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Effectiveness of trivalent influenza vaccines against hospitalizations due to laboratory-confirmed influenza a in the elderly: Comparison of test-negative design with register-based designs

Jussi Halme [a,b], Ritva K. Syrjänen [b,*], Ulrike Baum [c], Arto A. Palmu [b]

[a] Faculty of Medicine and Health Technology, Tampere University, Arvo Ylpön katu 34, 33520 Tampere, Finland
[b] Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Biokatu 6, 33520 Tampere, Finland
[c] Department of Health Security, Finnish Institute for Health and Welfare, Mannerheimintie 166, 00300 Helsinki, Finland

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ABSTRACT

Introduction: Measuring influenza vaccine effectiveness (IVE) seasonally is important and has been conducted utilizing several observational study designs. The active test-negative design has been most widely used and the validity of passive register-based studies has been debated. We aimed to explore the potential differences, advantages, and weaknesses of different study designs in estimating influenza vaccine effectiveness.

Methods: We compared three study designs in estimating IVE against hospitalization in the elderly aged 65 years or more over three influenza seasons 2015/16, 2016/17 and 2017/18. Designs compared were active test-negative design (TND), register-based cohort design and register-based case-control design with different selection criteria for cases and controls.

Results: Adjusted IVE estimates for the three consecutive seasons 2015–18 in active test-negative design were 82% (95% confidence interval 26, 96), 21% (-179, 77), 15% (-113, 66). For case-control design, estimates from different analyses ranged in 2015/16 from 47% (-16, 76) to 52% (-48, 84), in 2016/17 from 10% (-42, 43) to 29% (-20, 58), and in 2017/18 from −27% (-91, 15) to 1% (-40, 30). In the cohort design, the adjusted IVE estimates were 48% (-9, 75), 29% (1, 49), 13% (-21, 37) for the three seasons.

Conclusions: The register-based cohort design produced results more concordant with the active test-negative design than the case-control design. Furthermore, the register-based cohort design yielded most precise estimates with narrower confidence intervals. In Finland with the availability of near real-time nationwide register data, the register-based cohort design is the method of choice to continue the annual surveillance of influenza vaccine effectiveness.

1. Introduction

Burden of annual influenza epidemics is significant for health care and economy [1]. Severe influenza is especially frequent in the elderly and vaccination is the most effective way to prevent severe outcomes. In Finland, influenza vaccination is recommended and provided free of charge in the public health care to all individuals aged 65 years or more by the National Immunization Program (NIP). Vast majority of influenza vaccine doses annually consumed in Finland are given within the public health care, especially in the elderly. At the time of the study, trivalent influenza vaccines were used in Finland, until the end of season 2017/18. Thereafter tetravalent vaccines have replaced them.

Estimating influenza vaccine effectiveness (IVE) is important for understanding the effects of current vaccination program against burden of influenza. Influenza viruses go through changes every year and IVE varies from year to year, which is why it should be monitored yearly. Influenza vaccination is recommended for risk groups in Europe, so randomized placebo-controlled trials for estimating IVE in these target groups would not be ethical. Therefore, only observational study designs are conducted for the estimation of IVE for each season [2]. The test-negative design (TND) has been the standard choice in most studies estimating IVE, especially in Europe. Large register-based cohort studies are another study design frequently used to estimate IVE [3]. In Finland there are comprehensive real-time health registers that have already been used in cohort studies of IVE since 2012 [4,5]. It has been debated

* Corresponding author at: Finnish Institute for Health and Welfare, Finn-Medi 1, Biokatu 6, 33520 Tampere, Finland.
E-mail address: ritva.syrjanen@thl.fi (R.K. Syrjänen).

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how reliable these registers are and how reliable these estimates are compared to active hospital-based TND studies. Validation studies for vaccinations [6] and selected outcomes [7–8] have been conducted, but the validation studies in IVE estimation are pending.

The primary objective of this study was to compare active test-negative design to designs in which data are collected passively through registers. We aimed to explore the potential differences in results and to discuss the advantages and weaknesses of each design in estimating the influenza vaccine effectiveness in the elderly. We also explored accuracy of the register data compared to confirmed data from the hospital-based active test-negative study.

2. Methods

2.1. Study design

We compared three study designs to measure influenza vaccine effectiveness (IVE) against laboratory-confirmed influenza A (LCI-A) among the elderly aged 65 years and above in the city of Tampere, Finland, during three influenza seasons 2015/16, 2016/17 and 2017/18.

- The first sub-study was an active, hospital-based TND study for measuring IVE against LCI-A in elderly hospitalized with severe acute respiratory infection (SARI), by comparing influenza positives and negatives in vaccinated and nonvaccinated subjects.
- The second sub-study was a register-based, hospital-based case-control study, designed to mimic the TND study and to explore different variations in definitions for cases and controls.
- The third sub-study was a register-based, population-based cohort study for measuring IVE against LCI-A in association with hospitalization.

2.2. Study setting and population

The source population for all sub-studies comprised all permanent residents aged ≥65 years in Tampere. The elderly population of Tampere increased from 41 360 to 43 624 during the study period, 98% were Finnish speaking.

Acute infections of community-dwelling inhabitants requiring hospitalization were mostly treated in two hospitals: the most severe cases were treated in Tampere University Hospital of the Pirkanmaa Hospital District (the only tertiary care hospital in the area) and the less severe cases were treated in the city hospital (primary/secondary care). In 2018, the city hospital was merged with the university hospital.

2.3. Influenza seasons and vaccinations 2015–2018 in Finland

The three influenza seasons in Finland differed from each other. Fig. 1 shows the distributions of influenza A and B findings reported to National Infectious Diseases Register (NIDR) during the seasons. Table 1 presents the proportions of influenza A subtypes and influenza B lineages according to the virological surveillance [9–11]. More detailed description of the study seasons is given in Supplementary data 1. As influenza B was mainly absent in the first two seasons, we focused our analysis on Influenza A only.

Table 1 presents the vaccines used in the NIP. The vaccination coverage in elderly increased from 43% to 47% in the whole country during the study seasons while it remained 45% in Tampere.

2.4. Sub-study 1 – Active test-negative design (TND) study

As part of the EU-funded multi-center I-MOVE + project (Horizon2020, GA 634446), we conducted an active, hospital-based TND study to estimate the IVE against in LCI community-dwelling elderly, hospitalized with SARI in Tampere during three influenza seasons 2015–2018. The study was conducted each season in 4–7 hospital units representing infectious diseases and internal, respiratory, general, and geriatric medicine.

