proBNP, and GDF-15 to the model, HR 1.32 (95% CI, 1.23-1.41; $P < .0001$) per 50% higher IL-6 level at 2 months (Figure 1). Relative hazard for all-cause mortality according to continuous month 2 IL-6 level, adjusted for baseline IL-6 level, are shown in Figure 2.

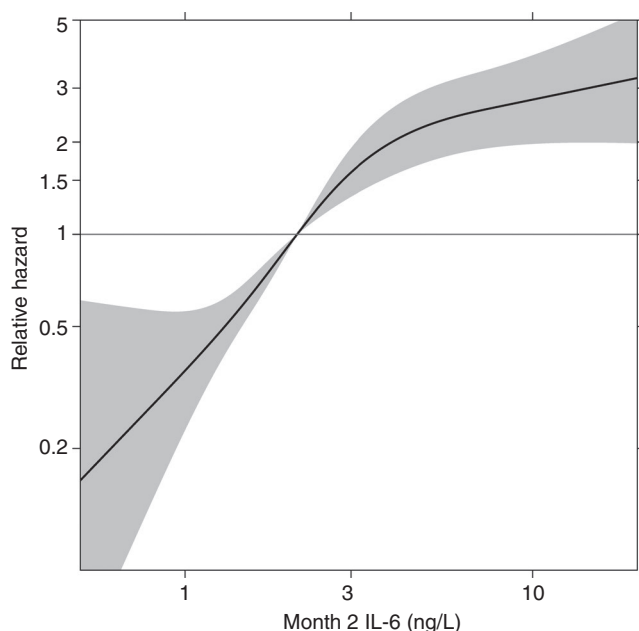


FIGURE 2 Relative hazard for all-cause mortality according to month 2 IL-6, adjusted for baseline IL-6, in the ARISTOTLE serial biomarker substudy. Reference point is median IL-6 at month 2, 95% confidence bounds are shown.

In the RE-LY substudy cohort, during follow-up, after 3 months, there were a total of 65 strokes or systemic embolic events with a yearly event rate of 1.53%, 113 major bleeding events with a yearly event rate of 2.91%, and 163 deaths with a yearly event rate of 3.79%. In Cox proportional hazards model adjusted for baseline IL-6 level, randomized treatment, and baseline characteristics, no significant association was seen between the risk of stroke or systemic embolism and increasing IL-6 level at 3 months. For major bleeding events, a significant association was seen with increase of IL-6 level at 3 months, HR 1.13 (95% CI, 1.02-1.26; $P = .0250$) per 50% higher IL-6 level at 3 months. The association remained essentially unchanged after extending the multivariable adjustment by adding the cardiovascular biomarkers troponin I, NT-proBNP, and GDF-15 to the model, HR 1.12 (95% CI 1.01-1.24; $P = .0390$) per 50% higher IL-6 level at 3 months. For all-cause mortality, a significant association was also seen with increase of IL-6 level at 3 months, HR 1.12 (95% CI, 1.02-1.23; $P = .0150$) per 50% higher IL-6 level at 3 months. The association remained essentially unchanged after extending the multivariable adjustment by adding the cardiovascular biomarkers troponin I, NT-proBNP, and GDF-15 to the model, HR 1.11 (95% CI, 1.01-1.22; $P = .0290$) per 50% higher IL-6 level at 3 months (Figure 1). Relative hazard for all-cause mortality according to continuous month 3 IL-6 level, adjusted for baseline IL-6 level, is shown in Figure 3.

