Short title: Influenza vaccination and myocardial infarction

Clinical Impact of Influenza Vaccination after ST- and Non-ST-segment elevation Myocardial Infarction

- Insights from the IAMI trial

Ole Fröbert, MD [1], Matthias Götberg, MD [2], David Erlinge, MD [2], Zubair Akhtar, MPH [3], Evald H. Christiansen, MD [4], Chandini R. MacIntyre, MBBS, PhD [5], Keith G. Oldroyd, MBChB, MD [6], Zuzana Motovska, MD [7], Andrejs Erglis, MD [8], Rasmus Moer, MD [9], Ota Hlinomaz, MD [10], Lars Jakobsen, MD [4], Thomas Engstrøm, MD [11], Lisette O. Jensen, MD [12], Christian O. Fallesen MD [12], Svend E Jensen MD [13], Oskar Angerås MD [14], Fredrik Calais, MD [1], Amra Kåregren, MD [15], Jörg Lauermann, MD [16], Arash Mokhtari, MD [2], Johan Nilsson, MD [17], Jonas Persson, MD [18], Per Stalby, MD [19], Abu K.M.M. Islam, MD [20], Afzalur Rahman, MD [20], Fazila Malik, MBBS [21], Sohel Choudhury, PhD [21], Timothy Collier, MSc [22], Stuart J. Pocock, PhD [22] and John Pernow, MD [23].

1) Örebro University, Faculty of Health, Department of Cardiology, Örebro, Sweden
2) Department of Cardiology, Skane University Hospital, Clinical Sciences, Lund University, Lund, Sweden
3) International Centre for Diarrhoeal Disease Research, Bangladesh, (icddr,b) Dhaka, Bangladesh
4) Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark
5) The Kirby Institute, UNSW Medicine, University of New South Wales, Sydney, New South Wales, Australia
6) Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom and West of Scotland Heart and Lung Centre, Golden Jubilee National Hospital, Clydebank, Glasgow, United Kingdom, UK
7) Cardiocenter, Third Faculty of Medicine, Charles University, Prague, Czech Republic and University Hospital Kralovske Vinohrady, Prague, Czech Republic
8) Pauls Stradins Clinical University Hospital, University of Latvia, Riga, Latvia
9) LHL-sykehuset Gardermoen, Oslo, Norway
10) International clinical research center, St. Anne University Hospital and Masaryk University, Brno, Czech Republic
11) Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
12) Department of Cardiology, Odense University Hospital, Odense, Denmark
13) Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark and Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
14) Sahlgrenska University Hospital, Gothenburg, Sweden and Institute of Medicine, Department of molecular and clinical medicine, Gothenburg University, Gothenburg, Sweden
15) Västmanlands sjukhus Västerås, Västerås, Sweden
16) Department of Cardiology, Jönköping, Region Jönköping County, and Department of Health, Medicine and Caring, Linköping University, Linköping, Sweden
17) Cardiology, Heart Centre, Department of Public Health and Clinical Medicine, Umeå University, Umea, Sweden
18) Division of Cardiovascular Medicine, Department of Clinical Sciences, Karolinska Institutet, Danderyd University Hospital, Stockholm, Sweden

19) Department of Cardiology, Karlstad Central Hospital, Karlstad, Sweden
20) National Institute of Cardiovascular Diseases, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh
21) National Heart Foundation Hospital & Research Institute, Dhaka, Bangladesh
22) Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK
23) Cardiology Unit, Department of Medicine Solna, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

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Address for correspondence

Ole Fröbert MD, PhD, FESC

Örebro University, Faculty of Health

Department of Cardiology

Södra Grev Rosengatan

701 85 Örebro

Sweden

Phone: +46 19 602 54 13

Fax: +46 19 602 54 38

Email: ole.frobert@regionorebrolan.se

Abstract

Background. Influenza vaccination early after myocardial infarction (MI) improves prognosis but vaccine effectiveness may differ dependent on type of MI.