Detailed methods of the TND study are described in the Supplementary data 2. In brief, a study nurse affiliated at the Finnish Institute for Health and Welfare (THL) systematically contacted new patients hospitalized with defined screening diagnoses (Supplementary Table S1), assessed the eligibility, and obtained a written informed consent. The patient was eligible to be enrolled if he or she was aged 65 years or more, was a permanent resident in the city of Tampere, could communicate in Finnish, had no contraindication for influenza vaccine, did not have the first symptom or sign 48 h or more after admission or <48 days after prior hospitalization, had not tested positive for influenza during the season before the symptom onset and was a SARI patient (Fig. 2). A SARI patient

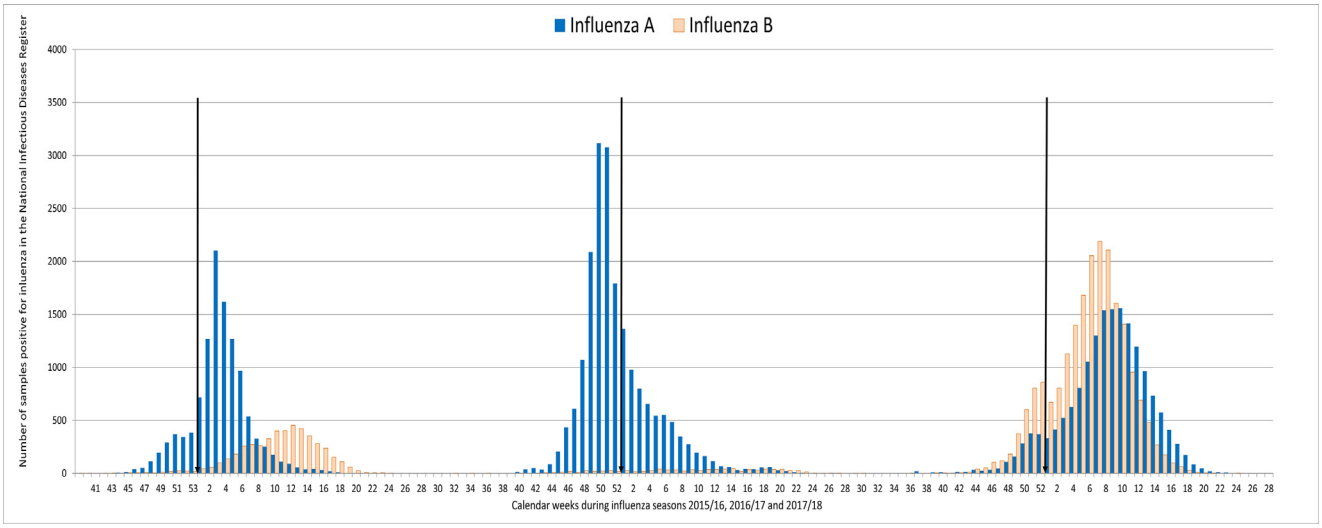

Fig. 1. Numbers of positive influenza A and B findings in the Finnish National Infectious Diseases Register (NIDR) in epidemiological seasons 2015/16, 2016/17 and 2017/18 by calendar week.

Table 1
Circulating influenza strains and influenza vaccinations in Finland during epidemic seasons 2015/16, 2016/17 and 2017/18.

Season	The distribution of circulating subtypes and lineages[a]				Vaccination coverage in population aged 65 years and more		Vaccines used in the National Immunisation Programme (NIP)	Vaccinations in NVR of Tampere elderly having visits in HILMO register[b]
	A(H1N1) pdm09	A(H3N2)	B Victoria	B Yamagata	Whole country	City of Tampere		
2015/2016	79%	7%	15%	0%	43.2%	44.6%	Fluarix®, split virion[c]	88%
							Vaxigrip®, split virion[d]	11%
2016/2017	0%	94%	0%	6%	47.4%	45.2%	Influvac®, subunit vaccine[e]	99.9%
2017/2018	3%	41%	3%	54%	47.3%	45.1%	Influvac®, subunit vaccine[e]	99%
							Agrippal®, subunit vaccine[f]	1%

[a] The distribution of subtypes and lineage are based on the samples obtained for virological surveillance from sentinel sites during visits due to acute respiratory infection or influenza like illness [9–11]. [b]Influenza vaccination records present in the National Vaccination Register (NVR) of residents of Tampere aged ≥ 65 years having at least one event registered in the hospital discharge register (HILMO) during the study periods. All vaccines were trivalent, inactivated, unadjuvanted, egg propagated vaccines, the normal dose 0.5 ml for intramuscular use containing 15ug/ml of hemagglutinin/strain. [c]GlaxoSmithKline Biologicals, [d]Sanofi Pasteur, [e]BGP Products B.V/Abbott Agrippal®, [f]Seqirus S.r.l./Seqirus Vaccines Ltd.

was defined as a person hospitalized with at least one systemic symptom or sign (fever or feverishness, malaise, headache, myalgia, or deterioration of general condition) and at least one respiratory symptom or sign (cough, sore throat, shortness of breath), starting <8 days before sampling.

The study nurse collected information on the symptoms of the current disease and background factors by interview and from the electronic medical records of the hospital. The influenza vaccinations were verified in the medical records of the Tampere health care center, or the vaccination site reported by the participant.

The study nurse collected a respiratory specimen from the study participant. If an influenza sample had already been obtained for clinical purposes after symptom onset, the residue of the specimen was requested from the local laboratory and used for the study purposes. All respiratory specimens were tested for influenza A and B at the virological laboratory at THL in Helsinki. The participants with positive influenza A tests were considered cases. Participants with a negative influenza test were considered controls, if their symptom onset coincided with those of the influenza A cases.

2.5. Sub-study 2 - Register-based case-control study

The data for this sub-study were retrieved from the Finnish national registers, described in the Supplementary data 3. We obtained from the hospital discharge register (HILMO) records of every hospital visit for all residents in Tampere aged 65 years or more at the start of the visit. Records were limited to the timeframe of influenza seasons 2015–2018 defined according to the study periods in the active TND study. If the residents had visited other hospitals than the university or city hospital, e.g., when travelling, the records were included. The influenza vaccination records were retrieved from the National Vaccination Register (NVR).

