D2.2

Systematic review of the sources of confounding, bias and strategies to manage their impact in influenza vaccine effectiveness studies

777363 - DRIVE

Development of robust and innovative vaccine effectiveness

WP2 –Study tools

Lead contributor	Gaël Dos Santos (GSK)
	gael.x.dos-santos@gsk.com
Other contributors	Josephine van Boxmeer (Seqirus), Miriam Levi (UNIFI), Anke Stuurman (P95), Topi Turunen (FISABIO)
Reviewers	Margarita Riera (P95), Helene Bricout (SP), Mendel Haag (Seqirus), Clotilde El Guerche-Seblain (SP), Ritva Syrjänen (THL), Laurence Torcel-Pagnon (SP)

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1. PUBLISHABLE SUMMARY

This deliverable presents the background, methodology, results and conclusions from the literature review that was conducted to identify potential biases, confounding factors and effect modifiers in influenza vaccine effectiveness (IVE) studies as well as strategies used to manage their impact in IVE studies. The review includes articles published in peer-reviewed journals and grey literature and builds further on the most recent guidelines or technical reports on the topic.

The results section summarizes what was found during the data extraction. Since the search criteria focused on IVE studies, there was no specific search conducted into the various biases and covariates. Therefore, the biases and covariates described in this section were the result of available information in the papers that were included according to the criteria described in Chapter 6 and the information is not exhaustive. First, various forms of bias are described: selection bias, frailty bias, healthy vaccinee bias, repeated vaccination bias, misclassification bias and biases due to type of study design and patient characteristics across recipients of different vaccine types. Secondly, the effect of various covariates on IVE studies are described: underlying medical conditions (in particular, obesity), concomitant administration of vaccines, use of antivirals or statins, the level of vaccine match, full vs. partial vaccination and intra-seasonal waning effectiveness. Lastly, an extensive overview of IVE estimates derived from meta-analyses and literature reviews is described for various subgroups alongside the estimates for the general population: for elderly, healthy adults, healthcare workers, healthy children, pregnant women and other even more specific populations.

Each chapter aims to provide recommendations. These recommendations serve as guidance and inform other deliverables of the DRIVE project such as the update of the generic protocols and the statistical analysis plans.

2. LIST OF ABBREVIATIONS

ADL	Activities of daily living
APR	Adjusted prevalence ratio
ARI	Acute respiratory illness
DRIVE	Development of Robust and Innovative Vaccine Effectiveness
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EU	European Union
GP	General Practitioner
HCW	Healthcare workers
ICD	International Classification of Diseases
ILI	Influenza-like illness
IVE	Influenza vaccine effectiveness
OR	Odds ratio
PICO	Population, Intervention, Comparison, Outcome
PPV	Positive predictive value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
RCT	Randomized controlled trials
RR	Relative risk or risk ratio
RT-PCR	Real Time Polymerase Chain Reaction
SM	Screening method
TIV	Trivalent inactivated vaccine
TND	Test-negative (study) design
VE	Vaccine effectiveness
WHO	World Health Organization
WP	Work Package

3. INTRODUCTION

Understanding the impact of influenza vaccines is important for public health professionals and policy-makers. Such data can be used to inform vaccination policy (such as determining whether a vaccine is effective in risk groups of severe disease or identifying preferred vaccine product classes) and other public health measures (e.g. use of antivirals when vaccine effectiveness is low). The assessment can be performed using randomized controlled trials (RCTs) of vaccine efficacy but is most often based on observational studies of vaccine effectiveness. The latter assess the performance of vaccines under real-world conditions but are likely to bear some limitations due to biases and inadequate adjustment for confounding factors.

The objectives of the DRIVE project are to build a sustainable study network for brand-specific influenza vaccine effectiveness (IVE) studies in the EU and to develop a governance model for transparent, scientifically robust public-private collaboration. DRIVE's Work Package (WP)2 provides tools to inform protocol development and study design for seasonal IVE studies. Other outputs of WP2 include mapping of available sources of vaccine brand information, Standard Operating Procedures and annual study tenders.

This document (DRIVE deliverable 2.2) provides the results of a literature review focusing on bias, confounding factors and effect modifiers, relevant in the context of IVE evaluation. It provides background information and a summary of the current knowledge together with a rationale for this work. This is followed by the search strategy and methodology to identify and extract findings. The results section starts off with a flow chart on included and excluded papers, followed by three separate sections of results from the data extraction. The first section summarises bias identified in articles from the literature search, the second section reports covariates (both confounders and effect modifiers), and the third section provides an extensive overview of IVE estimates derived exclusively from meta-analyses and systematic literature reviews. For each section, the references are numbered and can be found in the full reference list in the end, which is organised by section (chapter and paragraph).

4. BACKGROUND & OBJECTIVES

In observational vaccine effectiveness (VE) studies, confounding due to differences in disease risk or in care-seeking behaviour between vaccinated and unvaccinated subjects, and the difference in the probability of being vaccinated, can substantially bias VE estimates. The main purpose for collecting covariate data is to measure and control for potential confounders, either in study design (e.g. by matching) or in data analysis. Besides vaccination history and outcomes, investigators in VE studies need to collect data on other covariates of the study participants.

Another purpose of measuring covariates is to stratify VE estimates based on subpopulations of interest. Depending on the objectives of the evaluation, existing influenza VE programmes often produce stratified VE estimates, e.g., by age group, with the intent to account for effect modifier. Other groups of interest might be pregnant women or persons with underlying medical conditions.

A bias is a systematic error that leads to an incorrect effect estimate of the exposure on the outcome. Examples are selection bias and confounding bias.

A confounder is a variable that influences both the exposure and the outcome. Confounding can be subdivided into positive confounding, which leads to bias away from the null hypothesis (higher VE estimate), and negative confounding, which leads to bias toward the null hypothesis (lower VE estimate).

An effect modifier is a variable that differentially modifies the size of an effect of the exposure on the outcome.

This document summarizes the results of a systematic literature review to identify potential biases, confounding factors and effect modifiers in IVE studies, and to identify systematic literature reviews of IVE studies. The findings have been also put into perspective in light of existing guidelines [1] and technical reports [2-4] as well as articles published in peer-reviewed journals and grey literature.

The first part of the review describes sources of bias, the confounding factors and effect modifiers, their impact on IVE estimates and how to avoid them based on the publications that came up using the search criteria (as described in Section 5, Methodology). It also describes other covariates that were identified in those publications; however, those covariates that are near-universally agreed upon and commonly included in IVE analyses and have been well described in the above guidelines, are not described in detail. Covariates collected for purposes other than assessing bias and confounding are out of scope of this deliverable.

The second part of this review focuses on systematic reviews and/or meta-analyses that evaluated the effectiveness of influenza vaccines by target groups of vaccination. The aim is to provide an overview of the potential variability of vaccine effectiveness depending on the population considered for inclusion.

Ultimately this work intends to provide useful collated information that can help to design observational studies, improving the detection and control for biases and confounders in order to minimize the risk of generating erroneous findings IVE studies or facilitating the interpretation of results generated. It will also inform the updates of DRIVE D4.1 (*Methodology guidelines for concerted analysis of data and control of confounding factors*), D1.1 (*Multistakeholder Research Agenda*) and D4.6 (*Guideline for interpretation of influenza vaccine effectiveness results*).