Methods. A total of 2571 participants were prospectively enrolled in the IAMI trial and randomly assigned to receive in-hospital inactivated influenza vaccine or saline placebo. The trial was conducted at 30 centers in 8 countries from October 1, 2016 to March 1, 2020. Here we report vaccine effectiveness in the 2467 participants with ST-segment elevation MI (STEMI, n=1348) or non-ST-segment elevation MI (NSTEMI, n=1119). The primary endpoint was the composite of all-cause death, MI, or stent thrombosis at 12 months. Cumulative incidence of the primary and key secondary endpoints by randomized treatment and NSTEMI/STEMI was estimated using the Kaplan-Meier method. Treatment effects were evaluated with formal interaction testing to assess for effect modification.

Results. Baseline risk was higher in participants with NSTEMI. In the NSTEMI group the primary endpoint occurred in 6.5% of participants assigned to influenza vaccine and 10.5% assigned to placebo (hazard ratio [HR], 0.60; 95% CI, 0.39-0.91), compared to 4.1% assigned to influenza vaccine and 4.5% assigned to placebo in the STEMI group (HR, 0.90; 95% CI, 0.54-1.50, P=0.237 for interaction). Similar findings were seen for the key secondary endpoints of all-cause death and cardiovascular death. The Kaplan-Meier risk difference in all-cause death at 1 year was more pronounced in participants with NSTEMI (NSTEMI: HR, 0.47; 95% CI 0.28-0.80, STEMI: HR, 0.86; 95% CI, 0.43-1.70, interaction P=0.028).

Conclusions. The beneficial effect of influenza vaccination on adverse cardiovascular events may be enhanced in patients with NSTEMI compared to those with STEMI.

Keywords. Influenza vaccination; Percutaneous coronary intervention; Myocardial infarction.
Trial registration. URL: http://www.clinicaltrials.gov Unique identifier: NCT02831608.

Introduction

Inflammation is a key factor in atherosclerosis development and susceptibility to cardiovascular events. Influenza vaccination leads to rapid activation of the immune system and robust upregulation of genes involved in interferon signaling and antigen presentation pathways [1] while lowering of pro-inflammatory cytokines [2], and inducing anti-inflammatory and plaque stabilizing effects [3]. In the investigator-initiated, randomized, double-blind Influenza vaccination After Myocardial Infarction (IAMI) study we demonstrated that in-hospital influenza vaccination reduced the risk of the composite primary endpoint of all-cause death, acute myocardial infarction (MI), or stent thrombosis at 12 months in patients with MI or high-risk stable coronary heart disease [4]. Influenza vaccination also significantly reduced the risk of all-cause death and cardiovascular death, both key secondary endpoints. Our findings were in line with early smaller clinical trials of influenza vaccine vs. no vaccine or placebo in high-risk patients with cardiovascular disease [5-7].

Influenza **severity** and vaccine effectiveness differ depending on a number of risk factors including age, sex, diabetes mellitus, and smoking status [8 9 10 11]. The inflammatory environment may differ in patients with ST-segment elevation MI (STEMI) and non-ST-segment elevation MI (NSTEMI) potentially influencing vaccine effectiveness. In a prospective nested case-control study in Sweden, a substudy embedded in a large systematic risk factor screening program of healthy individuals, showed that biomarkers differed according to MI type among individuals who later developed a first MI. Patients with STEMI had higher levels of proteins associated with atherosclerosis severity and plaque development primarily mediated via altered phospholipid metabolism while patients with NSTEMI had higher levels of proteins associated with inflammation and macrophage activation, including interleukin (IL)-6, C-reactive protein, chemerin, and cathepsin X and D [12]. By contrast, admission levels of inflammatory markers were higher in patients with STEMI compared to patients with NSTEMI [13, 14]. In a study of 51 patients with MI levels of major histocompatibility complex class I related chain A and B microparticles were higher in patients with STEMI than in those with NSTEMI. Major histocompatibility complex class I molecules activate natural killer cells but this activation is inhibited by influenza virus [15]. Up to the age of 70 years in patients suffering from myocardial infarction, STEMI is more prevalent than NSTEMI while the latter is more prevalent later in life [16] implying that age-related chronic inflammation could play a role. It was

recently hypothesized that a possible influenza-independent effect of influenza vaccine in cardiovascular disease is driven by vaccine immunity and modulation of an ongoing immunoinflammatory response [17].

