Time of day for vaccination, outcomes, and relative effectiveness of high-dose vs. standard-dose quadrivalent influenza vaccine: a post-hoc analysis of the DANFLU-1 randomized clinical trialRunning title: Circadian timing of vaccination in the DANFLU-1 trial

Jacob Christensen, Niklas Dyrby Johansen, Kira Hyldekær Janstrup, Daniel Modin, Kristoffer Grundtvig Skaarup, Joshua Nealon, Sandrine Samson, Matthew Loiacono, Rebecca Harris, Carsten Schade Larsen, Anne Marie Reimer Jensen, Nino Emanuel Landler, Brian L. Claggett, Scott D. Solomon, Gunnar H. Gislason, Lars Køber, Martin J. Landray, Pradeesh Sivapalan, Jens Ulrik Stæhr Jensen, Tor Biering-Sørensen

PII: S0163-4453(24)00210-X

DOI: https://doi.org/10.1016/j.jinf.2024.106276

Reference: YJINF106276

To appear in: *Journal of Infection*

Accepted date: 13 September 2024

Please cite this article as: Jacob Christensen, Niklas Dyrby Johansen, Kira Hyldekær Janstrup, Daniel Modin, Kristoffer Grundtvig Skaarup, Joshua Nealon, Sandrine Samson, Matthew Loiacono, Rebecca Harris, Carsten Schade Larsen, Anne Marie Reimer Jensen, Nino Emanuel Landler, Brian L. Claggett, Scott D. Solomon, Gunnar H. Gislason, Lars Køber, Martin J. Landray, Pradeesh Sivapalan, Jens Ulrik Stæhr Jensen and Tor Biering-Sørensen, Time of day for vaccination, outcomes, and relative effectiveness of high-dose vs. standard-dose quadrivalent influenza vaccine: a post-hoc analysis of the DANFLU-1 randomized clinical trialRunning title: Circadian timing of vaccination in the DANFLU-1 trial, *Journal of Infection,* (2024) doi:https://doi.org/10.1016/j.jinf.2024.106276

Time of day for vaccination, outcomes, and relative effectiveness of high-dose vs. standard-dose quadrivalent influenza vaccine: a post-hoc analysis of the DANFLU-1 randomized clinical trial

Jacob Christensen[1,2], MB; Niklas Dyrby Johansen[1,2], MD; Kira Hyldekær Janstrup[1,2], PhD; Daniel Modin[1,2], MD; Kristoffer Grundtvig Skaarup[1,2], MD; Joshua Nealon[3], PhD; Sandrine Samson[3], PhD; Matthew Loiacono[4], PhD; PhD; Rebecca Harris[5], MBioch, MSc, PhD; Carsten Schade Larsen[6], MD, DMSc; Anne Marie Reimer Jensen[1,2], MD; Nino Emanuel Landler[1,2], MD, PhD; Brian L. Claggett[7], PhD; Scott D. Solomon[8], MD; Gunnar H. Gislason[9], MD, PhD; Lars Køber[10], MD, DMSc; Martin J. Landray[11,12], PhD; Pradeesh Sivapalan[13,14], MD, PhD; Jens Ulrik Stæhr Jensen[13,14], MD, PhD; Tor Biering-Sørensen[1,2,15,16], MD, MSc, MPH, PhD

1) Center for Translational Cardiology and Pragmatic Randomized Trials (CTCPR), Copenhagen University Hospital – Herlev and Gentofte, Copenhagen, Denmark

2) Cardiovascular Non-Invasive Imaging Research Laboratory, Department of Cardiology, Copenhagen University Hospital - Herlev and Gentofte.

3) Sanofi, Lyon, France

4) Sanofi, Swiftwater, United States of America

5) Sanofi, Singapore

6) Department of Clinical Medicine, Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark

7) Harvard Medical School, Cardiovascular Division, Brigham and Women's Hospital, Boston, United States of America

8) Harvard Medical School, Boston, United States of America

9) Department of Cardiology, Gentofte University Hospital, Copenhagen, Denmark

10) Department of Cardiology, Rigshospitalet - Copenhagen University Hospital, Copenhagen, Denmark

11) Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Public Health, University of Oxford, Oxford, United Kingdom

12) Big Data Institute, University of Oxford, Oxford, United Kingdom

13) Department of Medicine, Respiratory Medicine Section, Copenhagen University Hospital—Herlev and Gentofte, Copenhagen, Denmark

14) Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

15) Steno Diabetes Center Copenhagen

16) Department of Cardiology, Copenhagen University Hospital - Rigshospitalet

Funding: Sanofi

Trial Registration: Clinicaltrials.gov: NCT05048589

Running title: Circadian timing of vaccination in the DANFLU-1 trial

Abstract word count: 200

Manuscript word count: 2,563

Keywords:

- Influenza, Human
- Vaccination
- Pragmatic Clinical Trial
- Outcome Assessment, Health Care

Correspondence:

Tor Biering-Sørensen, MD, PhD, MPH

Center for Translational Cardiology and Pragmatic Randomized Trials, Department of Cardiology, Copenhagen University Hospital, Herlev & Gentofte, Denmark

Gentofte Hospitalsvej 8, 3.th., 2900 Hellerup, Denmark.