References

[1] World Health Organization. Evaluation of influenza vaccine effectiveness - A guide to the design and interpretation of observational studies. Geneva: World Health Organization; 2017.

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5. METHODOLOGY

This section describes the study protocol and method applied to build the literature search, the guidelines followed, data sources used, and the stepwise approach to select the relevant findings.

The primary focus of this deliverable is on the sources of bias, confounders, and effect modifiers. Therefore, using a standardised approach, we extracted all studies that evaluated bias, confounders and effect modifiers in the context of seasonal influenza VE evaluation. In addition, using the same search strategy, we took the opportunity to also extract all systematic reviews and meta-analyses that provided VE estimates, and summarised the finding by study population.

5.1. Systematic review objectives

The objectives of the systematic review were:

- To summarize relevant information from all published manuscripts identified through our search strategy and included through our inclusion criteria that contain data dealing with bias, confounding, and effect modification in the context of seasonal influenza VE assessment
- To report the observed impact of the biases as described in the published manuscripts
- To provide an overview of VE estimates, summarised by population group and derived from systematic literature reviews and meta-analyses identified through search criteria as listed below
- To propose recommendations on ways to account for bias, confounding and effect modification when implementing VE studies.

5.2. Approach and framework

The PICO (Population, Intervention, Comparison, Outcome) framework [1] was used to identify relevant studies from the literature.

The PICO framework includes:

- **Population:** All ages above 6 months, from any setting and inclusive of healthy individuals and those with pre-existing medical conditions.
- **Interventions:** The interventions of interest were any seasonal influenza vaccine administration (e.g., inactivated, adjuvanted or unadjuvanted vaccines, Live Attenuated Influenza Vaccines (LAIV), high dose, cell culture).
- **Comparator groups:** People who received a non-influenza vaccine or who were not vaccinated (unexposed comparator group).
- **Outcome measures:** Prevention influenza in any medical setting (primary care, emergency room visits, hospitalization, admission to Intensive Care Unit (ICU) and mortality attributable to influenza measured as VE.

The systematic literature review followed Cochrane guidelines, and Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [2].

- The review was systematic.
- The time period was limited to 1976–2018.
- All identified studies, literature or other records were independently screened by two reviewers for eligibility using a three-stage sifting approach to reviewing the title, abstract and full text.
- Any disagreements were resolved by discussion or involvement of the core group.
- The number of search hits identified and screened out was recorded at each stage (and the reason for exclusion at the full-text stage was reported).
- The result section includes a study flow diagram showing the number of studies identified, screened, included, excluded and reasons why excluded using PRISMA checklist.

The PRISMA flow diagram [2] was used to guide the different steps of the literature search and ultimate numbers are reported in the result sections.

5.3. Geographical scope

- Worldwide
- Source manuscripts from journals/reports in English (peer-reviewed as well as non-peer-reviewed).

5.4. Limits

The following limits were applied:

- Publication date: 01 Jan 1976 to 15 Mar 2018.
- Language: English.

5.5. Sources of data

The core of our review was a PubMed literature search. The PubMed search was complemented with a search in the Cochrane library and Embase as well as grey literature. The searches were conducted as described below.

5.5.1. PubMed

To find relevant articles on seasonal influenza vaccination in subjects with or without existing comorbidities, three search strings were defined, namely on:

#1. Influenza

Influenza [TI] *OR* fu [TI]

#2. Vaccination

vaccin*[TIAB] *OR* immuniz*[TIAB] *OR* immunis*[TIAB]

#3. “Estimate”

effect*[TIAB] *OR* impact*[TIAB] *OR* estim* [TIAB] *OR* confound*[TIAB] *OR* bias*[TIAB]

#4. #1 AND #2 AND #3

#5. (#4 NOT (Addresses[ptyp] *OR* Autobiography[ptyp] *OR* Bibliography[ptyp] *OR* Biography[ptyp] *OR* Case Reports[ptyp] *OR* Clinical Conference[ptyp] *OR* Clinical Trial, Phase I[ptyp] *OR* Clinical Trial, Phase II[ptyp] *OR* Comment[sb] *OR* Congresses[ptyp] *OR* Consensus Development Conference[ptyp] *OR* Consensus Development Conference, NIH[ptyp] *OR* Dataset[ptyp] *OR* Dictionary[ptyp] *OR* Directory[ptyp] *OR* Editorial[ptyp] *OR* Electronic Supplementary Materials[ptyp] *OR* Festschrift[ptyp] *OR* Guideline[ptyp] *OR* Historical Article[ptyp] *OR* Interactive Tutorial[ptyp] *OR* Interview[ptyp] *OR* Lectures[ptyp] *OR* Legal Cases[ptyp] *OR* Legislation[ptyp] *OR* Letter[ptyp] *OR* News[ptyp] *OR* Newspaper Article[ptyp] *OR* Patient Education Handout[ptyp] *OR* Periodical Index[ptyp] *OR* Personal Narratives[ptyp] *OR* Portraits[ptyp] *OR* Practice Guideline[ptyp] *OR* Published Erratum[sb] *OR* Retracted Publication[sb] *OR* Retraction of Publication[sb] *OR* Scientific Integrity Review[ptyp] *OR* pubmed books[filter])) Filters: Publication date from 1976/01/01; Humans; English

The combination of these search strings (i.e. #1 AND #2 AND #3) yielded 5599 hits (dd. 15-03-2018).

5.5.2. Cochrane library

The following search strings were used to search for relevant articles in the Cochrane library (http://onlinelibrary.wiley.com/cochranelibrary/search).

Influenza

1# influenza:ti *OR* flu:ti

Vaccination

2# vaccin*:ti,ab *OR* immuniz*:ti,ab *OR* immunis*:ti,ab

"Effect"

3#("effect*: ti,ab *OR* impact*: ti,ab *OR* estim*: ti,ab *OR* confound*: ti,ab *OR* bias*: ti,ab)

4# (#1 and #2 and #3)

The combination of these search strings (i.e. #1 AND #2 AND #3) yielded 29 hits (dd. 15-03-2018).

5.5.3. Embase

The following search strings were used to search for relevant articles in Embase.

#1 Influenza

(influenza*:ti,ab *OR* flu:ti,ab)

#2 Vaccination

(vaccin*:ti,ab *OR* immuniz*:ti,ab *OR* immunis*:ti,ab)

#3"Effect"

("effect*: ti,ab *OR* impact*: ti,ab *OR* estim*: ti,ab *OR* confound*: ti,ab *OR* bias*: ti,ab)

#4(#1 and #2 and #3)

The combination of these parts (i.e. #1 AND #2 AND #3) yielded 6899 hits (dd. 15-03-2018). We

expected some overlap between the databases. Therefore, deduplication took place prior to abstract screening.

Combining the results yielded 12527 hits which ultimately resulted in 7595 hits after deduplication.