Given the differences in inflammatory activation, we hypothesized that the effect of influenza vaccination would differ depending of type of MI. The purpose of this substudy of the IAMI study was to determine outcomes in two prespecified subgroups – participants with STEMI or NSTEMI.

Methods

The IAMI study was a randomized, double-blind, placebo-controlled, investigator-initiated trial designed to evaluate effectiveness of influenza vaccine following MI or percutaneous coronary intervention (PCI) in high-risk patients with coronary artery disease. The trial was conducted at 30 centers in 8 countries (Sweden, Denmark, Norway, Latvia, the UK, Czech Republic, Bangladesh and Australia) from October 1, 2016 to March 1, 2020. Participants were enrolled during the influenza season from September through February in northern hemisphere sites, and from May through September in the southern hemisphere (Bangladesh and Australia).

A detailed account of the procedural details of the IAMI trial has been published elsewhere [4, 18]. This study is an exploratory analysis of prespecified subgroups. The trial was approved by the ethical review board and national regulatory authority of each participating site. The trial is registered at ClinicalTrials.gov as NCT02831608 and at the European Union Drug Regulating Authorities Clinical Trials Database as 2014-001354-42.

Inclusion criteria were age >18 years, STEMI (symptoms for at least 30 minutes before hospital admission, time from onset of symptoms of less than 24 hours) or NSTEMI, or stable coronary artery disease in patients >75 years of age who had at least one additional risk criterion and a completed coronary angiography or PCI. In Bangladesh, inclusion criteria did not include coronary angiography or PCI. Main exclusion criteria were influenza vaccination during the ongoing influenza season, or intention to be vaccinated during that influenza season.[4] Participants were not revaccinated within the trial setting but were allowed to obtain influenza vaccination outside of the study on their own behalf.

We randomly assigned participants in a 1:1 ratio to receive either influenza vaccine or placebo by study nurses not otherwise involved or participating in the study within 72 hours of coronary angiography/PCI or, in Bangladeshi centers, hospital admission. The study participants and all other study personnel were blinded to group assignment. Randomization lists were generated with a permuted block design prepared by a data scientist not involved in the trial and stratified according to trial site (block size 6).

We used 0.5 mL standard dose influenza vaccine containing 15 μg of hemagglutinin per strain consistent with WHO recommendations according to season and hemisphere; trivalent

inactivated vaccine (Vaxigrip) in the 2016 northern hemisphere season and quadrivalent inactivated vaccine (Vaxigrip Tetra or FluQuadri) in the following seasons. Influenza vaccine was provided by Sanofi Pasteur, which had no role in the design or conduct of the study or in preparation or review of the manuscript. Placebo was 0.5 mL sterile 0.9% normal saline solution.

The primary endpoint was the composite of all-cause death, MI, or stent thrombosis at 12 months post-randomization. In this study, we also report the key secondary effectiveness endpoints of all-cause death and cardiovascular death. All primary and secondary endpoints were adjudicated by an independent event committee of experienced cardiologists who were blinded to the trial group assignments.

STATISTICAL ANALYSIS

Baseline characteristics in relation to NSTEMI/STEMI patients were summarized using frequencies and percentages, mean and standard deviation, or median and interquartile range as appropriate. Differences in baseline characteristics across the groups were tested using a chi squared test for categorical variables or a two sample t-test for quantitative variables.

Cumulative incidence of the primary endpoint at 12 months by randomized treatment and NSTEMI/STEMI was estimated using the Kaplan-Meier method. Patients who were lost to follow-up at 12 months were censored on the day of randomization. The treatment effect of vaccination versus placebo for the primary endpoint was evaluated in NSTEMI and STEMI patients with formal interaction testing to assess for effect modification. Hazard ratios (HR) and 95% confidence intervals (CIs) were estimated using a Cox proportional hazards model (checked visually**)** including a treatment-subgroup interaction term. Absolute differences and 95% CIs at 12 months were calculated using the Kaplan–Meier method and Greenwood standard errors. This was repeated for the key secondary endpoints of all cause and cardiovascular mortality. We used the Kaplan–Meier method to obtain estimates and standard errors at 1 year and then used these for the test of homogeneity. Kaplan-Meier cumulative incidence plots by randomized treatment were generated for NSTEMI and STEMI patients. All analyses were performed using Stata version 16.1 (College Station, Texas).