3.4 | Discriminative value of a second IL-6 measurement

In ARISTOTLE, addition of information from a second IL-6 measurement at 2 months to a model consisting of baseline IL-6 level and

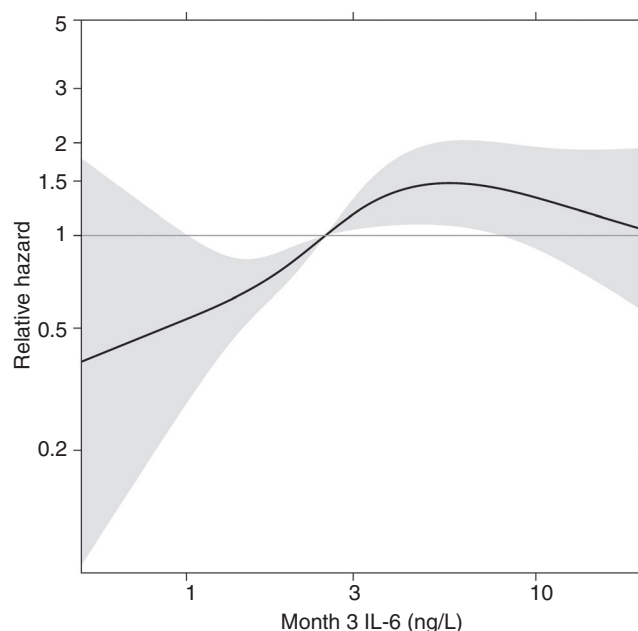


FIGURE 3 Relative hazard for all-cause mortality according to month 3 IL-6, adjusted for baseline IL-6, in the RE-LY serial biomarker substudy. Reference point is median IL-6 at month 3, 95% confidence bounds are shown.

study treatment improved the C index for all-cause mortality substantially from 0.65 to 0.70. Even in presence of clinical characteristics and other cardiovascular biomarkers, the C index for all-cause mortality was improved from 0.74 to 0.76 when adding information from the second IL-6 measurement to the model (Table 1).

In RE-LY, addition of information from a second IL-6 measurement at 3 months to a model including baseline IL-6 level and study treatment improved the C index for major bleeding events from 0.58 to 0.61. In presence of clinical characteristics and other cardiovascular biomarkers, the improvement of the C index for major bleeding events was attenuated, from 0.72 to 0.73. For all-cause mortality, the addition of information from a second IL-6 measurement at 3 months to the model did not result in improvement of the C index (Table 2).

3.5 | Outcomes in relation to study treatment and biomarker subgroups

There were no significant interactions between study treatment and biomarker levels regarding any of the evaluated outcomes in either trial (Figures S1 and S2).

4 | DISCUSSION

The main finding in the present substudy based on two separate cohorts of patients with AF on effective oral anticoagulation treatment suggests that a persistent systemic inflammatory activity, assessed by repeated IL-6 measurements, is associated with mortality

independent of established clinical risk factors and other strong cardiovascular biomarkers. This study also added new insights into the variability of IL-6 concentrations in patients with AF, with small changes in median IL-6 concentration up to 1 year. Together, these results suggest that a single IL-6 measurement, irrespective of when measured, adds relevant information on risk of mortality. However, no consistent associations were seen with repeated measurements of IL-6 and stroke or systemic embolism and bleeding, respectively.

To our knowledge, neither the degree of changes in IL-6 over time nor their associations with different clinical events and mortality have previously been studied in larger cohorts of patients with AF, particularly not in presence of established clinical risk factors and other strong cardiovascular biomarkers. We adjusted for troponin I, NT-proBNP, and GDF-15, on top of clinical variables, biomarkers previously shown to be independent and powerful markers of adverse outcomes in patients with AF.^{18,20} Recently, a small study of 117 Japanese patients with AF, randomized to rivaroxaban or dabigatran, serially measured IL-6 up to 12 months to assess the anti-inflammatory effects of the two oral anticoagulants. They reported similar IL-6 levels as in the present study with no significant association between IL-6 levels and bleeding events. However, neither association to stroke nor mortality was assessed due to the extreme low number of events.²¹