5.5.4. Grey literature search

The review was primarily based on a literature search of peer-reviewed articles in PubMed, Embase and the Cochrane library. In addition, a search in grey literature was conducted to identify data for the remaining gaps. The following sources were screened to identify possibly relevant data:

- WHO (http://www.who.int)
- WHO flunet: (http://www.who.int/influenza/gisrs_laboratory/flunet/en/)
- CDC (http://www.cdc.gov)
- European Centre for Disease Prevention and Control ([ECDC] http://www.ecdc.europa.eu)
- Flu News Europe: (http://www.flunewseurope.org/)
- Cidrap: http://www.cidrap.umn.edu/
- Google search, combining search terms for VE and influenza vaccine and/or vaccine efficacy and influenza vaccine.

When relevant, the figures or tables were copied with the source quoted, without any modifications.

5.6. Selection procedure

After removal of duplication, from the articles retrieved from PubMed, Cochrane library and Embase, the relevant references were selected by a three-step selection procedure, as follows:

1. Screening of title and abstract (first selection step)

This step yielded the articles that are to be assessed in full-text. The major topics of the articles were assessed based on relevancy for the objectives, by the title and abstract. In this step, articles that seemed to contain relevant data for the objectives were selected for full-text screening, while articles that did not seem to contain relevant data were not selected for full-text assessment. If in doubt, the articles were checked full-text in the second selection step.

To identify the eligible articles during the first step, the following inclusion and exclusion criteria were applied.

- Inclusion criteria:
    - Experimental studies, observational studies and systematic reviews (+/- meta-analyses), modelling studies which report bias, confounders, effect modifiers in the context of influenza vaccine effectiveness evaluation.
- Reasons for exclusion were the following:
    - Studies which only evaluated non-specific outcomes such as influenza-like illness or all-cause mortality, without any relevant information on bias, confounders or effect modification were excluded upfront
    - Studies dealing exclusively with pandemic influenza without relevant information on bias, confounders, or effect modifiers

- Influenza vaccine study focusing exclusively on safety endpoints
- Animal or cell culture studies
- Studies with immunogenicity or safety endpoints exclusively
- Non-pertinent publication types (e.g. letters to the editor, editorials or comments)
- Case reports / case series.

2. Screening of full article (second selection step):

In this step the full-text of articles selected in step 1 were assessed. First it was determined whether the paper contained relevant content for (one of) the review objectives. If so, information was extracted from the full-text articles using a predefined template for a consistent approach to data extraction throughout the systematic review.

3. Screening during data-extraction phase (third selection step):

Further scrutiny of the article during the data-extraction phase might led to exclusion. For example, from articles presenting similar results from identical datasets, only one was included (usually this would be the most recently published article).

The process of selection and inclusion and exclusion of articles was registered in an electronic database (Rayyan QCRI) [3]. In this way, a clear overview on all selection steps was maintained at all phases. Reasons for exclusion of the papers during the full-text screening selection procedure is reported in section 6.

References

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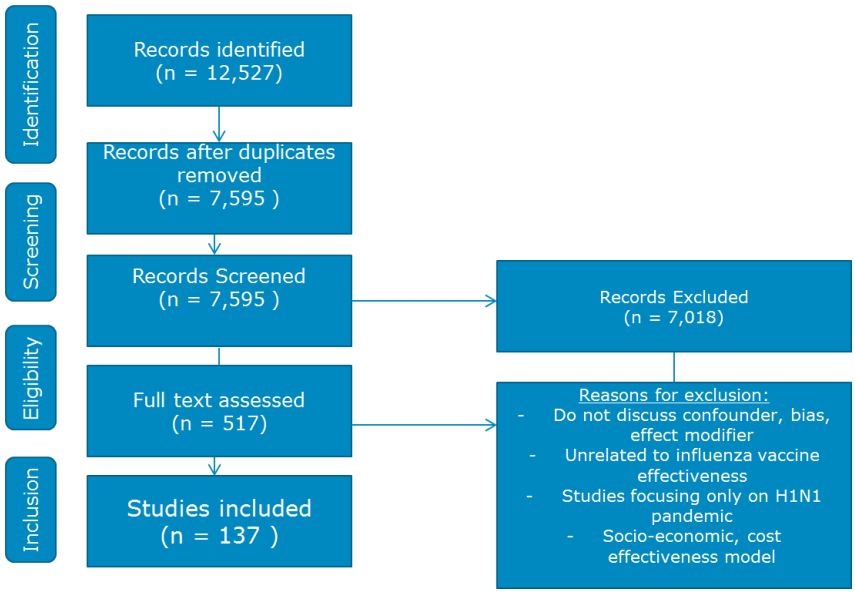
6. RESULTS

The selection process for inclusion and exclusion of studies is reported below. The process for identifying relevant articles is shown in Figure 1.

Figure 1 Flow chart for identification of relevant articles



6.1. Summary of data for bias

6.1.1. Selection Procedure

From the selection procedure, no exclusion was applied based on the type of bias and the screening phase was purposely broad to not neglect any potential biases relevant in the context of IVE assessment (Table 1). During the extraction phase biases were classified according to their description in the respective papers and grouped as such in the respective chapters presented in this section.

Table 1: Selection Procedures for bias during the full text screening phase

	Identified	Included	Excluded	Reason for exclusion
Selection bias	23	17	6	Doesn't discuss bias, n=1 Doesn't discuss selection bias, n=4 No full-text found, n=1
Healthy vaccines bias/ confounding by indication	30	6	24	Do not discuss healthy bias/Confounding by indication, n=24
Frailty bias	30	13	17	Do not discuss frailty bias, n=17
Misclassification bias	13	12	1	Wrong outcome (narcolepsy), n=1
Study design: Choice of controls	7	7	0	-
Results from Different study designs	14	4	10	Different outcomes across designs, n=1 Statistical techniques, n=3 Screening method only, n=1 Genetic drift, n=1 Narrative comment, n=2 Impact of lab test sensitivity/ specificity, n=1
Patient characteristics across recipients of different vaccine types	8	4	4	Population not relevant (in military), n=2 No relevant information, n=1 Narrative review, n=1

6.1.2. Selection bias

Selection bias is bias in the estimated association or effect of an exposure on an outcome that arises from the procedures used to select individuals into the study or the analysis (e.g. differential enrolment in a test negative design [TND] study based on exposure status). The selection of patients into a study using the test-negative design is dependent on whether a patient seeks medical care for treatment, agrees to participate in the study and is tested for influenza [1]. Selection bias leads to low external validity of the study results [2]. Five studies dealing with selection bias in the context of seasonal influenza vaccine effectiveness assessment were found. Three of these dealt with selection bias resulting from differences in healthcare seeking between the vaccinated and the unvaccinated, which biases the relationship between vaccination and influenza status [3-5]. Two studies address selection bias due to differential diagnostic testing [6;7].

6.1.2.1. Selection bias: the vaccinated are less likely to seek medical care, due to reduced disease severity

In some cases, vaccination may not fully prevent disease but may reduce its severity to a point where an individual chooses to not seek medical care. In such cases, the VE against medically-attended influenza is not equal to the VE against symptomatic influenza [5]. Ainslie et al. [3] have shown that if vaccinated

persons are less likely to seek medical care due to reduced disease severity, then VE against medically attended influenza acute respiratory illness (ARI) overestimates VE against symptomatic influenza [3]. When communicating results, clearly stating the outcome against which VE is measured is important to avoid misinterpretation of the VE.