Hospitalization was defined as an admission at a hospital ward for more than one day or one day with registration code for inpatient care. Consecutive visits or admissions in different hospital units were included in a hospitalization episode if the start date of the next visit/admission was registered at the same or the next day as the end date of the previous visit/admission. Exclusion criteria are shown in Fig. 3.

Acute hospitalizations were defined as hospitalization episodes starting with a visit to the hospital emergency department or arrival to hospital as an emergency patient. As we aimed to focus on community-acquired influenza, transfers between hospitals and other health or social care institutions were excluded.

To compare the register-based case-control results with the active TND study we used an ICD-10 code-based definition for SARI. Because the SARI case definition in the active TND study was based on specific symptoms and signs, which are often not available as ICD-10 codes in the register, we explored several combinations of ICD-10 codes to mimic the TND study case definition (Supplementary Table S2). The SARI definition selected included the ICD-10 codes J09-18, J20-22, J00-06 and J44.0. SARI hospitalization was defined as an acute hospitalization with a SARI-compatible diagnosis code recorded within 2 days of the start of hospitalization episode.

Different variations of case and control definitions were explored to see how well the register-based results would match the active TND study results in the hospital setting and how the hospital-based results relate to those of the population-based cohort study (Table 2). From the NIDR, we only got the positive sample results. A positive influenza finding was considered as related to the hospitalization episode, if the sample was obtained during the hospitalization or within 7 days before its start. Hospitalizations qualified as control episodes, if the subject had no LCI related to hospitalization during the season. For each analysis by season, only one control hospitalization per subject was randomly picked.

As a separate analysis, to mimic the active TND study as precisely as possible, we limited the study population to only those hospitalized in the university hospital (any season) or city hospital (season 2015/16) and excluding e.g., those hospitalized outside Tampere or at special hospitals for cardiological diseases or palliative care, because the active TND study was conducted in selected hospital units only. We also used a more sensitive definition for acute hospitalization, based on the local codes for the emergency and observation units as arrival units in these hospitals, to mimic the screening and recruitment procedure used in the active TND study (Supplementary data 2).

2.6. Sub-study 3 -Register-based cohort study

This study design is the same that has been routinely used in evaluating seasonal IVE in Finland since 2012 [5]. The study cohort for three influenza seasons 2015–2018 was defined according to the data extracted from the Finnish Population Information System. We included all residents of Tampere who were alive at the first of October and 65–100-years old at the end of December during the study season. Individuals who moved out of Tampere during the season were excluded from the analysis. Follow-up of study subjects as unvaccinated and vaccinated according to the NVR was conducted for each influenza season between October 1st and May

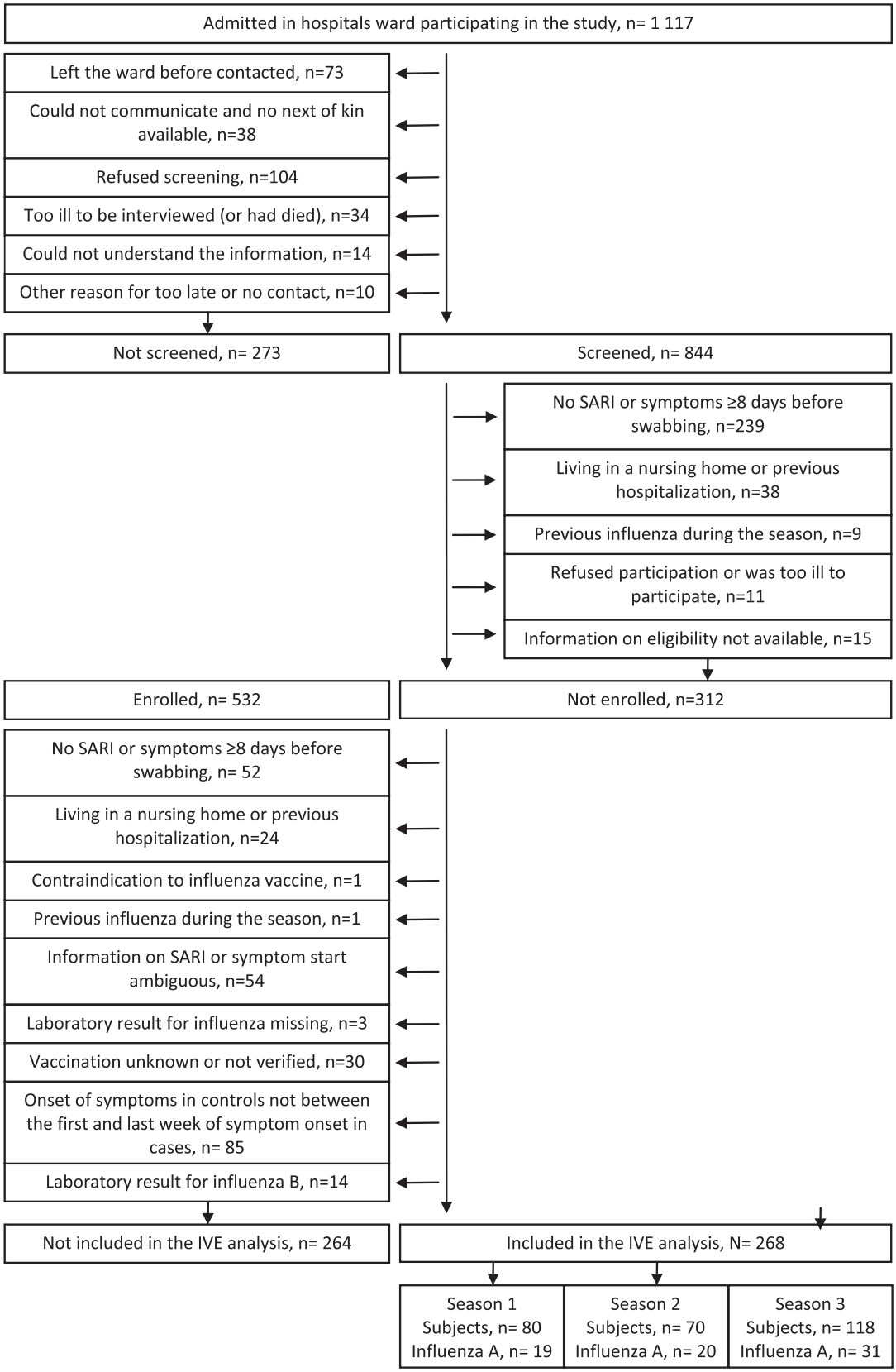

Fig. 2. Flow chart of the data included in the active, hospital-based TND (test negative design) study during the epidemiological seasons 2015/16, 2016/17 and 2017/18. The subjects were screened according to a daily list of patients admitted in the study hospitals with pre-defined admission diagnoses (Supplementary Table S1).