The lack of association with a repeated IL-6 measurement and stroke or systemic embolism in the present study is in line with our previous finding of single IL-6 measurements in patients with AF.¹⁰ Hence, the usefulness of IL-6 in clinical practice for improved prognostication of risk for stroke or systemic embolism in patients with AF seems limited.^{9,10} For bleeding events, the results were inconsistent between the two substudy cohorts, with a significant

Outcome	Model	C Index Model Excluding IL-6 Month 2 Level	C Index Model Including IL-6 Month 2 Level
Stroke/Systemic embolism	0	0.55	0.58
	A	0.64	0.65
	B	0.73	0.72
	C	0.74	0.74
Major bleeding events	0	0.54	0.57
	A	0.66	0.66
	B	0.66	0.67
	C	0.67	0.67
All-cause mortality	0	0.65	0.70
	A	0.69	0.72
	B	0.74	0.76
	C	0.74	0.76

TABLE 1 C index, before and after addition of IL-6 level at 2 months to models including baseline IL-6 level, baseline characteristics, and other biomarkers at baseline in the ARISTOTLE serial biomarker substudy

Note: Model 0 included month 2 IL-6, baseline IL-6, and randomized treatment. Model A for stroke/systemic embolism included month 2 IL-6, baseline IL-6, randomized treatment, heart failure, diabetes, previous stroke/systemic embolism/transient ischemic attack, hypertension, history of vascular disease, gender, and age. For all-cause mortality and major bleeding events, systolic blood pressure and cystatin C and smoking were also included. For major bleeding events, hemoglobin and use of NSAIDs/antiplatelets were also included. Model B: Same covariates as in model A with addition of Troponin I and NT-proBNP. Model C: Same covariates as in model B with addition of GDF-15.

TABLE 2 C index, before and after addition of IL-6 level at 3 months to models including baseline IL-6 level, baseline characteristics, and other biomarkers at baseline in the RE-LY serial biomarker substudy

Outcome	Model	C Index Model Excluding IL-6 Month 3 Level	C Index Model Including IL-6 Month 3 Level
Stroke/Systemic embolism	0	0.58	0.58
Major bleeding events	0	0.58	0.61
	A	0.69	0.70
	B	0.69	0.71
	C	0.72	0.73
All-cause mortality	0	0.65	0.65
	A	0.70	0.70
	B	0.73	0.73
	C	0.74	0.74

Note: Model 0 included month 3 IL-6, baseline IL-6, and randomized treatment. Model A for stroke/systemic embolism included month 3 IL-6, baseline IL-6, randomized treatment, heart failure, diabetes, previous stroke/systemic embolism/transient ischemic attack, hypertension, history of vascular disease, gender, and age. For all-cause mortality and major bleeding events, systolic blood pressure and cystatin C and smoking were also included. For major bleeding events, hemoglobin and use of NSAIDs/antiplatelets were also included. Model B: Same covariates as in model A with addition of Troponin I and NT-proBNP. Model C: Same covariates as in model B with addition of GDF-15. Shrinkage was applied to the models to obtain approximately 1 degree of freedom per 10 events.

association only in the RE-LY substudy. This may be due to chance or that these cohorts were not identical, with differences in baseline characteristics and study treatments. It may also imply that the associations are weak. The association between a second IL-6 measurement and mortality was more robust. Repeated measurements of cardiovascular biomarkers reflecting cardiac stress and dysfunction (ie, troponin and NT-proBNP) have previously shown to improve the prognostication of cardiovascular events and mortality in patients with AF.²² In the present study, a second IL-6 measurement provided independent prognostic information for mortality after adjustment for baseline IL-6 level, clinical risk factors, and these strong cardiovascular biomarkers. The significant but weaker association of a second IL-6 measurement and mortality in the RE-LY cohort may be due to the smaller sample size. The persistent association between higher IL-6 and higher risk of mortality could possibly reflect an overall burden of disease and a persistent state of low-grade systemic inflammation in patients with AF.