E-mail: tor.biering@gmail.com

Abstract

Objectives: Morning influenza vaccination enhances antibody response. In this posthoc analysis of the DANFLU-1 trial, we sought to evaluate the association between time of day for vaccination (ToV) and outcomes, and whether ToV modified the relative effectiveness of high-dose (QIV-HD) vs. standard-dose (QIV-SD) quadrivalent influenza vaccine. Methods: DANFLU-1 was a pragmatic feasibility trial of QIV-HD vs. QIV-SD. Outcomes included hospitalizations and mortality. For subgroup analysis, the population was dichotomized at median ToV into two groups (early and late). Results: The study population included 12,477 participants. Mean age was 71.7±3.9 years with 5,877 (47.1%) female participants. Median ToV was 11.29AM. Earlier ToV was associated with fewer respiratory hospitalizations independent of vaccine type, which persisted in adjusted analysis (IRR 0.88 per 1-hour decrement (95% CI 0.78- 0.98, p=0.025). No effect modification by continuous or dichotomous ToV was found. In subgroup analysis, effects consistently favored QIV-HD against hospitalizations for pneumonia or influenza (early: IRR 0.30; late: 0.29), all-cause hospitalizations (early: IRR 0.87; late: 0.86), and mortality (early: HR 0.53; late: 0.50). Conclusion: In this exploratory post-hoc analysis, earlier ToV was associated with fewer respiratory hospitalizations. The relative effectiveness of QIV-HD vs. QIV-SD was not modified by ToV. Further research is needed to confirm findings.

Background

The circadian rhythmicity of the adaptive immune system is becoming an increasingly active field of research.[1,2] As a likely consequence of this phenomenon, previous studies have found variations in antibody responses to influenza vaccination according to the time of day for inoculation.[3,4] Of note, several studies have demonstrated that morning vaccination is associated with an increased antibody response compared with afternoon vaccination in older adult populations.[4,5] Importantly, increased response is linked with superior protective effect against clinical outcomes.[6,7] Since older adults aged ≥65 years are at increased risk for severe influenza-associated illness, hospitalization, and death compared with younger persons,[8,9] and additionally respond less well to influenza vaccination,[10,11] the relationship between circadian timing of influenza vaccination and clinical outcomes warrant further investigation.

In addition to timing of vaccination, high-dose influenza vaccine has been shown to induce a higher serological response than standard-dose vaccine in older adults, yielding a response similar to that of standard-dose vaccination in younger adults.[12] As such, the high-dose influenza vaccine, with four-times the antigen content of the standard-dose, has demonstrated superior efficacy against lab-confirmed influenza infection,[6] as well as consistently improved effectiveness against severe clinical outcomes.[13]

The recent study titled *Feasibility of randomizing Danish citizens aged 65-79 years to high-dose quadrivalent influenza vaccine vs standard-dose quadrivalent influenza vaccine in a pragmatic registry-based setting,* or DANFLU-1 in short, was a pragmatic, open-label, randomized feasibility trial of the relative effectiveness of high-dose quadrivalent influenza vaccines (QIV-HD) vs. standard-dose quadrivalent influenza vaccines (QIV-SD) in adults aged 65-79 years.[14] In the trial, QIV-HD was associated with a lower incidence of

hospitalizations for influenza or pneumonia, all-cause hospitalizations,[15] and all-cause mortality compared with QIV-SD, which now remains to be replicated in a fully powered randomized trial. Given that the clinical effect of high-dose influenza vaccines likely relies upon an increased serological response, and considering that the time of day can influence the serological response to influenza vaccination, it might be of interest to test whether this timing effect also extends to the relative effectiveness of QIV-HD compared with QIV-SD. In this post-hoc analysis of the DANFLU-1 trial, we therefore sought to investigate whether time of day for vaccination (ToV) was associated with hospitalizations and mortality. Furthermore, we sought to investigate whether ToV modified the effects of QIV-HD compared with QIV-SD against clinical outcomes.

Methods

Study design and population

The study design and main results of the DANFLU-1 trial have been described in detail elsewhere.[16] The trial enrolled participants aged between 65-79 years regardless of medical history; the only exclusion criterion was allergy toward the study vaccines. Enrolment of participants was mediated by a private vaccination provider tasked with organising influenza vaccination sessions as part of the Danish national vaccination programme. The study was monitored by a central site which oversaw registry-based data collection and safety monitoring. Presence of disease at baseline was obtained from the National Patient Registry using International Classification of Diseases, 10th Edition, and Anatomical Therapeutic Chemical codes. These codes are provided in the supplemental material of a prior publication.[14] Written informed consent was obtained from all participants. The trial was registered at Clinicaltrials.gov: NCT05048589 and was approved by the Regional Danish Committee on Biomedical Research Ethics (H-21035316) and the Danish Medicines Agency (EudraCT no. 2021-003170-31).

Randomisation

Using centralized blocked randomization, participants were randomly assigned in a ratio of 1:1 to receive either QIV-SD or QIV-HD. Treatment assignment was not blinded, but subsequent data collection of prespecified health data was performed passively through patient-registries, thus minimizing the risk of differential ascertainment bias.

Study procedures

QIV-SD contained 15 μg of hemagglutinin antigen for each influenza strain, while QIV-HD (Fluzone High-Dose Quadrivalent [United States and Canada]/Efluelda [Europe]; Sanofi) contained 60 μg of hemagglutinin antigen for each strain. Both vaccines contained the four influenza strains recommended by the World Health Organization for the 2021–2022 Northern Hemisphere influenza season. Information regarding randomization group, administered vaccine, and ToV were obtained at inclusion. Vaccination took place between 07:05 (hour:minute) and 20:08. All other trial data were obtained subsequently by the central site from nationwide health registries. The follow-up period for prespecified outcomes was defined as starting from 14 days post-vaccination until May 31st, 2022.

Outcomes

We evaluated the same prespecified outcomes with the same methodology as a prior analysis of the DANFLU-1 trial.[15] These included hospitalization due to 1) pneumonia or influenza, (2) respiratory disease, (3) cardiorespiratory disease, (4) cardiovascular disease; (5) all causes, and (6) all-cause mortality. All outcomes were assessed as intention-to-treat.