6.1.2.2. Selection bias: differences in likelihood to seek medical care among the vaccinated and non-vaccinated

Individuals differ in how actively they seek medical care when faced with a respiratory infection. Hashim et al. [4] found that vaccinated individuals were consistently more likely than non-vaccinated individuals to consult a general practitioner (GP) for an acute respiratory infection; signaling that the likelihood to seek medical care may differ between these two groups, without providing insights on the expected impact of such bias [4].

6.1.2.3. Selection bias: Differential diagnostic testing

Fukushima et al. (2017) describe the effect of differential diagnostic testing on selection bias [7]. Whether or not a clinician orders a diagnostic test in routine clinical setting depends on their judgment regarding the likelihood of the patient having influenza (outcome) or having received influenza vaccination (exposure). Including only subjects with clinician-ordered tests in a TND study would result in a selection bias. For example, if clinicians order the diagnostic test for those with severe Influenza-like illness (ILI) and those who did not receive the vaccine, the proportion of non-vaccinees among cases is likely to increase, resulting in overestimation of VE. Once this type of selection bias is introduced, the extent and direction of the bias is impossible to predict [7]. Ferdinands et al. simulated a case-control study through Monte Carlo methods and found differential diagnostic testing (in which vaccinated children are tested less frequently than unvaccinated children) to be a source of bias that overestimates the true VE [6]. This source of bias was second in magnitude only to test specificity in the simulation study. To avoid this type of bias, pre-specified sampling strategies should be used to systematically recruit subjects from the source population to be tested for influenza [7].

6.1.2.4. Selection bias: identifying bias by looking at non-laboratory-confirmed outcomes outside the influenza season

The importance of controlling for residual bias when assessing vaccine effectiveness has been evaluated in several studies with practical recommendations provided [8-10]. In these studies, the authors notably pointed out the lack of precautionary measures to adequately measure the mortality benefits associated with influenza vaccination and highlighted that a frailty selection bias is likely to lead to a significant overestimation of the true effect of vaccination on influenza associated mortality. They questioned the findings that *(1)* the vaccine was purported to reduce 50% of all deaths, despite findings from national vital statistics studies that found ~5% of winter deaths were related to influenza in an average season, and *(2)* largest differences in mortality rates between vaccinated and unvaccinated persons are observed before influenza season, when the vaccine cannot be producing a true benefit [11]. According to the researchers, the main source of bias is likely a small subset of frail and terminally ill seniors who are less likely to become vaccinated during the preceding autumn months because of their deteriorating health, which is also magnified by the use of non-specific endpoints such as all-cause mortality in winter [10].

One important aspect reported by the authors to consider for cohort study analysis is to stratify the analysis per calendar month to segregate time (i.e., before influenza, during influenza and after seasonal influenza) to detect systemic and important mis-measurements. A pragmatic example supported this assumption when authors compared findings derived from a standard cohort study methods and stratified analysis per calendar time [11;12]. Using unstratified analysis, an apparent 50% VE for all-cause mortality over the entire winter was observed. Using the stratified analysis, they found no evidence that the vaccine prevented more deaths in the influenza period than in surrounding time

periods. The authors flagged the finding that vaccination apparently prevented mortality more effectively before the influenza season than during influenza season unambiguously demonstrates vaccination selection bias [10].

Detecting selection bias [13-17] by comparing VE in periods when influenza was circulating to periods adjacent to this time frame has subsequently been done in studies for other non-laboratory confirmed outcomes as well, such as ILI [16] and hospitalization for pneumonia [15-17].

Fireman et al. [14] proposed a method to adjust for this detected selection bias, through the so-called "differences in differences method" which examines a ratio of odds ratios. If influenza vaccine prevents mortality, then "there should be a detectable difference between 2 differences: *1)* the difference in the odds of prior vaccination between decedents and survivors that is observed on days when flu is circulating and *2)* the difference in the odds of prior vaccination between decedents and survivors that would be expected on the same calendar dates if flu were not circulating" [14] . To this end, they fitted a logistic regression model with a novel case-centered specification. This method has been subsequently applied in other studies [13;18].

Wong et al. [17] used instrumental variable (IV) analysis to compare mortality and pneumonia-and-influenza hospitalizations during and after the influenza season. Census subdivision-specific influenza vaccine coverage was used as the IV, so IV analysis compared groups of patients that differed in the likelihood of having received influenza vaccination. Results from the IV analysis were less biased than those from standard logistic regression [17].

In summary, it has been underscored that specific outcomes should be used to maximize the specificity and analysis per calendar time should be carried out using the virus surveillance data to identify the epidemic period for each season. If non-specific outcomes are used, comparing VE during and outside the influenza season can flag the presence of residual bias.

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6.1.3. Healthy vaccinee bias and confounding by indication

Thirty papers dealing with healthy vaccinees bias or confounding by indication in the context of IVE assessment have been identified, of which 24 were ultimately included as relevant to inform the discussion of bias in such context.

“Confounding by indication” is likely to be present if patients with underlying chronic diseases are more prone to receive the vaccination as compared to a healthy study participant. If no adequate statistical adjustment is made (for example on underlying medical conditions or comorbidities), this leads to an underestimation of VE since the less healthy population is at higher risk of adverse health outcomes.

The opposite situation is called “healthy vaccinee bias” and refers to a situation when patients, who are in better health, are more likely to adhere to the annually recommended influenza vaccination [1]. If not accounted for (for instance, by adjusting for comorbidities or indicators of health seeking behaviour), healthy vaccinee bias is expected to lead to an overestimation of VE.

Using a systematic review, researchers have investigated the frequency and impact of confounding by indication and healthy vaccinee bias in the context of observational studies assessing influenza vaccine effectiveness [2]. Looking at the baseline characteristics of the eligible studies for their work, Remschmidt, et al. [2] found that the majority of studies showed evidence of confounding by indication

rather than for healthy vaccinee bias. They have identified 23 relevant studies reporting 11 different outcomes of which 19 (83%) showed high risk of bias: 3 studies showing a combined form of confounding/bias [3-5], 2 for healthy vaccinee bias [6;7], and 14 due to confounding by indication (but no health vaccinee bias) [8-21].

Interestingly, adjustment for confounders increased VE on average by 12 % (95 % CI: 7–17 %; all-cause mortality), 9 % (95 % CI: 4–14 %; all-cause hospitalization) and 7 % (95 % CI: 4–10 %; influenza-like illness) and 9 still showing some residual confounding despite adjustment.

They concluded that both confounding by indication and healthy vaccinee bias are likely to operate simultaneously in observational studies on influenza VE. They more specifically recommended that cohort studies using administrative databases with unspecific outcomes should no longer be used to measure the effects of influenza vaccination. Alternatively, other study designs, including TND studies [22] and well-controlled observational studies using influenza-specific laboratory-confirmed outcomes, would be preferable to obtain more reliable estimates of influenza vaccine effectiveness.

Previous work proposed some useful approaches to verify further that residual confounding by healthy vaccinee effects is still present in the adjusted data. The baseline estimates should be calculated in the peri-influenza season period (i.e., outside influenza seasons) when the virus is (virtually) not circulating and therefore no vaccine effect should be present [1;23]. Any VE obtained during this control period reaching the statistical significance would be attributable to unmeasured confounding, whereas successful adjustment would have removed the effect. The caveat being that this may not be possible when using laboratory-confirmed endpoints due to the absence of cases outside the influenza period.