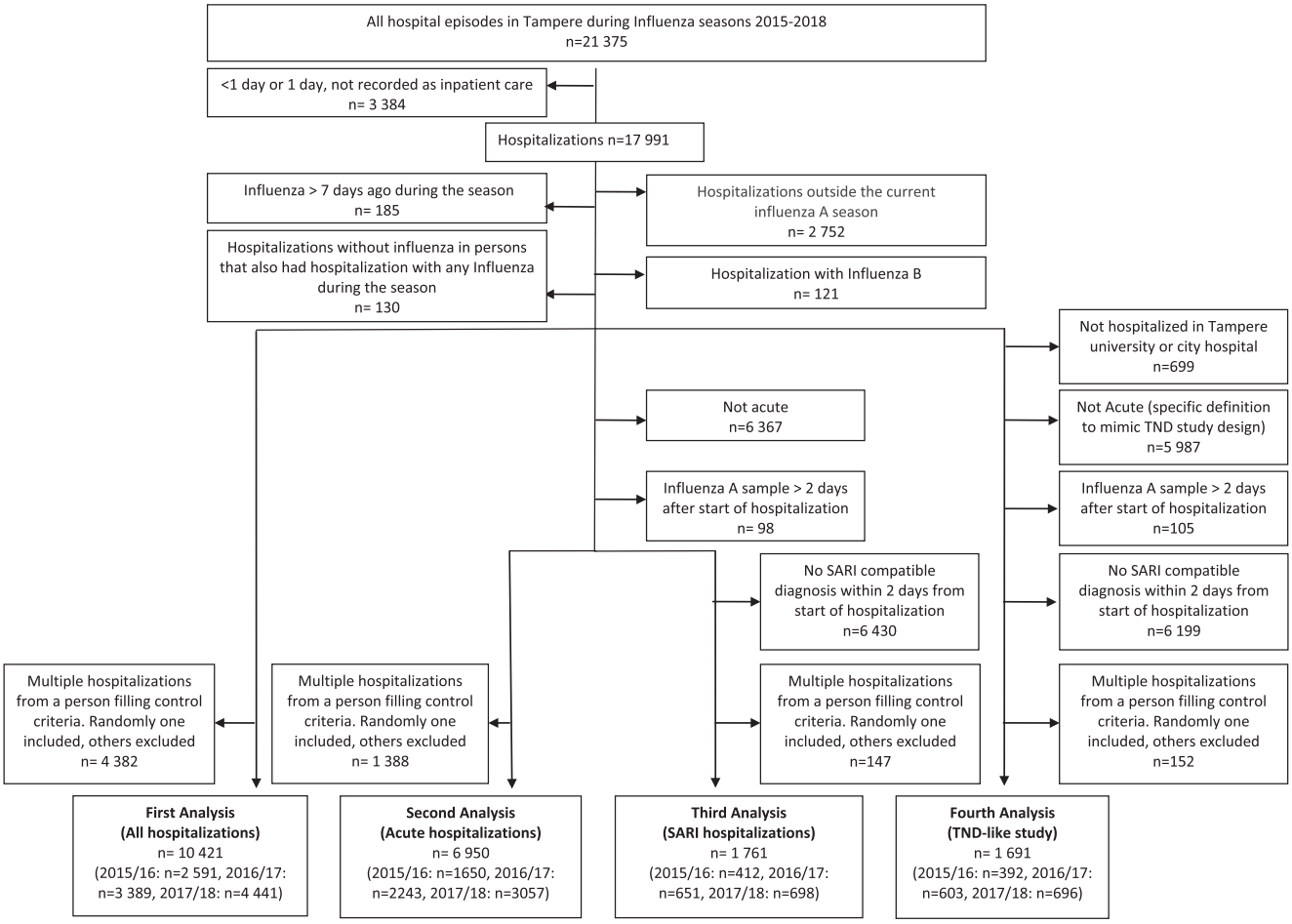

Fig. 3. Flow chart of the data included in the different analyses with variations of case and control definitions in the register-based, hospital-based case-control study during the epidemiological seasons 2015/16, 2016/17 and 2017/18.

Table 2
Case, control and exposure definitions in sub-studies and analysis sets.

Analysis	Study population	Case definition	Control definition	Exposure definition
Active TND study	Hospitalizations with SARI[a]	Hospitalized patient fulfilling clinical SARI-criteria with LCI-A[b]	Hospitalized patient fulfilling clinical SARI-criteria with negative sample for any LCI[c]	Vaccination for influenza >14 days before symptom onset
Register-based case-control study	All hospitalizations	Any hospitalization with LCI-A[b]	One random hospitalization without any LCI[c] per season per study subject	Vaccination for influenza >14 days before admission date
	Acute hospitalizations	Any acute hospitalization with LCI-A[b] within 7 days before and 2 days after the start of hospitalization	One random acute hospitalization without any LCI[c] per season per study subject	Same as in analysis 1
	SARI hospitalizations[d]	SARI hospitalization and LCI-A[b] within 7 days before and 2 days after the start of hospitalization	One random SARI hospitalization without any LCI[c] per season per study subject	Same as in analysis 1
	Selected SARI hospitalizations[d, e]	Same as in analysis 3	Same as in analysis 3	Same as in analysis 1
Cohort study	All residents of Tampere	Being inpatient at hospital at the time or within 7 days from LCI-A[b]	NA	>14 days had passed since the vaccination date

[a] SARI defined with data actively collected according to the protocol of the active test-negative design (TND) study, including one systematic and one respiratory sign or symptom occurring <8 days before influenza sampling.
[b] LCI-A, laboratory-confirmed influenza A.
[c] LCI, laboratory-confirmed influenza.
[d] SARI defined as any register-based ICD-10 code for acute respiratory infection (J09-18, J20-22, J00-06 and J44.0) recorded within 2 days after hospitalization.
[e] The hospitalizations were further restricted to mimic the active TND study design as precisely as possible, by limiting the hospitalisations to selected units, with admission through specific units.