Statistical analysis

ToV was assessed as a continuous variable with 1-hour increments. To investigate whether ToV was associated with outcomes, hospitalizations were assessed as recurrent events using negative binomial regression and further with Cox proportional hazards regression in time-to-event analysis. All-cause mortality was assessed using Cox proportional hazards regression. All regressions were adjusted for vaccine type received. Multivariable regression was

employed to further adjust for potential confounders including sex, age, diabetes mellitus, chronic obstructive pulmonary disease, asthma, chronic lung disease, chronic cardiovascular disease, chronic kidney disease, immunodeficiencies, and cancer. In an effort to reduce the risk that potential associations between continuous ToV and outcomes were driven by few events among participants vaccinated later in the day, three pairs of unadjusted and adjusted analyses were performed, the first of which included all hours of day, while the latter two were restricted to only include the hours of day with at least 500 and 100 vaccinations, respectively. In subgroup analysis, the population was dichotomized into two groups according to the median ToV which was 11.29 AM. These groups were denoted the early and late vaccination groups, respectively. Baseline clinical characteristics according to these were reported, and results were presented as mean with standard deviation (SD) for parametric continuous variables, median with interquartile interval (IQI) for non-parametric continuous variables, and counts with percentages for categorical variables, respectively. To assess whether relative effectiveness of QIV-HD vs. QIV-SD differed according to continuous ToV, splines were constructed using negative binomial regression models for hospitalizations and logistic regression models for all-cause mortality. Regression models for each vaccine type (ie. QIV-HD and QIV-SD) were constructed separately and used to fit incidence rates for hospitalizations and odds for mortality for each hour of continuous ToV. For hospitalizations, incidence rate ratios between QIV-HD and QIV-SD were calculated by dividing fitted incidence rates on a per-hour basis, and the pertaining standard errors were calculated by taking the square root of the sum of the two individual standard errors squared. A similar approach with odds ratios was taken for all-cause mortality. For the spline terms, the number of knots resulting in the lowest Akaike information criterion were chosen for each regression model. Effect modification by ToV was tested using interaction terms between ToV and vaccine type in separate regression models fitted on both randomization groups collectively.

The findings of this post-hoc analysis should be considered hypothesis-generating as the DANFLU-1 trial was not specifically powered for this analysis, and no adjustment for multiplicity has been applied. A two-sided statistical significance threshold of 0.05 was used. Analyses were performed using R version 4.3.3 (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

Results

The trial population was enrolled between October 1st, 2021, and November 20th, 2021, and consisted of 12,477 participants with a mean age of 71.7 years (SD 3.9 years), and of whom 5,877 (47.1%) were female. Additional baseline characteristics of the trial population have been published in previous works.[15] Baseline characteristics of the early and late vaccination groups are displayed in **table 1**. Subjects in the late vaccination group were generally more likely to be female and had higher overall prevalence of comorbidities including pulmonary disease, cardiovascular disease, chronic kidney disease, and diabetes.

The distribution of vaccinations according to ToV can be seen in **figure 1**. Earlier continuous ToV was associated with lower incidence of respiratory hospitalizations, both in recurrent event analysis (IRR 0.89 per 1-hour decrement (95% CI 0.79-1.00), $p = 0.048$) and in time-to-event analysis (HR 0.85 per 1-hour decrement (95% CI 0.78-0.94), $p = 0.003$) regardless of vaccine type (**table 2** and **figure 1**). These associations persisted in fully adjusted analyses (IRR 0.88 (95% CI 0.78-0.98), $p = 0.025$, and HR 0.87 (95% CI 0.79-0.97), $p = 0.010$). When restricted to the hours of day with at least 500 and 100 vaccinations, effects sizes remained consistent (**figure 1**). No other outcomes were associated with earlier ToV. Number of events and incidence rates according to continuous ToV for all outcomes can be seen in **supplemental table 1**.

Relative effectiveness of QIV-HD vs. QIV-SD

In recurrent event analysis, no significant effect modification by dichotomous or continuous ToV was found for any of the outcomes assessed. In the entire population, QIV-HD compared with QIV-SD was associated with a lower incidence of hospitalizations for pneumonia or influenza (IRR 0.30 (95% CI 0.14-0.64), $p = 0.002$) and all-cause

hospitalizations (IRR 0.87 (95% CI 0.76-0.99), $p = 0.032$). In subgroup analysis, estimates consistently favored QIV-HD across both the early and late vaccination group for both hospitalizations for pneumonia or influenza (early group: IRR 0.31 (95% CI 0.09-1.09); late group: IRR 0.29 (95% CI 0.11-0.76); p for interaction = 0.93) and all-cause hospitalizations (early group: IRR 0.87 (95% CI 0.73-1.05); late group: IRR 0.86 (95% CI 0.71-1.03); p for interaction = 0.89) (**figure 2**).

In time-to-event analysis, QIV-HD compared with QIV-SD was associated with a lower rate of hospitalizations for pneumonia or influenza (HR 0.36 (95% CI 0.17-0.73), $p = 0.005$) and all-cause mortality (HR 0.51 (95% CI 0.30-0.86), $p = 0.012$) in the entire population. In subgroup analysis, effects consistently favored QIV-HD across the early and late group for both hospitalizations for pneumonia or influenza (early group: HR 0.40 (95% CI 0.13-1.28); late group: HR 0.33 (95% CI 0.13-0.83); p for interaction = 0.80) and all-cause mortality (early group: HR 0.53 (95% CI 0.24-1.19); late group: HR 0.50 (95% CI 0.25-0.99); p for interaction = 0.90) (**figure 3**).

Splines illustrating incidence rate ratios and odds ratios for QIV-HD vs. QV-SD according to continuous ToV can be seen in **figure 4**. Effects consistently favored QIV-HD regardless of continuous ToV for all outcomes assessed excluding cardiovascular hospitalizations.