The present ARISTOTLE substudy demonstrated a smaller increase in mean IL-6 concentration at 2 months in the group treated with apixaban compared with the group treated with warfarin. One potential explanation for this could be a possible link between factor Xa and the expression of pro-inflammatory cytokines such as IL-6.^{23,24} Apixaban, a direct and specific oral factor Xa inhibitor, may more strongly inhibit the induction of pro-inflammatory cytokines by factor Xa than the more general vitamin K antagonist properties of warfarin. Further, administration of warfarin has been linked to an increase in IL-6 in experimental animal models.²⁵ Taken together, this may provide a hypothesis for the minor, but still statistically significant, difference in mean IL-6 concentration between the treatment arms in the ARISTOTLE substudy. However, these results need to be interpreted with caution as there were no significant interactions between study treatments and

IL-6 level regarding any of the evaluated outcomes. In the RE-LY substudy, no significant difference was seen in mean IL-6 concentration between treatment groups during follow-up.

Strengths of this study include the analyses of two large, prospective, closely monitored cohorts with rigorously adjudicated outcomes, and that influence of confounding factors were minimized by adjustments for a wide range of established conventional risk factors and conditions, and for other strong predictive cardiovascular biomarkers. This study also has some limitations. It is a clinical trial cohort of AF patients on oral anticoagulation treatment with at least one additional risk factor for stroke and the results may therefore not be generalizable to the broader AF population. The numbers of strokes were modest and there were too few deaths to conduct robust analyses regarding different causes of deaths. Information of other inflammatory conditions was not collected. Further, albeit a statistically significant difference, the clinical value of a slightly lower IL-6 concentration observed in the apixaban treated group compared with the warfarin treated group is not fully clear. It is also worth noting that, as there is variability of IL-6 within patient, repeated measurements will improve risk prediction because an averaged measurement always characterizes a marker better through reduction of measurement error. Also, the setting of the present study does not permit conclusions regarding the mechanism behind the association between IL-6 and mortality in patients with AF and the technique to analyze IL-6 is at present not broadly available for clinical use.

5 | CONCLUSION

Persistent systemic inflammatory activity, assessed by repeated IL-6 measurements, is associated with mortality independent of

established clinical risk factors and other strong cardiovascular biomarkers in patients with AF on oral anticoagulation treatment. IL-6 levels were fairly stable over time in both study cohorts suggesting that a single IL-6 measurement, irrespective when measured, adds relevant information on risk of mortality. No significant interactions were seen between effects of study treatment (apixaban, dabigatran, or warfarin) and biomarker level regarding any of the outcomes.