Results

Due to the coronavirus disease 2019 pandemic, the data safety and monitoring board recommended on April 7, 2020 that it would not be feasible for the trial to continue and recruitment in the trial was stopped. In total, 2571 patients provided written informed consent and underwent randomization; 2532 received influenza vaccination or placebo and were included in the modified intention-to-treat analysis. In this study, only participants with a diagnosis of NSTEMI (n=1119) or STEMI (n=1348) were included, 2467 in total.

Participants with STEMI were more often male and smokers, were somewhat younger, but were less likely to have diabetes mellitus, hypertension, hyperlipidemia, or a previous cardiovascular event than participants with NSTEMI. Also, participants with STEMI more often had 1-vessel disease, were more often treated with PCI, but had lower left ventricular ejection fraction at discharge (Table 1).

In the NSTEMI group the primary composite endpoint occurred in 37 participants (6.5%) assigned to influenza vaccine and 58 (10.5%) assigned to placebo (HR 0.60; 95% CI 0.39 to 0.91) (Table 2, Figure 1), compared to 27 participants (4.1%) assigned to influenza vaccine and 31 (4.5%) assigned to placebo (HR 0.90; 95% CI 0.54 to 1.50) in the STEMI group (P=0.237 for interaction on HR scale and P=0.237 for interaction in Kaplan-Meier risk difference at 1 year). Similar findings were seen for the key secondary endpoints of all-cause death and cardiovascular death. However, the Kaplan-Meier risk difference in all-cause death at 1 year was more pronounced in participants with NSTEMI (HR 0.47; 95% CI 0.27 to 0.80, interaction P=0.028). There were 8 non-cardiovascular deaths (3 in the vaccine group and 5 in the placebo group, Table 2) and because of relatively few events and balance between groups we chose not to perform a competing risk analysis.

Because treatment practices differed between Bangladeshi sites, where PCI is not standard treatment following myocardial infarction, and the other participating countries, where PCI is standard, we analyzed outcomes for Bangladesh separately. In Bangladesh 620 patients were enrolled and all were enrolled in the last study season only (2019-20). In NSTEMI participants the primary composite endpoint occurred in 22/179 participants (12.3%) assigned to influenza vaccine and 35/180 (19.4%) assigned to placebo (HR 0.59; 95% CI 0.35 to 1.01), compared to 12/117 (9.3%) assigned to influenza vaccine and 13/117 (10.0%) assigned to placebo (HR 0.93;

95% CI 0.43 to 2.05) in the STEMI group (p=0.35 for interaction on HR scale and p=0.22 for interaction in Kaplan-Meier risk difference at 1 year).

Serious adverse events were rare, and the incidence was similar in the vaccine and placebo groups while injection site reactions were reported significantly more often in participants assigned to influenza vaccine as previously reported [4].