Discussion

In this post-hoc analysis of a pragmatic, randomized trial with >12,000 participants, we found that earlier ToV was associated with lower incidence of respiratory hospitalizations, independent of vaccine type and sex, age, and comorbidities. In addition to this, we found that the relative effectiveness of QIV-HD vs. QIV-SD against hospitalizations for pneumonia or influenza, all-cause hospitalizations, and all-cause mortality did not vary with ToV. These exploratory findings suggest that earlier time of day for influenza vaccination might be associated with a lower risk of respiratory hospitalizations, and furthermore that the effects of QIV-HD compared with QIV-SD are independent of time of day for vaccination. These findings remain to be tested in future research.

To our knowledge, this is the first study to investigate time of day for influenza vaccination against clinical outcomes as previous studies have focused on antibody response. Our findings indicated an association between earlier ToV and lower incidence of hospitalizations for respiratory disease, which might suggest a potential benefit of morning vaccination, though it should be regarded as an exploratory finding. Though the circadian rhythmicity of the adaptive immune system remains less extensively studied than that of its innate counterpart, the underlying mechanisms are believed to involve trafficking of T and B lymphocytes, regulation of T cell activation and proliferation, and so-called clock genes influencing gene expression, among other things.[17] Diurnal variations in these likely precipitate the varying antibody responses following vaccination observed in prior studies. As a proposed technique to utilize this phenomenon, circadian timing of vaccinations, or chronovaccination,[18] has been a subject of interest for some time, as it potentially constitutes an easily implementable and low-cost method to boost vaccine effectiveness. In support of

this, several studies investigating different pathogens have found an increased serological response to morning vaccination compared with afternoon or evening vaccination.[4,19–21] Beyond increased serological responses, a recent observational study also indicated a potential benefit of morning vaccination against SARS-CoV-2 in relation to clinical outcomes including breakthrough infection and hospitalization for COVID-19.[22] Our results are in line with this, but can, as previously mentioned, only serve as hypothesis-generating findings. Interestingly, though there is mounting evidence in favor of morning vaccination in general, Wang et al. found evidence for the contrary, as they observed increased anti-Spike antibody response to SARS-CoV-2 vaccination in subjects vaccinated later in the day.[23] As the authors note, this difference might be due to lack of prior exposure to SARS-CoV-2 in their study population, which exclusively consisted of seronegative subjects. Contrarily, influenza vaccination will typically involve some degree of memory mechanisms of the adaptive immune system,[24] which might favor vaccination earlier in the day. However, Burton et al. have found memory B cell response to influenza vaccination to be impaired in persons aged above 67 years compared with younger individuals,[25] and, as previously discussed, individuals at this age have still been shown to respond better to morning influenza vaccination. In extension of previous studies, to investigate whether morning vaccination for influenza is superior to later vaccination, future studies might test its protective effect toward clinical outcomes such as those assessed in the present study.

In addition to investigating ToV against clinical outcomes, this is the first study to investigate whether ToV modifies the effect of high-dose influenza vaccine compared with standard-dose vaccine in relation to clinical outcomes. The present study found no evidence of this and thus indicated that the effect of QIV-HD is independent of ToV. A possible explanation might be that ToV affects antibody response to both QIV-HD and QIV-SD equally, thus

leaving the relative effectiveness of QIV-HD unaffected. In addition, the findings did not indicate an additive effect of morning vaccination and high-dose vaccine against clinical outcomes. It could, however, be of interest for future studies to test whether time of day affects the dose-response relationship between influenza vaccines and antibody titers.

Limitations

As with all post-hoc analyses, the present study has several important limitations. The DANFLU-1 trial was not specifically powered for the analyses performed in this work, and results should therefore be regarded in this context. The association between ToV and respiratory hospitalizations are likely primarily driven by relatively few outcomes in subjects vaccinated later in the day, and though adjusted analysis was performed, the finding might still be subject to residual confounding. The exploratory nature of the findings is therefore important to underline. In addition, no adjustments for multiplicity were applied, meaning there is a risk that our results are chance findings. Regarding outcomes, the Danish national registries are not primarily maintained for research purposes and may therefore be subject to some extent of imprecision. Such imprecision, if present, would however be equally present between the randomization arms and should therefore not invalidate findings regarding the relative effectiveness of QIV-HD vs. QIV-SD.

Conclusion

In this exploratory post-hoc analysis of the large-scale, pragmatic, randomized DANFLU-1 trial of QIV-HD vs. QIV-SD, we found that earlier time of day for influenza vaccination might be associated with a lower incidence of hospitalizations for respiratory disease. Furthermore, we found that the relative effectiveness of QIV-HD vs. QIV-SD against hospitalizations for pneumonia or influenza, all-cause hospitalizations, and all-cause mortality did not vary with time of day for vaccination. Further research is needed to confirm these findings and understand possible mechanisms.

References

1. Wang C, Lutes LK, Barnoud C, Scheiermann C. The circadian immune system. *Sci Immunol*. 2022;7(72):eabm2465. doi:10.1126/sciimmunol.abm2465

2. Scheiermann C, Kunisaki Y, Frenette PS. Circadian control of the immune system. *Nat Rev Immunol*. 2013;13(3):190-198. doi:10.1038/nri3386

3. Langlois PH, Smolensky MH, Glezen WP, Keitel WA. Diurnal Variation in Responses to Influenza Vaccine. *Chronobiology International*. 1995;12(1):28-36. doi:10.3109/07420529509064497

4. Long JE, Drayson MT, Taylor AE, Toellner KM, Lord JM, Phillips AC. Morning vaccination enhances antibody response over afternoon vaccination: A cluster-randomised trial. *Vaccine*. 2016;34(24):2679-2685. doi:10.1016/j.vaccine.2016.04.032