31st and was analyzed for each season separately. Each person included in the cohort was considered at risk for the outcome from the start of the season to the end of the season, until the measured outcome occurred or loss to follow-up (death), whichever occurred first, as described earlier by Baum et al. [5]. Hospitalization was defined as being a hospital inpatient according to HILMO at the time of positive influenza A sample or during the 7 following days. The positive influenza A findings were retrieved from NIDR.

2.7. Vaccinations

The subject was considered vaccinated, if >14 days had elapsed since the vaccine administration. During 0–14 days after vaccine administration, the subject was considered unvaccinated in the active TND study and in the register-based case-control study. In the cohort study these subjects were considered partially vaccinated.

Table 2 specifies the time point for defining the vaccination status in each of the analyses. For the active TND analysis, this was the date of symptom onset and in the register-based case-control studies it was the hospital admission. In the cohort study, the vaccination was managed as a time-dependent exposure, change of the exposure status from unvaccinated to partially vaccinated took place at the time of vaccine administration and the change from partially to full vaccinated took place 15 days after vaccine administration.

2.8. Laboratory testing

In the active TND study, the influenza viruses were identified from collected specimens by RT-PCR test in the virological laboratory at THL. The test determined the presence of influenza A and B viruses and confirmed the results by determination of the subtypes/lineages and in selected cases, by sequencing.

For register-based sub-studies, positive influenza samples were acquired from the NIDR. During the 3-season-long study period, there were two PCR tests and one antigen test in use for influenza testing in Tampere. The guideline to clinicians of Tampere university hospital for influenza sampling during the study period was that every patient admitted to hospital ward with influenza-like symptoms, should be tested for influenza with a PCR test, but in practice, the decision to test was on the discretion of the clinician [12].

2.9. Data analysis

For the active TND study and the register-based case-control study, IVE was estimated as 1 – OR (odds ratio, expressed as percentage). IVEs were estimated crude and, for register-based case-control study, adjusted for age, sex, calendar time (the season divided to three phases according to the number of cases, 25%, 50% and 25% of cases in each), one-year vaccination history, presence of at least one underlying chronic condition and nights hospitalized in the past five years. The TND study was adjusted only for age, sex and calendar time. Distributions of continuous variables for background characteristics between cases and controls were compared with Mann-Whitney's *U* test. Categorical variables were compared with Pearson's chi-squared test.

For the cohort study, a Cox proportional hazards model with time since the beginning of the study period as the underlying timescale was used. IVE was estimated as 1- the ratio comparing the hazard of influenza A in vaccinated and unvaccinated. Adjustment was made for age, sex, one-year vaccination history, presence of at least one underlying condition and nights hospitalized in the past five years.

In the cohort study, the data analyses were performed in R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). All other analyses were performed with SPSS version 27 (IBM SPSS STATISTICS).

2.10. Ethical aspects

The active TND study was conducted according to Declaration of Helsinki and submitted for ethical review to Pirkanmaa hospital district regional Ethics Committee. In the TND study, written or oral witnessed informed consents from the participants were collected. In season 2015/16, also written informed consent from the next of kin was allowed.

For the register-based studies, the protocol was evaluated by the institutional ethics committee at THL. In addition, permissions for the use of data from the national registers have been obtained from the register controllers at THL, including permission to combine register data with the active study data. No informed consent is required in Finland for register-based studies.

3. Results

3.1. Results of the sub-study 1 – Active TND study

The study was conducted between December 8, 2015, and May 13, 2016, November 21, 2016, and April 3, 2017, and December 8, 2017, and May 16, 2018. Of almost 10 800 citizens of Tampere aged ≥65 years admitted into either of the two hospitals in Tampere (university hospital and city hospital), 1 117 were admitted to the participating wards with at least one of the screening diagnoses listed in Table 1. Of them, 585 (52%) could not be screened or did not fulfil eligibility criteria for reasons specified in the flowchart (Fig. 2). Of the 532 enrolled participants, for 264 the study eligibility criteria could not be verified from the medical records, or they did not qualify as controls for the analysis (Fig. 2).

Of the 268 SARI patients included in the analysis, 170 (63%) were vaccinated against influenza >14 days before the onset of the SARI symptoms. Only one patient was vaccinated <14 days before the symptom onset. Thus, the vaccination coverage was somewhat higher than in the target population (Table 1, Table 3).

Table 3 presents the background profiles of the cases and controls each season. No systematic trends for differences between them could be observed. During the first season, all but one of the A strains were A/H1N1, and during the two other seasons, all were A/H3N2. Twelve of the 70 influenza A cases were not found in the NIDR; 9 because the sample was taken by the study nurse and not by the hospital and 3 because the positive finding at the hospital laboratory was not recorded in the NIDR. Two additional influenza A positive cases were detected at the hospital and reported into the NIDR but remained negative in further analyses at the THL laboratory. Thus, the estimated sensitivity of the NIDR data was 83% (58/70) and specificity 99% (false positives 2/total negatives 198) in detecting influenza among hospitalized elderly, compared to the actual test results. Of the 198 samples obtained by the hospital clinicians, 97% were PCR-based and only 3% were antigen-based. The hospital clinicians sampled 62% of the vaccinated and 65% of unvaccinated study participants.

The crude and adjusted IVE estimates are given in Table 4. During the study seasons 2015/16, 2016/17 and 2017/18, the adjusted estimates were 82% (95% confidence interval (CI) 26, 96), 21% (-179, 77) and 15% (-113, 66), respectively.

3.2. Results of the sub-study 2 - register based case-control study

A total of 21 375 hospital episodes were identified in the HILMO register for the source population during the study seasons. 17 991 of these were defined as inpatient hospitalizations, from which 3

Table 3
Clinical characteristics of hospitalized cases and controls in the register-based case-control study and active test-negative design (TND) study by analysis and season.