Table 1, baseline and discharge characteristics

Patient Characteristic	Statistic	Overall (n=2467)	NSTEMI (n=1119)	STEMI (n=1348)	P-value
Randomised allocation					
Placebo	n (%)	1234 (50.0)	551 (49.2)	683 (50.7)	
Influenza vaccine	n (%)	1233 (50.0)	568 (50.8)	665 (49.3)	0.480
Sex					
Male	n (%)	2016 (81.7)	889 (79.4)	1127 (83.6)	
Female	n (%)	451 (18.3)	230 (20.6)	221 (16.4)	0.0078
Age at randomization	Mean (SD)	59.7 (11.2)	60.2 (11.0)	59.3 (11.2)	0.046
	Median (IQR)	60 (52, 67)	60 (52, 68)	59 (52, 67)	
Smoking					
Never smoked	n/N (%)	894/2400 (37.3)	457/1095 (41.7)	437/1305 (33.5)	
Former smoker	n/N (%)	641/2400 (26.7)	330/1095 (30.1)	311/1305 (23.8)	
Current smoker	n/N (%)	865/2400 (36.0)	308/1095 (28.1)	557/1305 (42.7)	<0.0001
Medical History/Comorbidities					
Diabetes	n/N (%)	523/2452 (21.3)	319/1111 (28.7)	204/1341 (15.2)	<0.0001
Hyperlipidemia	n/N (%)	801/2451 (32.7)	492/1112 (44.2)	309/1339 (23.1)	<0.0001
Hypertension	n/N (%)	1205/2447 (49.2)	677/1114 (60.8)	528/1333 (39.6)	<0.0001
Previous MI	n/N (%)	345/2448 (14.1)	219/1113 (19.7)	126/1335 (9.4)	<0.0001
Previous PCI	n/N (%)	246/2459 (10.0)	148/1114 (13.3)	98/1345 (7.3)	<0.0001
Previous CABG	n/N (%)	64/2460 (2.6)	47/1116 (4.2)	17/1344 (1.3)	<0.0001
Killip class ≥2	n/N (%)	95/2266 (4.2)	45/942 (4.8)	50/1324 (3.8)	0.241
Left main coronary artery disease	n/N (%)	123/2442 (5.0)	69/1099 (6.3)	54/1343 (4.0)	0.011
Coronary findings and treatment					
1-vessel disease	n/N (%)	1122/2391 (46.9)	433/1071 (40.4)	689/1320 (52.2)	
2-vessel disease	n/N (%)	501/2391 (21.0)	211/1071 (19.7)	290/1320 (22.0)	
3-vessel disease	n/N (%)	334/2391 (14.0)	181/1071 (16.9)	153/1320 (11.6)	
normal/atheromatosis	n/N (%)	55/2391 (2.3)	48/1071 (4.5)	7/1320 (0.5)	
N/A	n/N (%)	379/2391 (15.9)	198/1071 (18.5)	181/1320 (13.7)	<0.0001
PCI	n/N (%)	1829/2460 (74.3)	714/1114 (64.1)	1115/1346 (82.8)	<0.0001
Treated with CABG	n/N (%)	46/2455 (1.9)	34/1110 (3.1)	12/1345 (0.9)	<0.0001
Medical treatment only	n/N (%)	577/2454 (23.5)	354/1109 (31.9)	223/1345 (16.6)	<0.0001
LVEF at discharge					
Normal (≥50%)	n/N (%)	1119/1861 (60.1)	574/829 (69.2)	545/1032 (52.8)	
Slightly reduced (40-49%)	n/N (%)	515/1861 (27.7)	179/829 (21.6)	336/1032 (32.6)	
Moderately reduced (30-39%)	n/N (%)	186/1861 (10.0)	56/829 (6.8)	130/1032 (12.6)	
Severely reduced (<30%)	n/N (%)	41/1861 (2.2)	20/829 (2.4)	21/1032 (2.0)	<0.0001
Medication at discharge					
Aspirin	n/N (%)	2349/2397 (98.0)	1060/1085 (97.7)	1289/1312 (98.2)	0.338
P2Y12-inhibitor	n/N (%)	2326/2386 (97.5)	1021/1074 (95.1)	1305/1312 (99.5)	<0.0001
Beta-blocker	n/N (%)	1860/2387 (77.9)	842/1078 (78.1)	1018/1309 (77.8)	0.843
ACEi or ARB	n/N (%)	1668/2381 (70.1)	762/1071 (71.1)	906/1310 (69.2)	0.292
Statin	n/N (%)	2350/2394 (98.2)	1058/1083 (97.7)	1292/1311 (98.6)	0.119