5. Liu Y, Zhang H, Yuan G, et al. The impact of circadian rhythms on the immune response to influenza vaccination in middle-aged and older adults (IMPROVE): a randomised controlled trial. *Immun Ageing*. 2022;19(1):46. doi:10.1186/s12979-022-00304-w

6. DiazGranados CA, Dunning AJ, Kimmel M, et al. Efficacy of High-Dose versus Standard-Dose Influenza Vaccine in Older Adults. *N Engl J Med*. 2014;371(7):635-645. doi:10.1056/NEJMoa1315727

7. Dunning AJ, DiazGranados CA, Voloshen T, Hu B, Landolfi VA, Talbot HK. Correlates of Protection against Influenza in the Elderly: Results from an Influenza Vaccine Efficacy Trial. Staats HF, ed. *Clin Vaccine Immunol*. 2016;23(3):228-235. doi:10.1128/CVI.00604-15

8. Mullooly JP, Bridges CB, Thompson WW, et al. Influenza- and RSV-associated hospitalizations among adults. *Vaccine*. 2007;25(5):846-855. doi:10.1016/j.vaccine.2006.09.041

9. Thompson WW, Shay DK, Weintraub E, et al. Mortality Associated With Influenza and Respiratory Syncytial Virus in the United States. *JAMA*. 2003;289(2):179. doi:10.1001/jama.289.2.179

10. Grohskopf LA, Blanton LH, Ferdinands JM, Chung JR, Broder KR, Talbot HK. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2023–24 Influenza Season. *MMWR Recomm Rep*. 2023;72(2):1-25. doi:10.15585/mmwr.rr7202a1

11. CDC. *Past Seasons' Vaccine Effectiveness Estimates.*; 2023. https://www.cdc.gov/flu/vaccines-work/past-seasons-estimates.html

12. Tsang P, Gorse GJ, Strout CB, et al. Immunogenicity and safety of Fluzone® intradermal and high-dose influenza vaccines in older adults ≥65 years of age: A randomized, controlled, phase II trial. *Vaccine*. 2014;32(21):2507-2517. doi:10.1016/j.vaccine.2013.09.074

13. Lee JKH, Lam GKL, Yin JK, Loiacono MM, Samson SI. High-dose influenza vaccine in older adults by age and seasonal characteristics: Systematic review and meta-analysis update. *Vaccine: X*. 2023;14:100327. doi:10.1016/j.jvacx.2023.100327

14. Johansen ND, Modin D, Nealon J, et al. A Pragmatic Randomized Feasibility Trial of Influenza Vaccines. *NEJM Evidence*. 2023;2(2). doi:10.1056/EVIDoa2200206

15. Johansen ND, Modin D, Skaarup KG, et al. Effectiveness of high-dose versus standard-dose quadrivalent influenza vaccine against recurrent hospitalizations and mortality in relation to influenza circulation: A post-hoc analysis of the DANFLU-1 randomized clinical trial. *Clinical Microbiology and Infection*. Published online January 2024:S1198743X24000399. doi:10.1016/j.cmi.2024.01.017

16. Johansen ND, Modin D, Nealon J, et al. Feasibility of randomizing Danish citizens aged 65–79 years to high-dose quadrivalent influenza vaccine vs. standard-dose quadrivalent influenza vaccine in a pragmatic registry-based setting: rationale and design of the DANFLU-1 Trial. *Pilot Feasibility Stud*. 2022;8(1):87. doi:10.1186/s40814-022-01044-w

17. Cermakian N, Stegeman SK, Tekade K, Labrecque N. Circadian rhythms in adaptive immunity and vaccination. *Semin Immunopathol*. 2022;44(2):193-207. doi:10.1007/s00281-021-00903-7

18. Otasowie CO, Tanner R, Ray DW, Austyn JM, Coventry BJ. Chronovaccination: Harnessing circadian rhythms to optimize immunisation strategies. *Front Immunol*. 2022;13:977525. doi:10.3389/fimmu.2022.977525

19. de Bree LCJ, Mourits VP, Koeken VACM, et al. Circadian rhythm influences induction of trained immunity by BCG vaccination. *Journal of Clinical Investigation*. 2020;130(10):5603-5617. doi:10.1172/JCI133934

20. Zhang H, Liu Y, Liu D, et al. Time of day influences immune response to an inactivated vaccine against SARS-CoV-2. *Cell Res*. 2021;31(11):1215-1217. doi:10.1038/s41422-021-00541-6

21. Phillips AC, Gallagher S, Carroll D, Drayson M. Preliminary evidence that morning vaccination is associated with an enhanced antibody response in men. *Psychophysiology*. 2008;45(4):663-666. doi:10.1111/j.1469-8986.2008.00662.x

22. Hazan G, Duek OA, Alapi H, et al. Biological rhythms in COVID-19 vaccine effectiveness in an observational cohort study of 1.5 million patients. *Journal of Clinical Investigation*. 2023;133(11):e167339. doi:10.1172/JCI167339

23. Wang W, Balfe P, Eyre DW, et al. Time of Day of Vaccination Affects SARS-CoV-2 Antibody Responses in an Observational Study of Health Care Workers. *J Biol Rhythms*. 2022;37(1):124-129. doi:10.1177/07487304211059315

24. Wrammert J, Smith K, Miller J, et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature*. 2008;453(7195):667-671. doi:10.1038/nature06890

25. Burton AR, Guillaume SM, Foster WS, et al. The memory B cell response to influenza vaccination is impaired in older persons. *Cell Reports*. 2022;41(6):111613. doi:10.1016/j.celrep.2022.111613