CONFLICT OF INTEREST

Dr. Aulin reports institutional grants from Bristol-Myers Squibb/Pfizer and Boehringer Ingelheim. Dr. Hijazi reports lecture fees from Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer, and Roche Diagnostics; consulting fees from Boehringer Ingelheim, Bristol-Myers Squibb, Meda, Merck Sharp & Dohme, Pfizer, and Roche Diagnostics; and research grants from the Swedish Society for Medical Research [S17-0133] and the Swedish Heart-Lung Foundation [20170718]. Dr. Siegbahn reports institutional research grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, GlaxoSmithKline, and Roche Diagnostics; and consulting fees from Olink Proteomics. Dr. Andersson reports an institutional research grant from Bristol-Myers Squibb/Pfizer. Dr. Alexander reports institutional research grants and consulting fees/honoraria from Bristol-Myers Squibb; institutional research grants from Boehringer Ingelheim, CryoLife, CSL Behring, and Volumetrix; and consulting fees/honoraria from AbbVie, Bayer, Novo Nordisk, Pfizer, Portola, Quantum Genomics, XaTek, and Zafgen. Dr. Connolly reports consulting fees, speaker fees, and research grants from Boehringer Ingelheim, Bristol-Myers Squibb, Bayer, and Portola; consulting fees and research grants from Sanofi-Aventis; and research grants from Boston Scientific. Dr. Ezekowitz reports grants and consulting fees from Boehringer Ingelheim, Pfizer, and Bristol-Myers Squibb; and consulting fees from Boston Scientific, Anthos Therapeutic, and Alta Therapeutics. Dr. Gersh reports consulting fees/honoraria from Xenon Pharmaceuticals; serving on a data safety monitoring board for Armetheon Inc, Baxter Healthcare Corporation, CardioVascular Research Foundation, Janssen Research & Development, Medtronic, Mount Sinai St. Luke's, Teva Pharmaceuticals, and Thrombosis Research Institute; and other support from Boston Scientific, Cipla Limited, Janssen Scientific Affairs LLC, and St. Jude Medical Inc. Dr. Granger reports grants and personal fees from Boehringer Ingelheim, Bristol-Myers Squibb, Janssen Pharmaceutica Products, L.P., and Pfizer; grants from AKROS, Apple, AstraZeneca, Daiichi-Sankyo, GlaxoSmithKline, Medtronic Foundation, Novartis Pharmaceutical Company, and US Food & Drug Administration; and personal fees from Abbvie, Bayer Corp US, Boston Scientific Corp, CeleCor Therapeutics, Corveio, Espero BioPharma, Medscape, Medtronic Inc, Merck, National Institutes of Health, NovoNordisk, Rhoshan Pharmaceuticals and Roche Diagnostics. Dr. Hylek reports consultant/advisory board fees from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Janssen, Medtronic, Pfizer, and Portola. Dr. Lopes reports institutional research grant and consulting fees from Bristol-Myers Squibb, GlaxoSmithKline, Medtronic, Pfizer and Sanofi; and consulting fees from Bayer, Boehringer Ingelheim, Daiichi Sankyo,

Merck, and Portola. Dr. Yusuf reports consulting fees, lecture fees, and grant support from Boehringer Ingelheim, AstraZeneca, Bristol-Myers Squibb, Sanofi-Aventis, Bayer, and Cadila. Dr. Wallentin reports institutional research grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, GlaxoSmithKline, Roche Diagnostics, and Merck & Co; consulting fees from Abbott; and holds two patents (EP2047275B1 and US8951742B2) licensed to Roche Diagnostics. Dr. Oldgren reports fees to his institution from AstraZeneca, Boehringer Ingelheim, Bayer, Bristol-Myers Squibb, Daiichi Sankyo, Pfizer, Portola, Roche Diagnostics, and Sanofi.

AUTHOR CONTRIBUTIONS

Julia Aulin contributed to conception, design, statistical analysis, interpretation, and drafting of the manuscript. Ziad Hijazi, John H. Alexander, Stuart J. Connolly, Michael D. Ezekowitz, Bernard J. Gersh, Christopher B. Granger, John Horowitz, Elaine M. Hylek, Renato D. Lopes, Salim Yusuf, Lars Wallentin, and Jonas Oldgren contributed to conception, design, analysis, interpretation, and critical revision. Agneta Siegbahn was responsible for the biobank and the biochemical analyses and contributed to conception, design, interpretation, and critical revision. Ulrika Andersson was responsible for the statistical analysis plan and the statistical analyses and contributed to design, interpretation, and critical revision.

REFERENCES

1. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. *Stroke*. 1991;22:983-988.
2. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham heart study. *Circulation*. 1998;98:946-952.
3. Hart RG, Pearce LA, Aguilar MI. Meta-analysis: antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. *Ann Intern Med*. 2007;146:857-867.
4. Ruff CT, Giugliano RP, Braunwald E, et al. Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. *Lancet*. 2014;383:955-962.
5. Yin WH, Chen JW, Jen HL, et al. Independent prognostic value of elevated high-sensitivity C-reactive protein in chronic heart failure. *Am Heart J*. 2004;147:931-938.
6. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in coronary artery disease. *N Engl J Med*. 2000;343:1139-1147.
7. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973-979.
8. Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. *JAMA*. 2001;286:2107-2113.
9. Aulin J, Siegbahn A, Hijazi Z, et al. Interleukin-6 and C-reactive protein and risk for death and cardiovascular events in patients with atrial fibrillation. *Am Heart J*. 2015;170:1151-1160.
10. Hijazi Z, Aulin J, Andersson U, et al. Biomarkers of inflammation and risk of cardiovascular events in anticoagulated patients with atrial fibrillation. *Heart*. 2016;102:508-517.