Table 2, primary and key secondary endpoints

Endpoint	N	Number (%)[a] with event			Hazard Ratio[b] (95% CI)		P_{int}	KM Risk Difference[c] (95% CI)	P_{int}
		All	Vaccine	Placebo					
Primary Endpoint									
NSTEMI	1,119	95 (8.5)	37 (6.5)	58 (10.5)	0.60	(0.39-0.91)		-4.0% (-7.3%, -0.7%)	
STEMI	1,348	58 (4.3)	27 (4.1)	31 (4.5)	0.90	(0.54-1.50)	0.237	-0.5% (-2.6%, 1.7%)	0.077
All Cause Death									
NSTEMI	1,119	63 (5.6)	21 (3.7)	42 (7.6)	0.47	(0.28-0.80)		-3.9% (-6.6%, -1.2%)	
STEMI	1,348	33 (2.5)	15 (2.3)	18 (2.6)	0.86	(0.43-1.70)	0.179	-0.4% (-2.0%, 1.3%)	0.028
CV Death									
NSTEMI	1,119	56 (5.0)	19 (3.4)	37 (6.7)	0.49	(0.28-0.85)		-3.4% (-5.9%, -0.8%)	
STEMI	1,348	32 (2.4)	14 (2.1)	18 (2.6)	0.80	(0.40-1.61)	0.276	-0.5% (-2.2%, 1.1%)	0.066

[a] percentages are cumulative Kaplan-Meier at 1 year; [b] hazard ratio (vaccine v placebo); [c] KM=Kaplan-Meier risk difference at 1 year (vaccine v placebo); N=total number of patients; P_{int} = interaction p-value;