Table 1

	Early	**Late**
n	6,238	6,239
Male sex, n (%)	3,335 (53.5)	3,265 (52.3)
Age, years, mean±SD	71.7±3.9	71.8±3.9
Chronic obstructive pulmonary disease, n (%)	200 (3.2)	217 (3.5)
Asthma, n (%)	203 (3.3)	239 (3.8)
Chronic lung disease, n (%)	391 (6.3)	459 (7.4)
Arterial hypertension, n (%)	1,023 (16.4)	1,029 (16.5)
Diabetes mellitus, n (%)	563 (9.0)	599 (9.6)
Chronic cardiovascular disease, n (%)	1,229 (19.7)	1,311 (21.0)
Chronic kidney disease, n (%)	127 (2.0)	128 (2.4)
Cancer, n (%)	687 (11.0)	676 (10.8)
Immunodeficiency, n (%)	243 (3.9)	240 (3.8)
QIV-HD, n (%)	3,116 (50.0)	3,129 (50.2)

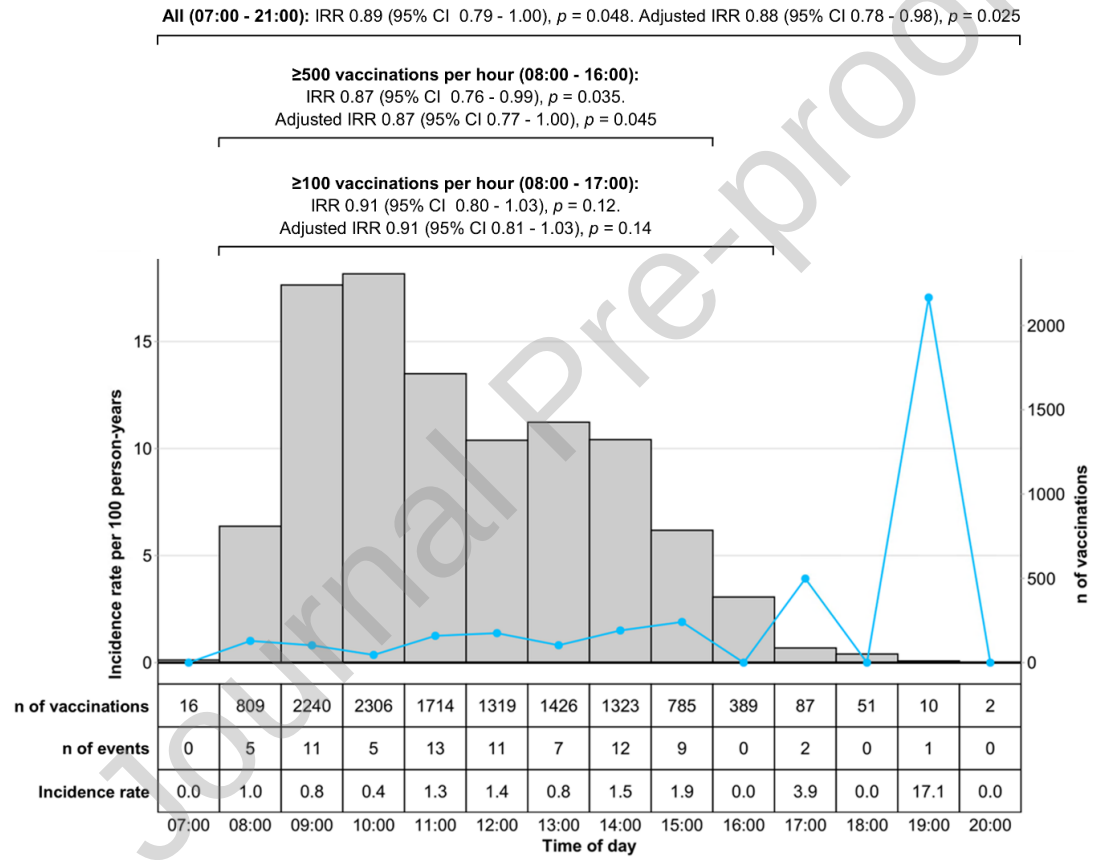
Chronic lung disease is a composite of several lung diseases including tuberculosis, sarcoidosis, cystic fibrosis, bronchitis, emphysema, bronchiectasis, interstitial pulmonary disease, and lung transplantation. Similarly, chronic cardiovascular disease is a composite of several cardiovascular diseases, including, but not limited to, heart failure, atrial fibrillation, ischemic heart disease, and cerebrovascular disease.

Table 2

	IRR (95% CI)	*p*	Adjusted IRR (95% CI)	Adjusted *p*
Recurrent event analysis				
Hospitalization for pneumonia or influenza	0.93 (0.80, 1.07)	0.32	0.91 (0.79, 1.05)	0.19
Hospitalization for respiratory disease	0.89 (0.80, 1.00)	0.048	0.88 (0.78, 0.98)	0.025
Hospitalization for cardiovascular disease	1.00 (0.93, 1.07)	0.94	1.02 (0.95, 1.10)	0.60
Hospitalization for cardiorespiratory disease	0.96 (0.91, 1.03)	0.26	0.98 (0.92, 1.04)	0.47
All-cause hospitalization	0.97 (0.95, 1.00)	0.07	0.98 (0.96, 1.01)	0.23
Time-to-event analysis				
Hospitalization for pneumonia or influenza	0.88 (0.77, 1.01)	0.07	0.90 (0.78, 1.03)	0.11
Hospitalization for respiratory disease	0.86 (0.77, 0.95)	0.003	0.87 (0.78, 0.97)	0.010
Hospitalization for cardiovascular disease	1.01 (0.94, 1.08)	0.84	1.01 (0.95, 1.09)	0.68
Hospitalization for cardiorespiratory disease	0.96 (0.91, 1.02)	0.16	0.97 (0.91, 1.02)	0.26
All-cause hospitalization	0.98 (0.95, 1.00)	0.10	0.98 (0.96, 1.01)	0.21
All-cause mortality	0.91 (0.82, 1.02)	0.09	0.92 (0.83, 1.03)	0.15