Characteristics	**All hospitalizations**			**Acute hospitalizations**			**SARI hospitalizations**			**TND-like study**			**Acute TND study**		
2015/16 season	Cases	Controls	P[a]	Cases	Controls	P[a]	Cases	Controls	P[a]	Cases	Controls	P[a]	Cases	Controls	P[a]
Total number	50	2541		34	1616		30	382		26	366		19	61	
Sex, male	34 (68%)	1195 (47%)	**0.003**	22 (65%)	754 (47%)	**0.037**	19 (63%)	190 (50%)	0.152	17 (65%)	187 (51%)	0.159	13 (68%)	25 (41%)	**0.037**
Age, median	75	78	**0.005**	74	79	**0.012**	76	80	**0.013**	76	80	0.057	73	81	**0.004**
Chronic conditions	34 (68%)	1463 (58%)	0.139	23 (68%)	921 (57%)	0.214	21 (70%)	224 (59%)	0.222	20 (77%)	218 (60%)	0.080	16/16(100%)	58 (95%)	0.366
Hospital nights 5 years[b]	3	3	0.656	3	3.5	**0.012**	3	4	0.191	3	4	0.382	NA	NA	NA
Vaccinated last season	13 (26%)	1036 (41%)	**0.035**	9 (27%)	671 (42%)	0.078	8 (27%)	167 (44%)	0.069	7 (27%)	163 (45%)	0.080	5/17 (29%)	34/54 (63%)	**0.015**
Vaccinated this season	12 (24%)	983 (39%)	**0.034**	8 (24%)	628 (39%)	0.069	7 (23%)	154 (40%)	0.066	6 (23%)	148 (40%)	0.080	6 (32%)	40 (66%)	**0.009**
2016/17 season															
Total number	201	3188		135	2108		126	525		115	488		20	50	
Sex, male	80 (40%)	1444 (45%)	0.129	52 (39%)	917 (44%)	0.257	50 (40%)	273 (52%)	**0.013**	45 (39%)	257 (53%)	**0.009**	9 (45%)	29 (58%)	0.324
Age, median	84	78	**<0.001**	84	80	**<0.001**	84	81	**0.005**	84	80.5	**0.011**	80.5	75	0.431
Chronic conditions	111 (55%)	1800 (57%)	0.731	71 (53%)	1143 (54%)	0.713	65 (52%)	292 (56%)	0.414	60 (52%)	279 (57%)	0.331	18 (90%)	48 (96%)	0.329
Hospital nights 5 years[b]	4	3	**0.009**	4	3	**<0.001**	4	4	0.496	4	4	0.395	NA	NA	NA
Vaccinated last season	77 (38%)	1383 (43%)	0.159	53 (39%)	874 (42%)	0.615	50 (40%)	235 (45%)	0.302	44 (38%)	221 (45%)	0.172	12 (60%)	29 (58%)	0.878
Vaccinated this season	64 (32%)	1213 (38%)	0.078	44 (33%)	749 (36%)	0.489	40 (32%)	198 (38%)	0.212	36 (31%)	190 (39%)	0.128	11 (55%)	31 (62%)	0.589
2017/18 season															
Total number	205	4236		146	2911		135	563		135	561		31	87	
Sex, male	83 (41%)	1806 (43%)	0.544	61 (42%)	1192 (41%)	0.842	58 (43%)	255 (45%)	0.625	58 (43%)	255 (46%)	0.601	12 (39%)	36 (41%)	0.795
Age, median	83	78	**<0.001**	83.5	80	**<0.001**	83	81	**0.019**	83	81	**0.020**	82	79	0.971
Chronic conditions	119 (58%)	2401/4235 (57%)	0.702	81 (56%)	1595 (55%)	0.871	75 (56%)	340 (60%)	0.304	75 (56%)	339 (60%)	0.301	29/29 (100%)	82/86 (95%)	0.237
Hospital nights 5 years[b]	3	3	0.761	3	3	**<0.001**	3	4	**<0.001**	3	4	**<0.001**	NA	NA	NA
Vaccinated last season	94 (46%)	1827/4235 (43%)	0.444	72 (49%)	1213 (42%)	0.068	66 (49%)	254 (45%)	0.429	66 (49%)	255 (46%)	0.472	19/28 (68%)	59/85 (69%)	0.877
Vaccinated this season	89 (43%)	1739 (41%)	0.502	72 (49%)	1145 (39%)	**0.016**	67 (50%)	239 (43%)	0.131	67 (50%)	241 (43%)	0.161	21 (68%)	61 (70%)	0.805

[a] Statistical significance of difference between groups shown as the p-value. Distributions of continuous variable were compared between cases and controls with Mann-Whitney's *U* test. Categorical variables were compared with Pearson's chi-squared test. [b]Nights spent in hospital during past 5 years, median. Denominators added if different from the total number of cases/controls because of missing data.

Table 4
Influenza vaccine effectiveness calculated in four analysis sets in the register-based case-control study, in the active test-negative design (TND) study and in the register-based cohort study during the epidemic seasons 2015/16, 2016/17 and 2017/18.

			Vaccinated		Unvaccinated			
Season	Analysis	N	Cases	Controls	Cases	Controls	Crude IVE, 95% CI (%)	Adjusted IVE, 95% CI (%)
2015/16	All hospitalizations	2591	12	983	38	1558	50 (4, 74)	47 (-16, 76)
	Acute hospitalizations	1650	8	628	26	988	52 (-8, 78)	50 (-28, 81)
	SARI hospitalizations	412	7	154	23	228	55 (-8, 81)	50 (-40, 83)
	TND-like study	392	6	148	20	218	56 (-13, 83)	52 (-48, 84)
	Active TND study	80	6	40	13	21	76 (27, 92)	82 (26, 96)[a]
			Cases	Attack rate[b]	Cases	Attack rate[b]		
	Cohort study	38464	13	124	43	200	58 (22, 78)	48 (-9, 75)
2016/17	All hospitalizations	3389	64	1213	137	1973	24 (-3, 44)	11 (-29, 39)
	Acute hospitalizations	2243	44	747	91	1361	12 (-27, 39)	10 (-42,43)
	SARI hospitalizations	651	39	197	87	328	23 (-16, 49)	22 (-28, 52)
	TND-like study	603	36	190	79	298	29 (-10, 54)	29 (-20, 58)
	Active TND study	70	11	31	9	19	25 (-114, 74)	21 (-179, 77)[a]
			Cases	Attack rate[b]	Cases	Attack rate[b]		
	Cohort study	39619	71	421	170	767	43 (25, 57)	29 (1, 49)
2017/18	All hospitalizations	4441	89	1739	116	2497	−10 (-46, 17)	1 (-40, 30)
	Acute hospitalizations	3057	72	1144	74	1767	−50 (-109, −8)	−27 (-91, 15)
	SARI hospitalizations	698	67	239	68	324	−34 (-95, 8)	−24 (-102, 24)
	TND-like study	696	67	241	68	320	−31 (-91, 10)	−20 (-95, 26)
	Active TND study	118	21	61	10	26	11 (-116, 63)	15 (-113, 66)[a]
			Cases	Attack rate[b]	Cases	Attack rate[b]		
	Cohort study	37771	96	584	128	617	5 (-24, 27)	13 (-21, 37)