Figure 1

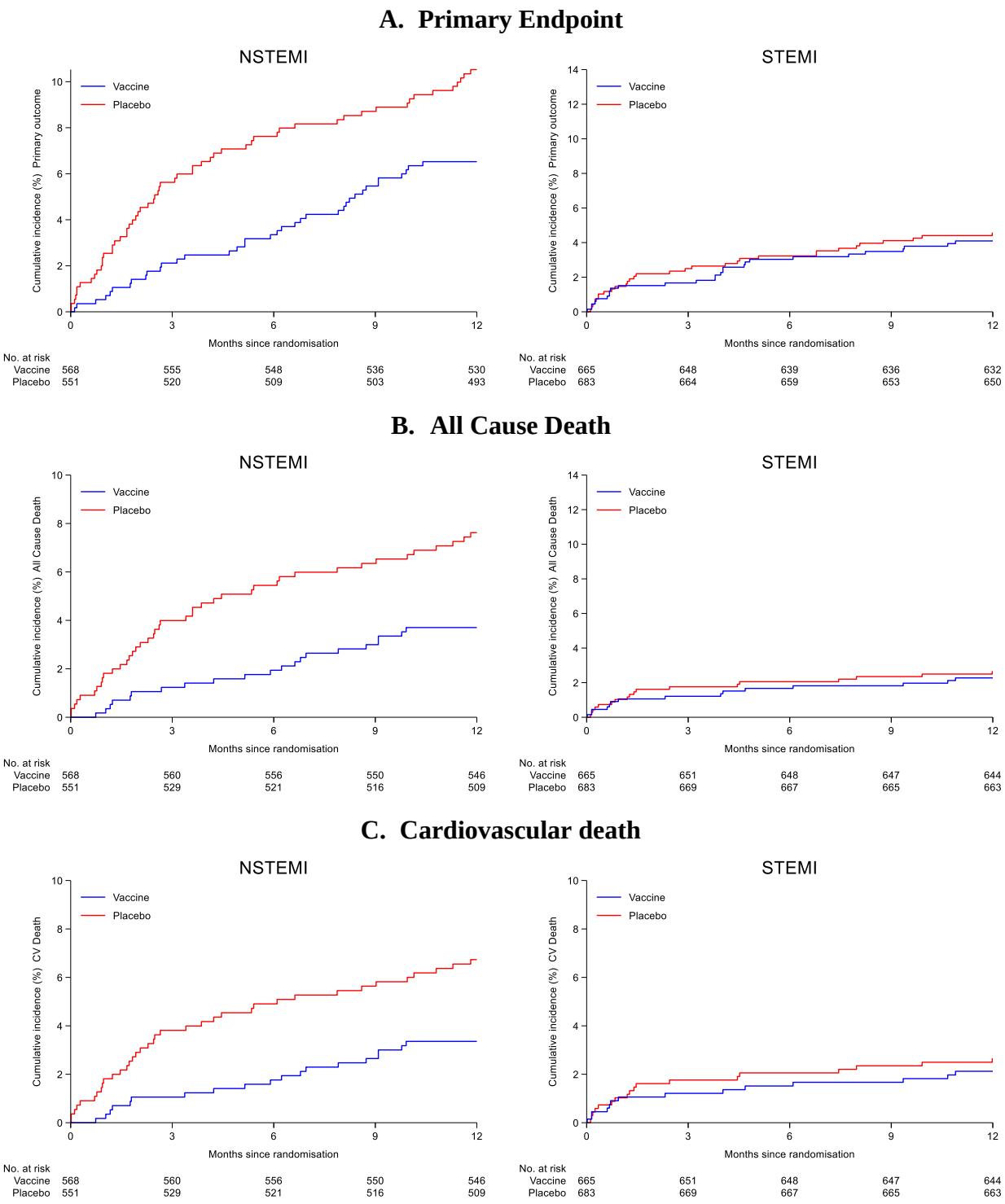

Figure 1. Kaplan-Meier event curves of the influenza vaccine and placebo groups in patients with NSTEMI (left panels) and STEMI (right panels) for the primary composite endpoint of all-cause death, myocardial infarction, or stent thrombosis in a time-to-event analysis (A); for all-cause death (B); and for cardiovascular death (C).

Discussion

In this IAMI trial substudy we found that the beneficial effect of influenza vaccination seemed to be more pronounced in participants with NSTEMI compared to participants with STEMI. The trial was not powered for investigating differences between NSTEMI and STEMI and our findings should be interpreted with caution. Although participants with STEMI were more frequently smokers and had a lower ejection fraction at discharge, participants with NSTEMI had a higher risk profile overall. Most notably, and largely driven by centers in Bangladesh where medical treatment alone without PCI is routine [19], only two-thirds of participants in the NSTEMI group were treated with PCI as opposed to more than 80% in the STEMI group. One explanation to a potential enhanced vaccine effectiveness in participants with NSTEMI could be a higher risk profile and more to gain from influenza vaccination. Notwithstanding, effectiveness of influenza vaccination declines with advancing age [20] and is likely lower in individuals with comorbidities [21] which implies that other factors may be of importance.

Observational studies on myocardial infarction and insights from inflammation research could help explain the surprising and apparent large differences in vaccine effectiveness on cardiovascular outcomes between groups. Previous findings point to NSTEMI and STEMI being distinct pathophysiological entities. Findings from pathology and optical coherence tomography studies, summarized by Pasterkamp et al., indicate that plaque rupture is almost twice as frequent in patients with STEMI versus patients with NSTEMI [22]. In patients with recurrent MI the majority have either repeated episodes of NSTEMI or STEMI but not both, indicating predilection to repeated episodes of occlusive thrombi or to repeated episodes of non-occlusive thrombi [23]. Whereas the medium-term prognosis is almost identical, patients with NSTEMI are at increased risk for death at any time after PCI, while the mortality of STEMI patients is higher during the first 30 days after PCI but lower thereafter [24].

Anti-inflammatory therapies with canakinumab, a selective IL-1β monoclonal antibody, or colchicine reduce the risk of future cardiovascular events following a recent MI but do not reduce all-cause mortality [25, 26]. While outcome data concerning type of MI from large anti-inflammatory therapy trials have not been published a recent small trial randomized a balanced number of patients with NSTEMI/unstable angina or STEMI to colchicine or placebo. In that study, colchicine also reduced cardiovascular events but only three out of a total of 36 events occurred in patients with STEMI [27].