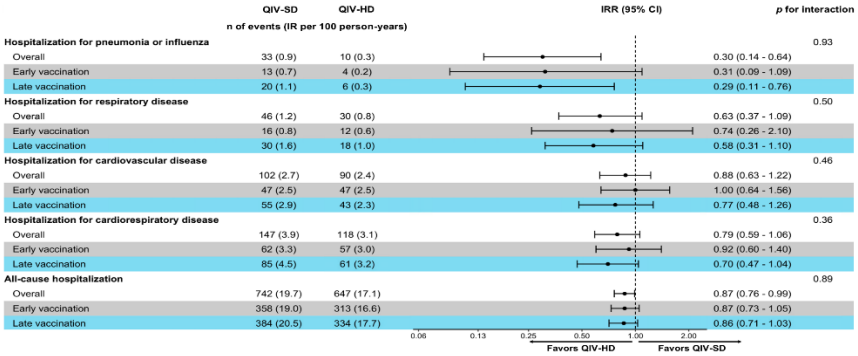
Recurrent event analysis and time-to-event analysis for hospitalizations and all-cause mortality (the latter only examined as time-to-event) according to continuous time of vaccination. All effect sizes are per 1-hour decrements. All analyses are adjusted for vaccine type, while fully adjusted analyses are further adjusted for sex, age, diabetes mellitus, chronic obstructive pulmonary disease, asthma, chronic lung disease, chronic cardiovascular disease, chronic kidney disease, immunodeficiencies, and cancer.

Figure 1



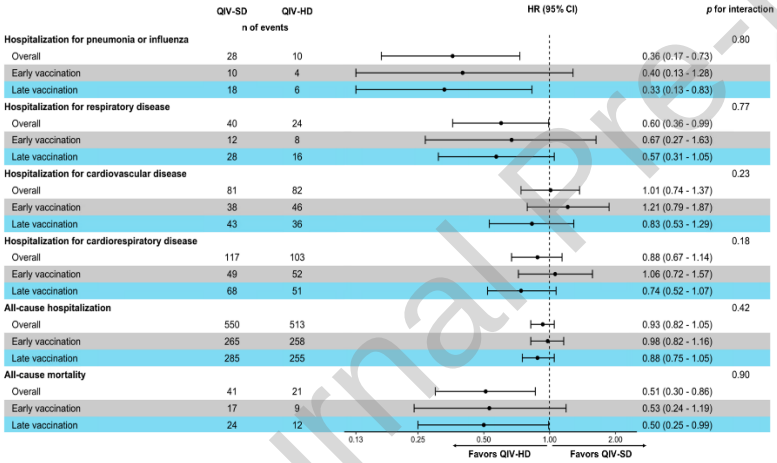
Incidence rates of respiratory hospitalizations per 100 person-years according to continuous ToV (blue line). A histogram illustrating the distribution of vaccinations according to ToV is further presented (gray bars). Above the graph can be seen the results of three pairs of unadjusted and adjusted analyses, the first of which include all hours of day, while the latter two are restricted to the hours of day with at least 500 and 100 vaccinations, respectively. All incidence rate ratios are per 1-hour decrements. All analyses are adjusted for vaccine type, while fully adjusted analyses are further adjusted for sex, age, diabetes mellitus, chronic obstructive pulmonary disease, asthma, chronic lung disease, chronic cardiovascular disease, chronic kidney disease, immunodeficiencies, and cancer. Number of vaccinations, number of events, and incidence rates according to ToV are reported in the table below the plot. IRR, incidence rate ratio. CI, confidence interval.

Figure 2



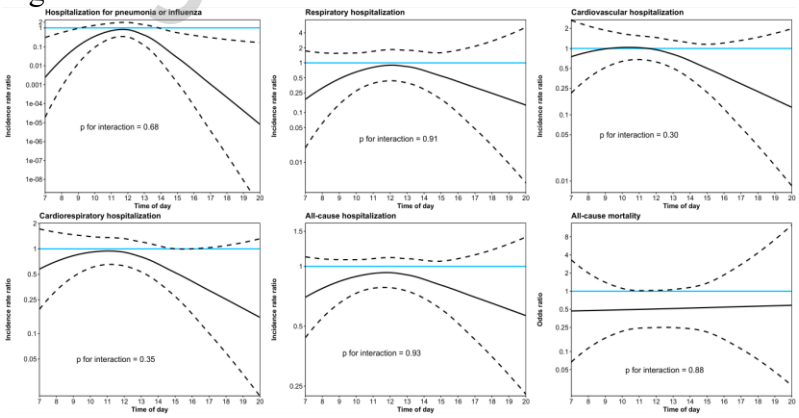
Recurrent event analysis illustrating relative effectiveness of QIV-HD versus QIV-SD for overall population and according to early or late time of vaccination. Outcomes were assessed using negative binomial regression and presented with incidence rate ratios. QIV-HD, high-dose quadrivalent influenza vaccine. QIV-SD, standard-dose quadrivalent influenza vaccine. IR, incidence rate. IRR, incidence rate ratio. CI, confidence interval.

Figure 3



Time-to-event analysis illustrating relative effectiveness of QIV-HD versus QIV-SD for overall population and according to early or late time of vaccination. Outcomes were assessed using Cox proportional hazards regression and presented with hazard ratios. QIV-HD, high-dose quadrivalent influenza vaccine. QIV-SD, standard-dose quadrivalent influenza vaccine. HR, hazard ratio. CI, confidence interval.