Some frameworks [2] have been developed to detect the presence of confounding by indication or healthy vaccinee bias in influenza VE studies (Table 2).

Table 2: Conceptual framework: Indicators and conclusions for presence of confounding by indication and healthy vaccinee bias in influenza vaccine effectiveness [2]

Indicator	Conclusion	References
Vaccinated study participants have a higher proportion of comorbidities than unvaccinated study participants, as indicated by baseline characteristics	High risk of confounding by indication in the unadjusted data set	[24;25]
Vaccinated study participants have a lower proportion of comorbidities than unvaccinated study participants, as indicated by baseline characteristics	High risk of healthy vaccinee bias in the unadjusted data set	[26;27]
Inclusion of comorbidities in the regression model increases vaccine effectiveness	Confounding by indication has led to underestimation of vaccine effectiveness in the unadjusted data set	[4]
Inclusion of comorbidities in the regression model decreases vaccine effectiveness	Healthy vaccinee bias has led to overestimation of vaccine effectiveness in the unadjusted data set	[4]
Significant effects of influenza vaccination appear outside the influenza season ("off-season estimates"), despite adjustment for comorbidities	Residual confounding by healthy vaccinee bias	[13;23;27]

Hak and collaborators [24] studied confounding by indication in observational studies in the context of prevention of influenza complications. They summarised methods to reduce confounding by indication and highlighted 3 statistical methods that are usually used for adjustments, as follows [28-35].

1. Statistical control of confounding factors in multivariable regression model [28;29]

   The first option (statistical control), is widely used and encompasses a stepwise approach starting with the identification of relevant covariates in the dataset. Then the univariate analysis is used to identify the potential confounders that reach the pre-defined statistical significance. Finally, the multivariate model is launched which includes the confounding variables that collectively influence the estimated association between the exposure and the outcome.

2. Sub-classification of patients on levels of the propensity score [30-32]

   An alternative approach (sub-classification of patients) is used in particular when the number of prognostic variables is numerous and refer to the propensity score method, initially introduced by Rubin and Rosenbaum [30-32]. Additional methods such as discriminant matching for multivariate normal covariates [34] and the use of "confounder score" [35] have been also presented elsewhere.

3. Pseudo-randomisation on levels of instrumental variables [33]

   The third option (pseudo-randomisation) aims to overcome the potential lack of balance on unobserved prognostic indicators (for example, health behaviour), using the instrumental variable. This technique originates from the field of econometrics and has so far not been extensively used in medical research. With this approach the patients are subdivided according to levels of a covariate that is associated with the exposure, but not associated with the outcome. This approach aims to lead to equal distribution of health characteristics in both non-exposed and exposed people and thus prevent potential confounding. Some applications have been reported previously [33]. Further medical studies and use of instrumental variables may be needed to verify the validity of the approach. Further insights are reported in the chapter on bias associated with study design (section 6.1.6).

Mori, et al. evaluated confounding in the context of influenza vaccine effectiveness [36] and presented useful methods for controlling confounding factors. They highlighted the importance of distinguishing two stages for controlling a confounding factor. The first stage is at the time of designing a study plan, and the second stage is at the time of data analysis.

The first step relates to the time of designing a study plan such as:

- Restriction of study subjects (restriction is a procedure that limits participation in the study to people who are similar in relation to the confounder)
- Matching a confounding factor with the comparative groups (matching refers to a procedure whereby controls are selected in such a way that the distribution of potential confounders among them will be identical to those of the cases)
- Randomization of the study subjects are methods for controlling a confounding factor at the stage of study design (randomization of study subjects with a reasonable sample size aims to ensure that the distribution of potential confounding variables will be similar among the groups to be compared). The RCT is a well-known intervention study using randomization of the study subjects, but will not be developed here as this is not in scope of observational studies.

The second stage is at the time of the data analysis such as:

- Stratification (stratified analysis) and regression modeling (multivariate analysis) are the methods for adjusting for a confounding factor at the stage of data analysis [37], allowing measurement of the strength of association separately within each well-defined category of confounding variable.

To illustrate these two stages, Mori et al. [36] summarised some works in which retrospective or prospective cohort studies have been carried out to evaluate influenza vaccines in which confounding by indication, and other confounding have been adjusted with a technique of restriction, matching, stratified analysis, or multivariate analysis.

In this chapter, the search revealed useful frameworks [2] to detect the presence of confounding by indication or healthy vaccinee bias in influenza VE studies. Furthermore, methods were presented to capture confounding by indication [24], using multivariable regression model [28;29], sub-classification of patients on levels of the propensity score [30-32], or pseudo-randomisation on levels of instrumental variables [33]. Lastly some works underscore the need to distinguish two stages for controlling a confounding factor; the first one relating to the time of designing a study plan and the second relating to the data analysis.

It is important to apply adequate statistical adjustment to account for confounding by indication (which is expected to underestimate the VE) and to account for the opposite, namely heathy vaccinee bias

(which is excepted to lead to an overestimation of VE), although both are likely to operate simultaneously. Authors highlighted the need to use specific endpoints for VE assessment and preferably alternative methods TND or well controlled observational studies using influenza-specific laboratory-confirmed outcomes are favoured.

In addition, to verify if residual confounding by healthy vaccinee effects is still present in the adjusted data, it has been recommended to assess the VE using the peri-influenza season period (i.e., outside influenza seasons) when the virus is (virtually) not circulating and therefore no vaccine effect should be present [1;23].

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6.1.4. Frailty bias

Thirty papers dealing with frailty in the context of seasonal IVE assessment have been identified, among which 13 were ultimately included as relevant to inform the discussion of bias in such context.

6.1.4.1. Defining frailty

A recent review focusing on the evolution of methods in the context of VE assessment provided insights to better understand the complexity of the frailty syndrome [1]. Frailty is a dynamic and multifactorial syndrome in older adults that represents a reduction in physiological reserve, limited ability to resist environmental stressors, and increased risk of functional decline [2;3]. Frailty is a state of increased vulnerability to adverse outcomes compared to others of the same age [4]. There are numerous ways to conceptualize and measure frailty.

Two leading models of frailty are the deficit accumulation model (Frailty Index [FI]) and the phenotypic model. With deficit accumulation, the more problems or "deficits" (broadly defined as illnesses, disabilities or symptoms) an individual has, the frailer the person is [5;6]. The phenotypic model is based solely on physical factors (weakness, slow walking speed, weight loss, fatigue and inactivity); individuals with 1–2 factors are "pre-frail" and those with 3 or more are "frail" [5].

Other measures of frailty, such the Edmonton Frail Scale and the Tilburg Frailty Indicator, consider varying numbers of factors that are associated with vulnerability in older adults. When these are compared head to head, all frailty measures are associated with adverse outcomes but the deficit accumulation model allows for precise quantification of a continuous gradient in frailty, which in turn

allows mathematical modelling of frailty in relation to outcomes and potential mediators [5;7-9]. Aging is undeniably complex, affecting many systems via a variety of mechanisms [10;11].