Differences in inflammatory activation between NSTEMI and STEMI could help explain a more pronounced effect of influenza vaccine in participants with NSTEMI in this study. In the IAMI study, the beneficial effect of influenza vaccination was much larger and occurred earlier (within weeks) than could be expected from a reduction in influenza illness. A possible explanation of the findings in IAMI could be an immunomodulating effect of influenza vaccine on immune homeostasis, limiting dysregulated post-infarction inflammation. Following influenza vaccination, a number of cytokines are up- or downregulated. Interleukin-2 rises three-fold 6 weeks after immunization while IL-6 and tumor necrosis factor-α (TNFα) are virtually unchanged one week after vaccination in healthy volunteers [28]. Interestingly, influenza vaccination also modulates cytokine signaling in response to various stimuli. In a randomized placebo-controlled study influenza vaccination in the week prior to coronary artery bypass surgery, a procedure resulting in a major inflammatory response, was associated with a substantial reduction in pro-inflammatory cytokine levels (IL-6, IL-8 and TNFα) while the level of the anti-inflammatory marker IL-10 was five-fold higher in the vaccinated patients [2]. Another possible mechanism of action involves the observation that antibodies elicited by influenza vaccines activate kinin B2 receptors, which are of importance in preventing arrhythmias, thrombosis, and reducing infarct size [29]. These findings suggest transcriptomic reprogramming of immune cells and one explanation could be that influenza vaccination post MI protects from residual myocardial inflammation by inducing a fine-tuned balanced immune response with both up- and downregulated pathways of inflammation. Such an immune response may be of greater importance in a more chronic inflammatory condition like NSTEMI. Documenting immunological effects of influenza vaccination in cardiac tissues require additional studies – e.g. by ^{68}Ga-DOTATATE PET imaging and immune stimulation assays [30].

This study has several limitations. In the IAMI study reporting of outcome in the NSTEMI and STEMI groups was prespecified but the study was not powered to assess differences between the two groups and our findings should be considered associative and not causative. The observational nature of this substudy implies risk of residual confounding and we noted significant differences in important baseline and discharge clinical variables. Blood was not collected in the IAMI study and potential differences in NSTEMI and STEMI with regard to inflammation described in previous studies could not be confirmed in this study.

In conclusion, the beneficial effect of influenza vaccination on adverse cardiovascular events may be enhanced in patients with NSTEMI compared to those with STEMI. It is conceivable

that these findings could be explained by differences in inflammatory activation in NSTEMI and STEMI. Our findings may inform future studies but influenza vaccination remains strongly recommended in all patients with cardiovascular disease.

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CRediT authorship contribution statement

Ole Fröbert: Conceptualization, Methodology, Validation, Writing – original draft, Investigation, Funding acquisition. **Matthias Götberg:** Validation, Supervision, Writing – review & editing, Investigation. **David Erlinge:** Validation, Supervision, Writing – review & editing, Investigation. **Zubair Akhtar:** Writing – review & editing, Investigation. **Evald H. Christiansen:** Validation, Supervision, Writing – review & editing, Investigation, Funding acquisition. **Chandini R. MacIntyre:** Validation, Supervision, Writing – review & editing, Investigation. **Keith G. Oldroyd:** Supervision, Writing – review & editing, Investigation. **Zuzana Motovska:** Writing – review & editing, Investigation. **Andrejs Erglis:** Writing – review & editing, Investigation. **Rasmus Moer:** Writing – review & editing¸ Investigation. **Ota Hlinomaz**: Writing – review & editing, Investigation. **Lars Jakobsen:** Writing – review & editing, Investigation. **Thomas Engstrøm:** Writing – review & editing, Investigation. **Lisette O. Jensen:** Writing – review & editing, Investigation. **Christian O. Fallesen:** Writing – review & editing, Investigation. **Svend E Jensen:** Writing – review & editing, Investigation. **Oskar Angerås:** Writing – review & editing, Investigation. **Fredrik Calais:** Writing – review & editing, Investigation. **Amra Kåregren:** Writing – review & editing, Investigation. **Jörg**

Lauermann: Writing – review & editing, Investigation. **Arash Mokhtari:** Writing – review & editing, Investigation. **Johan Nilsson:** Writing – review & editing, Investigation. **Jonas Persson:** Writing – review & editing, Investigation. **Per Stalby:** Writing – review & editing, Investigation. **Abu K.M.M. Islam:** Writing – review & editing, Investigation. **Afzalur Rahman:** Writing – review & editing, Investigation. **Fazila Malik:** Writing – review & editing, Investigation. **Sohel Choudhury:** Writing – review & editing, Investigation. **Timothy Collier:** Data Curation, Formal analysis, Writing – review & editing. **Stuart J. Pocock:** Formal analysis, Writing – review & editing. **John Pernow:** Validation, Supervision, Writing – review & editing, Investigation.

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