The influenza vaccine effectiveness (IVE) was calculated in the register-based case-control and TND studies as 1-odds ratio (OR) and in the cohort study as 1-ratio comparing the hazard of the outcome influenza A in vaccinated to that in the unvaccinated. 95% CI, 95% confidence interval. The estimates of register-based analyses are adjusted for age, sex, time, one-year vaccination history, presence of underlying chronic conditions, and nights hospitalized in the past five years. [a]The estimates for the hospital-based TND study are adjusted for age, sex, and time only. Age, 65–69, 70–74, 75–79, 80–84 or ≥85 years at the start of the symptoms or at hospitalization. Time, three phases of the epidemic according to the number of cases (25%, 50% and 25%) in each. [b]The attack rate is presented as the cumulative risk at the end of the study period multiplied by 1e + 05.

188 were excluded from all analyses for reasons shown in Fig. 3. After exclusions for the specific analyses, 10 421, 6 950, 1 761 and 1 691 subjects were selected for our four respective analyses (Fig. 3, Table 2).

Clinical characteristics of cases and controls by analysis and season are shown in Table 3. For all analyses and seasons (except "TND-like study" 2015/16), cases and controls were statistically significantly different by age. In 2015/16 cases were younger and the last two seasons, cases were older than controls. The groups were also statistically different in most of the analyses regarding the history of previous hospital nights within 5 years. Although in some analyses, cases had more previous hospital nights, in other analyses they had less, so there was no clear trend. Other variables also showed statistical difference between cases and controls in some analyses (Table 3).

Table 4 shows the crude and adjusted IVE estimates with CIs for different variations of case and control definitions in the register-based case-control study. For season 2015/16, adjusted IVE estimates ranged from 47% to 52%. 2016/17 estimates ranged from 10% to 29%. 2017/18 estimates ranged from −27% to 1%. None of the estimates were significantly different from 0% at the 5% significance level.

3.3. Results of the sub-study 3 - cohort

During the three study seasons 2015–18, after excluding subjects who moved out from the study site, the final cohort sizes ranged from 37 771 to 39 619 per season. Each season, 44% of study population was vaccinated. The attack rates and crude and adjusted IVE estimates for inpatient LCI-A are shown in Table 4. Adjusted IVE estimates were 48% (-9, 75) in 2015/16, 29% (1, 49) in 2016/17 and 13% (-21, 37) in 2017/18.

3.4. Comparison of the IVE estimates retrieved from the sub-studies

The IVE estimates for all designs and analyses tended to be quite similar. The IVE varied between the seasons more than between the study designs (Table 4). None of the designs gave systematically higher or lower estimates than others. In season 2015/16, the active TND estimated the IVE slightly higher than the two other designs, but this was not the case for other seasons. The register-based case-control design estimated the IVE slightly lower than TND and cohort designs in the season 2017/18, but this trend was not clearly seen during other seasons. Of register-based case-control analyses, "TND-like study" tended to produce closer estimates to the active TND than analyses with a broader spectrum of hospital controls, although this was not very clear, and the estimates were close to the active TND in season 2016/17 only.

Adjustment did not considerably change the IVE estimates or their concordance. With a few exceptions, adjustment tended to slightly move the IVE estimate towards zero. Only a few of the analyses had enough power to give statistically significant estimates, although cohort study produced the narrowest confidence intervals.

4. Discussion

The three different study designs, active hospital-based test-negative design, register-based population-based cohort design and register-based hospital-based case-control design, produced mostly similar results in estimating the IVE against influenza A

in three epidemiologic seasons 2015–2018. The IVE estimates differed more by season than by study design. The register-based cohort design yielded most precise estimates and produced results more concordant with the active test-negative design than the case-control design.

With a few exceptions, our results were in line with pooled European TND study results for IVE against hospitalized influenza A, published by the I-MOVE + project, which were in consecutive order 42 (22–54), 17 (1–31) and 24 (2–40) [13–15]. North American results were similar as well [16–18]. Also, results of the previously published cohort study conducted with the same design and comprising all residents ≥65 years old from Finland were very similar: 48 (41–51), 24 (19–28) and 16 (12,19) during the three study seasons [5]. These estimates with narrow confidence intervals support the cohort study estimates in the current study setting. Season 2015/16 was dominated by A(H1N1)pdm09 which has been associated with better IVE in the elderly than A(H3N2) which dominated during seasons 2016/17 and 2017/18. This explains the drop in IVE estimates between the first season and others. Suggested potential reasons for the lower protection especially against H3N2 in the elderly are mismatch between the circulating virus and the vaccine virus due to genetic drift and egg propagation, waning immunity, other complex host immunity issues and prior vaccinations [19].

The test-negative design is a relatively new, but nowadays a standard method used globally in studying IVE. Its theoretical basis has been well studied and validated for measuring influenza under several assumptions [20]. It is designed to minimize the effect of healthcare seeking behavior. Also, testing everyone for the outcome in a standardized manner reduces misclassification bias. However, it has been shown that the method does not necessarily remove selection or confounding by healthcare seeking completely and might create selection bias by limiting the study population only for subjects having been tested [21,22].

While active prospective TND studies are easier to conduct than active prospective cohort studies, they can still be logistically challenging and resource demanding, especially in countries like Finland where the IVE estimation is not integrated into the routine virological surveillance. It requires a lot of work with the patient enrolment and typically multiple study sites to recruit enough participants for statistical power and to be geographically and epidemiologically representative. Assessment of strain and product specific IVE estimates is often limited or not possible due to inadequate sample size. In our TND sub-study, the strengths were prospective, active hospital-based recruitment of patients and data collection by study staff specifically dedicated and trained to the study. Strict protocols were used for vaccination verification and highly specific and sensitive RT-PCR tests were used for testing, minimizing misclassification. The screening at the participating wards was systematic. In addition to data collected from the patients and/or their next of kin, all medical records of both hospitals and those of municipal Tampere health care center were available. The two participating hospitals were the only ones in Tampere and thus served the whole elderly population of the city, increasing the generalizability to the source population. Main weakness was the small sample size that restricted adjustment for potential confounders and offered little statistical power. In addition, our study had poor efficiency, because a major part of initially enrolled patients had to be excluded as shown in Fig. 2.