The Frailty Index, which measures the degree to which a person is frail, relates to the accumulation of deficits in all aspects of health and functional status [12], and predicts mortality risk as well as the risk of health care use and changes in health status [13;14]. From this perspective, other populations than elderly, including younger people who are immunosuppressed, have advanced cancer or organ failure, can also be seen as frail, especially when frailty is conceptualized as the accumulation of health deficits.

Of note, calculation of the Frailty Index incorporates the presence and severity of the multiple chronic conditions and functional status and has been shown to be a better predictor of overall health status compared to the type or number of chronic diseases, or self-report of fatigue or balance problems; frailty measures are thus beginning to be incorporated into vaccination studies [15]. Frailty accelerates this immunosenescence although the impact of frailty on immune response specific to influenza vaccine among older adults varies [16].

Frail individuals have been shown to mount lower immune responses to antigen stimulation [17]. Physical frailty, characterized by diminished strength, endurance, and reduced physiologic function [18], leads to increased risk of acute illness, falls, disability, hospitalization, institutionalization and mortality [5;18].

Some authors attempted to use dependency in activities of daily living (ADL) as a proxy for frailty but acknowledged that that control for confounding by frailty is likely to be only partially achieved [19]. While ADL dependence may serve as a proxy for frailty, they are not equivalent. Even a perfect representation of ADL dependence would not completely capture frailty and its confounding which support the importance to use standardised criteria to measure appropriately frailty.

6.1.4.2. Frailty and immune response to influenza vaccine

One study evaluating the influence of frailty syndrome on strain-specific antibody response and clinical effectiveness of vaccination with a trivalent inactivated vaccine (TIV) concluded that assessing frailty status in the elderly and may identify those who are less likely to respond to TIV and be at higher risk for seasonal influenza and its complications [20]. Another study focusing on the effect of frailty on hemagglutination inhibition (HAI or HI) titers response to influenza vaccine among community-dwelling adults ≥ 50 years of age, showed that immune responses were lower among those ≥65 years of age than those <65 years. Among those ≥65 years there were no significant differences between frail and non-frail individuals in seroprotection or seroconversion for any influenza strain [16].

6.1.4.3. Frailty among the vaccinated vs. unvaccinated

When studying the elderly population several authors emphasized the critical need to consider the frailty component. Using a cross-sectional study in Denmark, Hellfritzsch [21] found that the vaccinated group have a higher burden of disease and more markers of frailty than the unvaccinated group. Andrew et al. [22] illustrated the importance of accounting for frailty in the context of seasonal influenza vaccine effectiveness.

They reported a vaccine effectiveness against hospitalization going from 45.0% (95% CI, 25.7% – 59.3%) to 58.0% (95% CI, 34.2%–73.2%) when crude estimates were compared to fully adjusted values. This range was essentially driven by frailty, noting that the VE against hospitalisation was 77.6% (95% CI, 39.3%–91.7%) among the non-frail, 51.0% (95% CI, 5.2% – 74.7%) in the prefrail, 59.6% (95% CI, 8.0% - 82.3%) in the frail and -24.8% (95% CI, -1040.4% – 86.3%) in the most frail older adults.

6.1.4.4. Frailty and Test-negative design studies

Frailty has been reported as likely influential factors in the association of influenza vaccination and the risk of serious health outcomes [23]. One group aimed to evaluate to which extent the TND is valuable to control for the frailty bias in influenza VE [24]. Using the study populations from previously reported vaccine effectiveness studies [25] they collected additional data from comprehensive chart reviews to calculate a frailty index using a standardized measure of frailty, to determine if the case-positive, TND adequately controlled for frailty. The authors concluded that from their analysis, frailty did not appear to be a significant confounder in the test-negative study design since inclusion of a validated measure of frailty did not substantially change vaccine effectiveness estimates. They further suggested that the use of the test-negative study could be a suitable approach to adequately control for frailty without necessarily including a specific frailty index [24].

However, a more recent study from the SOS Network by Andrew et al. [22] in the 2011/12 influenza season suggests that frailty remains a significant confounder with VE declining while the level of frailty increases in the TND. In fact, frailty appears to account for a "frailty bias", which it in this case acting similarly as confounding by indication. In another study, researchers indicated that a main source of bias was likely to be a small subset of frail and terminally ill seniors who are less likely to become vaccinated during the preceding autumn months because of their deteriorating health (a form of health vaccinee bias) [26].

Taken together, these findings suggest that in the over 60/65 age group, frailty is a strong confounder and could significantly bias the vaccine effectiveness findings if not fully accounted for and accurately measured. In addition, even if it appeared beyond the frailty bias per se, the framework to detect residual bias in the context of influenza VE assessment [27], illustrated in light of the frailty bias, was found relevant to critically discuss the findings and further confidently conclude whether biases were satisfactory accounted for in the analyses.

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6.1.5. Misclassification bias

Misclassification bias is defined as classification of an individual, a value, or an attribute into a wrong category in a study. The systematic literature search identified 9 articles treating misclassification bias; 3 more were added from the grey literature search and other sources.

One study [1] was an overview on misclassification bias in influenza vaccine effectiveness studies. Four studies [2-5] used mathematical or simulation models to examine the effect of outcome misclassification arising from imperfect laboratory tests and its relative difference between traditional cohort and case-control studies and the test-negative design (TND). Three studies [6-8] focused on the test-negative design (TND), reviewing its theoretical basis and considering sources of bias, including misclassification. Two articles [9;10] addressed miscellaneous topics relating to misclassification in IVE studies. Finally, a guide from the World Health Organization (WHO) [11] discussed the mitigation of various biases from the point of view of study design and interpretation, and one article was added to cover the effect of antivirals of viral shedding, a presumed determinant of diagnostic test accuracy [12].

6.1.5.1. Types of misclassification

There are several potential causes of misclassification that can occur either at the level of the exposure (i.e. vaccination) or the outcome (e.g. ILI or laboratory-confirmed influenza) and be differential (i.e. misclassification of exposure associated with the evaluation of outcome or vice versa) or nondifferential [1]. The following summarizes the different types of misclassification discussed in the literature, along with their causes and measures to reduce misclassification.

Misclassification of exposure (vaccination) occurs if the patients' vaccination status is determined incorrectly e.g., because of error in recall or vaccination registry [1].

In most cases, exposure misclassification is *nondifferential* [1;8], i.e. biasing the observed effect of the vaccine towards null. It can be avoided by using sources of vaccination information that are accurate and complete [11]; for example, the use of medical records is preferable to self-reporting [1].

Differential exposure misclassification may occur in traditional case-control studies and retrospective cohort studies if vaccination status is determined by patient recall after performing the influenza test. This type of bias can be avoided in TND (where case status is unknown at the time of recruitment) [8] or prospective cohort studies (where population members are identified at the beginning based on vaccination status) [11]. De Smedt et al. showed that differential exposure misclassification arising from both low specificity and sensitivity can bias VE results in either direction with potentially large deviations from the true VE [5].

Misclassification of outcome (influenza diagnosis) occurs if the patients are incorrectly classified as having or not having influenza. It can stem from imperfect diagnostic tests, other reasons having to do with their positive predictive value (time in the epidemic window) or other reasons related to the diagnostic procedure (swabbing technique and sample quality, time from symptoms to testing, use of

antivirals), or the interpretation of inconclusive results.