Figure 4



Relative vaccine effectiveness of QIV-HD compared with QIV-SD illustrated with splines (solid black line) and 95% confidence interval (dotted black lines). Values below the blue line favor QIV-HD, while values above favor QIV-SD. Hospitalization outcomes were assessed with negative binomial regression models containing restricted cubic spline terms for continuous time of vaccination. Models for each randomization group (ie. QIV-HD and QIV-SD) were constructed

separately and used to fit incidence rates for each hour of continuous time of vaccination. Incidence rate ratios between QIV-HD and QIV-SD were calculated by dividing fitted incidence rates on a per-hour basis, and the pertaining standard errors were calculated by taking the square root of the sum of the two individual standard errors squared. For the spline terms, the number of knots resulting in the lowest Akaike information criterion were chosen for each regression model. For all-cause mortality, a similar approach with logistic regression was employed. Effect modification by time of vaccination was tested using interaction terms between time of vaccination and vaccine type in separate regression models fitted on both randomization groups collectively.

CRediT authorship contribution statement:

All authors contributed to the conceptualization and design of the study. JC, NDJ, and TB-S had access to the raw data and performed the analyses JC, NDJ, and TB-S wrote the first draft of the manuscript. All authors contributed to the interpretation of data and writing of the final version of the manuscript. All authors approved the final manuscript.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

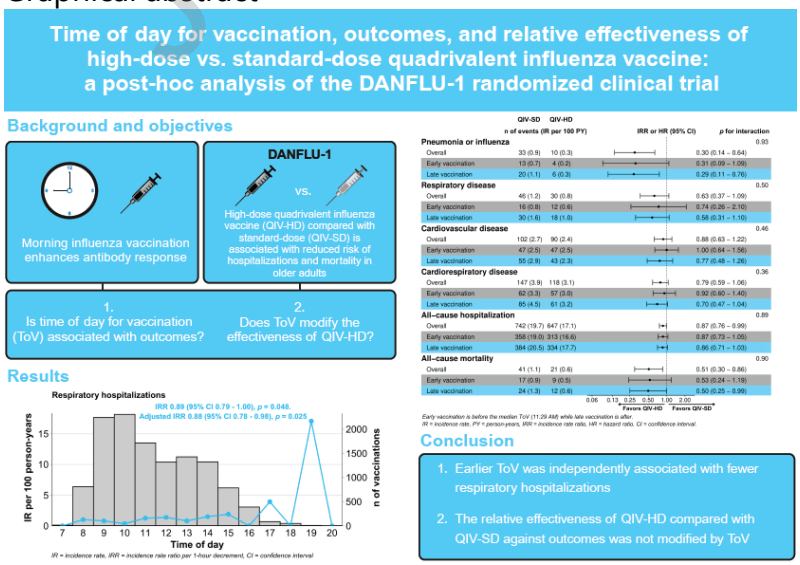
☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for *[Journal name]* and was not involved in the editorial review or the decision to publish this article.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

JN was previously employed by Sanofi and may own shares and/or stock options in the company. **SS**, **ML**, and **RCH** are full-time employees of Sanofi and may own shares and/or stock options in the company.
CSL is chief physician at Danske Lægers Vaccinations Service, part of European LifeCare Group, and has received speaker fees and served on advisory boards for GSK, MSD, Pfizer, Takeda, and Valneva.
BLC has received consulting fees from Amgen, Cardurion, Corvia, Myokardia, and Novartis.
SDS has received research grants from Actelion, Alnylam, Amgen, AstraZeneca, Bellerophon, Bayer, BMS, Celladon, Cytokinetics, Eidos, Gilead, GSK, Ionis, Lilly, Mesoblast, MyoKardia, NIH/NHLBI, Neurotronik, Novartis, Novo Nordisk, Respicardia, Sanofi, Theracos, US2. AI and consulted for Abbott, Action, Akros, Alnylam, Amgen, Arena, AstraZeneca, Bayer, Boehringer Ingelheim, BMS, Cardior, Cardurion, Corvia, Cytokinetics, Daiichi-Sankyo, GSK, Lilly, Merck, Myokardia, Novartis, Roche, Theracos, Quantum Genomics, Cardurion, Janssen, Cardiac Dimensions, Tenaya, Sanofi, Dinaqor, Tremeau, CellProThera, Moderna, American Regent, Sarepta, Lexicon, Anacardio, Akros, and Puretech Health.
LK has received speaker fees from Novo Nordisk, Novartis, AstraZeneca, Boehringer Ingelheim, and Bayer. **TB-S** is chief investigator of the Boston Scientific financed ”DANLOGIC-HF” trial, the Sanofi financed “NUDGE-FLU” trial, the Sanofi financed “DANFLU-1″ trial, the Sanofi financed “DANFLU-2″ trial and steering committee member of the Boston Scientific sponsored “LUX-Dx TRENDS Evaluates Diagnostics Sensors in Heart Failure Patients Receiving Boston Scientific's Investigational ICM System” trial, the Amgen sponsored GALACTIC-HF trial, the Boehringer Ingelheim financed EASi-KIDNEY trial and served on advisory boards for Sanofi, Amgen, CSL Seqirus and GSK and received speaker honorariums from Bayer, Novartis, Sanofi, GE Healthcare and GSK and received research grants from Boston Scientific, GE Healthcare, AstraZeneca, Novo Nordisk, and Sanofi and consulted for Novo Nordisk, IQVIA and Parexel.

The remaining authors have nothing to disclose.

Graphical abstract



Highlights

- Morning vaccination enhances the antibody response to influenza vaccine
- Post-hoc analysis of DANFLU-1; high- (HD) vs. standard-dose (SD) influenza vaccine
- Earlier vaccination was associated with fewer respiratory hospitalizations
- HD compared with SD lowered risk of hospitalizations and mortality in older adults
- Relative effectiveness of HD vs. SD was independent of circadian timing