The register-based cohort design offers a feasible and cost-effective way to produce seasonal IVE estimates. As described above, the registers cover the whole population, so the cohort can be defined as the whole source population. This removes selection bias and usually offers good statistical power due to a large cohort size. The cohort design as a follow up study is the only one that can directly estimate IVE as the relative reduction of infection hazard. Like all observational studies the cohort study is at risk for confounding bias. While adjusting for all known confounding factors might not be possible in small studies, big sample size offers possibility to adjust for many potential confounders. Different strategies can be used for adjustment. Baum et al. have studied this in Finnish setting and found a simple set of covariates that is likely to produce almost unconfounded estimates [5]. In this study we used the same covariates and would expect the residual confounding to be minimal as well.

Misclassification bias is another concern in register-based studies. Sources of possible misclassification are imperfect testing methods, healthcare seeking behavior, swabbing policies and register inaccuracies. In our study setting mostly PCR tests were used with close to 100% specificity. Influenza testing in real-life conditions is not as systematic as in the active TND study, and therefore, some cases were missed. In our study, in total 9 additional cases were discovered in the TND sub-study by the THL investigators, the test being not obtained by the hospital prior to enrolment. This lowers case detection, possibly equally for both vaccinated and unvaccinated, potentially only slightly underestimating IVE, depending on the outcome specificity. Misclassification due to missed tests could be differential, if vaccinated patients would be tested at different rate or their health seeking behavior would be different to the unvaccinated. In our TND study material, vaccinated and unvaccinated were tested at the same rate by the hospital clinicians. Nevertheless, if vaccinated patients would be more active in healthcare seeking, this would underestimate IVE.

The main concern in the register-based studies is information bias due to wrong or missing records in the registers. Thus, we expect exposure, outcome, and covariate misclassification. In our study we found that, in total 83% of confirmed cases in TND sub-study were found in the NIDR. Two false positives were found compared to THL's own retesting, but this was due to imperfect PCR test specificity rather than an issue with the registers. This would suggest good validity of the NIDR data.

Overall high outcome specificity would suggest low misclassification bias, even if outcome sensitivity is non-differentially imperfect [23–25]. This should apply for our cohort study as well, since cumulative risk for influenza during our study was small. Theoretical methods have been developed to adjust for misclassification, but we did not use such in this study [26].

Register-based case-control design offers another inexpensive and easy way to estimate IVE. Compared to active TND design, it also offers bigger sample sizes, improving statistical power and possibilities to adjust for potential confounders. It is simple to use different definitions for cases and controls with specifically defined ICD codes to study the exact exposure and outcome of interest. However, the registers don't have direct information about severity of chronic diseases or onset time of symptoms. NIDR doesn't include information about negative samples, so proper test-negative design was not possible. Instead, we had to settle for ''TND-like study'' described above. This more classical case-control design has also many weaknesses. Use of hospital-based controls might induce selection bias if they don't represent the source population where cases arise. Controls from different specialties of hospital might be inherently different than cases [27]. A recent study Balasubramani et al. compared active and register-based TND and found that the study populations differed significantly and tended to produce difference in IVE estimates. They concluded that the active designs estimates might be more generalizable, probably due to selection and other types of bias in register-based estimates [28]. In our study, the cases and controls should have risen from the same source population since virtually all inpatient care for Tampere residents is covered by the national registers. In addition, the background characteristics did not systematically differ between the cases and controls, except

for age. However, it is possible that some of the difference in estimates was due to selection bias, such as different health seeking behavior of controls. We did not use matched pairing of cases and controls which could have reduced bias. Confounding was adjusted for the same variables as in the cohort analysis, but adjustment had a minor effect on the results. Misclassification bias discussed above should be comparable to that in the cohort substudy.

Overall, despite relatively large sample size in some analyses, the confidence intervals were wide and overlapped with each other. This could mean that all the differences found between the analyses could be due to chance, and therefore, our results should be considered descriptive in nature. Additionally, systematic bias such as selection bias, misclassification or residual confounding were likely to have affected our estimates. Also, not having a category for partially vaccinated, could lead to slight underestimation of the IVE in TND and case-control studies compared to the cohort study.

This was the first study to directly compare register-based case-control and routinely used cohort design with robustly designed and conducted active TND in Finland. Although none of the substudies had sufficient statistical power to give precise estimates, we found reasonable consistence between the results of the compared designs. In future, the accuracy of Finnish health register data could be assessed more comprehensively by comparing the exposure and outcome data in registers to all appropriate actively collected IVE study data. In addition, we hope to get access to the data of influenza sample taking, to be able to collect data on the negative test results and to perform register-based TND studies in the future. It should be noted that the sample of this study was only elderly population. Comparisons made in this article may differ in younger age groups.

In conclusion, active hospital-based TND study and register-based, population-based cohort studies produced results comparable with each other and with other European studies. Furthermore register-based cohort design yielded most precise estimates with narrower confidence intervals. Register-based case-control design did not perform quite as well. This study further supports the use of register-based cohort design in Finland as routine estimation of IVE, since it requires a lot less effort compared to active studies and gives IVE estimates that are comparable to those observed in an active TND study.

Author contributions

All authors attest they meet the ICMJ criteria for authorship. The study was designed by JH, UB, RS, AP. Data analyses and interpretation were performed by JH, UB, RS, AP. Principal investigator of the active TND study was RS. Responsible for writing the manuscript was JH. All authors made a significant intellectual contribution to the development of this manuscript and approved the final version for submission.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: No financial conflicts related to the current work. Finnish Institute for Health and Welfare (THL) conducts Public-Private Partnership with vaccine manufacturers and has received research funding from Sanofi Inc., Pfizer Inc., and GlaxoSmithKline Biologicals SA. THL is a partner in a EU IMI funded project IMI Drive, which evaluates the effectiveness of influenza vaccination. RKS and AAP have been investigators in these studies, but they have received no personal remuneration.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.05.080. These data include Google maps of the most important areas described in this article.

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