Nondifferential outcome misclassification leads to an underestimation of the association between the exposure and outcome, which has also shown in mathematical models of cohort studies, traditional case-control studies and TND studies [2]. When using laboratory tests to confirm influenza status, specificity of the test and the resulting positive predictive value (PPV) are especially important [1]. Low test specificity is considered to introduce more bias than low sensitivity [4;5;11]. Suboptimal specificity has been identified as an influential source of bias in case-control studies [3] and the effect is likely more pronounced in TND [4;5]. Moreover, a modelling study [2] found that the major determinants of bias were the test specificity and the ratio of the attack rates of influenza and non-influenza-ILIs.

Low test sensitivity can also introduce bias. Sensitivity is reduced if viral shedding is low e.g., because of a long delay between symptom onset and swabbing [8]. The use of rapid diagnostic testing has been demonstrated to underestimate VE due to imperfect specificity and sensitivity in comparison to RT-PCR or viral culture [7].

Misclassification of diagnosis can thus be reduced by using highly specific diagnostic criteria (e.g. laboratory-confirmed influenza instead of ILI [7]) and tests (e.g. RT-PCR instead of rapid test with low specificity). Using ILI as an outcome measure in measuring IVE has been criticized also owing to the various ILI definitions and their unclear correlation with laboratory-confirmed influenza [10]. The increasingly common use of highly sensitive and specific RT-PCR assays mitigates potential bias arising from imperfect tests [3]. In this situation, misclassification of non-cases as cases may be rare and limited to data-entry errors and sample contamination [8].

Since PPV is also dependent on the prevalence of the illness, bias can be further reduced by focusing the study on the peak weeks of the influenza season when the prevalence is the highest [1], and by excluding persons during the time influenza is not circulating [6]. Since calendar time is correlated with both vaccine uptake and with incidence of non-influenza infections, analyses must control for calendar time [6;7]. Of note, calendar time is often reported in two distinct ways:

- Period of the year; i.e. related to the seasonality of flu and the peak in the prevalence during the season
- Number of days post-symptom onset; i.e. related to the viral shedding of an individual person.

Sensitivity of diagnostic testing in TND studies can be improved by restricting patients to those presenting within 4 days of symptom onset, by choosing controls who test positive for another respiratory virus (to ensure that the sample was of sufficient quality) and taking steps to optimize swabbing techniques [8]. For individual inconclusive test results, erring on the side of considering them negative is expected to cause less bias than considering them to be positive [4].

Differential misclassification of outcome (influenza diagnosis) occurs if false positives or negatives are relatively more pronounced in the vaccinated or unvaccinated group. This could be because vaccinated people are more or less likely to seek healthcare than unvaccinated, or if physicians are more or less likely to perform a laboratory test on vaccinated than unvaccinated people. It can bias IVE in either direction [1]. Physicians may order more tests of patients they suspect of having influenza, or of patients whose status they are unsure of. Either case may cause systematic misclassification [11]. Thus, enrolling study subjects within a routine clinical setting can introduce bias; [7] the effect of differential diagnostic testing has also been observed in a simulation model [3].

Another potential cause of differential misclassification would be if vaccination affected viral shedding and thus diagnosis of the outcome [8]. Differential outcome misclassification arising from both low specificity and sensitivity can bias VE, with TND performing worse than the other designs, particularly for low levels of disease specificity in the exposed [5].

To reduce differential misclassification, it should be ensured that the vaccinated and unvaccinated groups are treated equally [1], which is particularly important in traditional case-control and cohort studies. Specifically, all cases (or a representative sample) of the study population should be ascertained, and vaccination anamnesis should not influence the diagnosis of influenza [1]. Study protocols should specify the symptoms and other eligibility criteria for enrolling and testing patients in the study [11].

In one study where patients were enrolled from the same source population either in prospective sentinel surveillance (tested by RT-PCR) or tested on clinical grounds (by a variety of rapid antigen tests), IVE was lower in the latter group; the authors suggest that the effect may have been a combination of nondifferential outcome misclassification and selection bias arising from clinical testing [9].

While antiviral treatment was not identified as a cause of misclassification in the reviewed literature, it has been found to reduce viral shedding [12] and may therefore need to be considered; the same is true of poor quality of sample collection or storage.

The reviewed literature focused mostly on outcome misclassification, especially that arising from diagnostic tests [1;3;4;7;8]. Less research was encountered on exposure misclassification in the context of IVE studies, even as its impact on VE may be larger than that of outcome misclassification as highlighted by the simulation model of De Smedt et al. [5]. This model allowed to test different scenarios and showed that decreased exposure specificity (poorer identification of non-vaccinees) had greatest impact for influenza VE estimation and noted that exposure misclassification had a larger impact compared to disease misclassification, whereas previous research focused on disease misclassification only.

Misclassification of exposure and outcome in influenza vaccine effectiveness studies is likely to be primarily nondifferential, i.e. leading to underestimation or “dilution” of VE [1;8]. However, this requires significant assumptions such as the vaccination not affecting the outcome ascertainment. In addition, confounders such as age can result in errors in exposure and outcome ascertainment not being independent of each other [8]. Where differential misclassification occurs, it can lead to either under- or over-estimation of VE [1]. The impact of misclassification also depends on disease epidemiology and vaccination coverage, as demonstrated by two different scenarios (seasonal influenza and pertussis vaccination) [5].

Misclassification can be reduced by using accurate and complete sources of vaccination information, highly specific and sensitive outcomes (e.g. influenza infection confirmed by RT-PCR) and by accounting for the seasonality of influenza. Study protocols should ensure that the vaccinated and unvaccinated groups are treated equally in the study process. These measures should be implemented in all studies where applicable.

It is of particular interest if the choice of study design affects misclassification bias. According to some of the reviewed literature, case-control [2] and particularly TND studies [2;6] may be less susceptible to misclassification bias than cohort studies. Another study [4] has contested these findings, pointing out a possible methodological flaw of the former and presenting a model where TND studies appeared more prone to misclassification. However, the difference was trivial under typical conditions, and other advantages of TND (such as reduced confounding due to healthcare seeking behaviour [6]) may outweigh potential increase in misclassification.

A modelling study [5] emphasized that the impact of the misclassification parameters was found to be more noticeable than that of the different study designs, with the different study designs performing similarly when misclassification is limited. Altogether, the choice of study design does not appear to play a major role.

References

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6.1.6. Study design: Choice of controls in TND studies

In TND studies, cases are universally defined as those that test positive for influenza. Controls may be defined as all those that test negative for influenza or as subsets of this group. Three TND studies were found that used two control groups: those testing negative for influenza, and those testing negative for influenza but also testing positive for another respiratory virus [1-3]. Three other TND studies (Nunes et al., Sanduram et al. and Van Doorn et al. were found that used the two control groups described above and, additionally, a third pan-negative control group, i.e. controls testing negative for influenza and for other respiratory viruses [4-6]. Foppa et al. performed a simulation study where the number of pan-negative controls is high [7]. No traditional case-control studies were found that compared different definitions of controls.