11. Granger CB, Alexander JH, McMurray JJ, et al. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2011;365:981-992.
12. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139-1151.
13. Lopes RD, Alexander JH, Al-Khatib SM, et al. Apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation (ARISTOTLE) trial: design and rationale. *Am Heart J*. 2010;159:331-339.
14. Ezekowitz MD, Connolly S, Parekh A, et al. Rationale and design of RE-LY: randomized evaluation of long-term anticoagulant therapy, warfarin, compared with dabigatran. *Am Heart J*. 2009;157:805-810.e1-2.
15. Hijazi Z, Siegbahn A, Andersson U, et al. High-sensitivity troponin I for risk assessment in patients with atrial fibrillation: insights from the Apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation (ARISTOTLE) trial. *Circulation*. 2014;129:625-634.
16. Hijazi Z, Wallentin L, Siegbahn A, et al. N-terminal pro-B-type natriuretic peptide for risk assessment in patients with atrial fibrillation: insights from the ARISTOTLE Trial (Apixaban for the Prevention of Stroke in Subjects With Atrial Fibrillation). *J Am Coll Cardiol*. 2013;61:2274-2284.
17. Hohnloser SH, Hijazi Z, Thomas L, et al. Efficacy of apixaban when compared with warfarin in relation to renal function in patients with atrial fibrillation: insights from the ARISTOTLE trial. *Eur Heart J*. 2012;33:2821-2830.
18. Wallentin L, Hijazi Z, Andersson U, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. *Circulation*. 2014;130:1847-1858.
19. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med*. 1996;15:361-387.
20. Hijazi Z, Oldgren J, Siegbahn A, Granger CB, Wallentin L. Biomarkers in atrial fibrillation: a clinical review. *Eur Heart J*. 2013;34:1475-1480.
21. Kikuchi S, Tsukahara K, Sakamaki K, et al. Comparison of anti-inflammatory effects of rivaroxaban vs. dabigatran in patients with non-valvular atrial fibrillation (RIVAL-AF study): multicenter randomized study. *Heart Vessels*. 2019;34:1002-1013.
22. Hijazi Z, Oldgren J, Andersson U, et al. Importance of persistent elevation of cardiac biomarkers in atrial fibrillation: a RE-LY substudy. *Heart*. 2014;100:1193-1200.
23. Zuo P, Zuo Z, Wang X, et al. Xa induces pro-inflammatory cytokine expression in RAW 264.7 macrophages via protease-activated receptor-2 activation. *Am J Transl Res*. 2015;7:2326-2334.
24. Borensztajn K, Stiekema J, Nijmeijer S, Reitsma PH, Peppelenbosch MP, Spek CA. Factor Xa stimulates proinflammatory and profibrotic responses in fibroblasts via protease-activated receptor-2 activation. *Am J Pathol*. 2008;172:309-320.
25. Belij S, Miljković D, Popov A, et al. Effects of subacute oral warfarin administration on peripheral blood granulocytes in rats. *Food Chem Toxicol*. 2012;50:1499-1507.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Aulin J, Hijazi Z, Siegbahn A, et al. Serial measurement of interleukin-6 and risk of mortality in anticoagulated patients with atrial fibrillation: Insights from ARISTOTLE and RE-LY trials. *J Thromb Haemost*. 2020;18:2287-2295. <https://doi.org/10.1111/jth.14947>