Studies by Sundaram et al. (2013), Blyth et al. (2014) and Pierse et al. (2016), found little difference between VE estimates obtained using different control groups [1;3;5]. Sundaram et al. concluded use of influenza-negative controls did not generate a biased estimate of vaccine effectiveness due to an effect of vaccination [5]. Pierse et al. (2016) suggested that testing for other respiratory viruses is not needed to produce valid VE estimates [3]. Blyth et al. [1] noted that their results were contrary to results of an earlier study they conducted [2].

Kelly et al. (2011) found no difference in VE between the two control groups in a general practice setting [2]. In an emergency department setting, however, a trend towards higher VE was found when controls testing negative for influenza but positive for another respiratory virus were used (65% [95% CI 8 – 87]) compared to when those testing negative for influenza alone were used (51% [95% CI-21 to 80]). A significantly higher vaccine coverage existed among controls who tested positive for other respiratory viruses than for those who tested negative for those viruses. The authors suggest that these results may be due to "the difficulty of collecting nasal swabs from young children who are unwell", resulting in samples inadequate for viral detection and hence false-negatives for influenza. They therefore conclude that "the optimal comparison group consists of those testing positive for another respiratory virus, ensuring adequate sample collection in both cases and controls" [2].

Both studies with three control groups found VE was the highest in controls positive for other respiratory viruses and the lowest in the pan-negative control group [4]. Like Kelly et al. (2011), Nunes et al. (2014) found a higher vaccine coverage among controls who tested positive for other respiratory viruses than for those who tested negative for those viruses [4]. They argue that effect modification due to viral interference could explain the observed results. In this scenario, vaccinated individuals are at increased risk of ILI due to non-influenza respiratory viruses as compared to unvaccinated individuals, due to the non-specific immunity derived from influenza infection during an influenza epidemic (others, such as Van Doorn et al., argue this response may be too short lived to have an effect [6]).

Van Doorn et al. (2017), on the other hand, did not find a difference in vaccine coverage between the control groups [6]. Instead they found that pan-negative controls were older and had a higher prevalence of chronic diseases. They hypothesized that pan-negative controls may be more prone to seeking healthcare and therefore included a higher proportion of respiratory illness not caused by infection, which would make this group seemingly less valid as controls. In addition, they note that IVE estimates resulting from non-influenza positive controls are more consistent with other vaccine efficacy and vaccine effectiveness studies ("likely due to limiting controls without an infectious cause of respiratory disease" [6]). Furthermore, Van Doorn et al. (2017) noted the inclusion of controls positive for other viruses assumes that adequate laboratory tests are used for both cases and controls, thereby reducing false-negative controls and misclassification bias [6].

Foppa et al. [7] performed a simulation model for a hospital TND study. They defined patients with COPD, asthma and congestive heart failure as 'CP patients'. CP patients typically have a higher vaccine uptake and a higher rate of non-infectious respiratory exacerbations. If the study inclusion criteria are

broad enough to allow for the enrollment of subjects with non-infectious respiratory exacerbations in CPR patients (effectively increasing the number of pan-negative controls in the study), this will result in biased (higher) VE estimates. They showed that adjusting for CP status decreases the bias, but warned that CP status is not a binary characteristic, and if that heterogeneity is not fully characterized, adjustment for CP status will not result in full removal of the bias. They also mentioned that the over-representation of CP subjects among controls may be higher in inpatient as compared to primary care TND studies.

In summary, in some studies there was little impact of the choice of control group on the VE estimate. Others found that using controls negative for influenza but positive for another respiratory virus resulted in a higher VE and recommend the use of this control group. Proposed mechanisms are certainty of adequate sample collection, viral interference, and the exclusion of controls without an infectious cause of respiratory disease.

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6.1.7. Comparing results from different study designs

Vaccine effectiveness can be assessed through several study designs. Each design has its own strengths and weaknesses. Direct comparisons between designs may help understand how study design impacts VE estimates. Four studies comparing VE estimates obtained through different methodologies were found. One of these was conducted in Spain and compared a cohort vs. a nested TND study [1]; one study in Germany compared a TND study with a case-series [2]; and two studies compared VE estimates obtained through a TND study with the screening method (SM) in France [3] and Canada (Ontario) (Savage et al. 2015)[4].

6.1.7.1. TND vs. cohort

One study in Navarre, Spain, by Castilla et al. (2012) compared estimates obtained through a prospective cohort based on electronic records of physicians and laboratories with estimates obtained in a nested test-negative case-control analysis of swabbed patients [1]. The same cases were included in the two analyses. The obtained VE estimates were very similar; the authors conclude this supports the validity of the results and suggests good control of biases.

6.1.7.2. TND vs. case-series

One study in Germany by Uphoff et al. (2011) compared TND with a case-series [2]. For the TND, routinely collected virological surveillance data was used. For the case-series, all cases of pandemic influenza A/H1N1/2009 reported to the national mandatory surveillance system were used. For the case-series, VE was estimated by comparing the ratio of the cumulative force of infection to the number of cases during the unprotected (from day of vaccination to day 7 after vaccination) and the protected phase (from day 14 after vaccination) of vaccinated subjects. Similar VE point estimates in two age strata were obtained. They used different data sources and two different statistical methods but obtained similar point estimates of VE**.**

6.1.7.3. TND vs. screening method

Vilcu et al. used data from the French influenza surveillance system in primary care [3]. The same laboratory-confirmed influenza cases were used to estimate VE through the screening method (SM) and a TND study. VE estimates obtained through SM were more biased (as confounding factors were not taken into account) but also more precise (i.e. narrower confidence interval) early in the season than those obtained through the TND method. Assuming biases are constant over the years, the authors argue SM-VE estimates may be more appropriate to monitor early VE among populations at risk of severe or complicated influenza compared to previous seasons. They judge preciseness and early availability to be of higher importance than unbiased but imprecise estimates to perform comparisons across seasons and help national health authorities in evaluating the impact of each seasonal epidemic in at risk groups. However, they also state that the TND-VE estimates in the general populations are more readily comparable between countries, given the popularity of the design.

For the SM, Savage et al. (2015) used passive surveillance data on influenza cases in Ontario reported to the integrated Public Health Information System (iPHIS) [4]. For the TND study, they used data on ILI patients who were tested for influenza from the Sentinel Physician Surveillance Network in Ontario. In this study, SM-VEs were generally 20-35% lower than the TND-VEs. For half the iPHIS cases, immunisation status was missing; these cases were excluded. The authors argued that unvaccinated cases were more likely to have missing immunisation status than vaccinated cases, thereby biasing SM-VE estimates downwards. The authors stated that while the SM approach using existing surveillance data offers advantages in timeliness, ease and efficiency, there is a potentially important trade-off of reliability due to methodological issues related to completeness of vaccine information and case ascertainment.

In conclusion, data quality (missing data) and sample size (precision) and the purpose of the VE calculation (comparison to previous years in the same setting or comparison with other studies) are important factors to take into account when deciding whether TND, cohort or SM is the most appropriate method to obtain VEs in a specific setting. The two studies described comparing estimates obtained from a TND study vs. a cohort and a TND study vs. a case-series obtained similar results between the two study designs. This is likely an indication of good control of biases.

References

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6.1.8. Patient characteristics across recipients of different vaccine types

Certain characteristics may be associated both with receipt of a specific *type* (or brand) of influenza vaccine and with the outcome, resulting in confounding similar to confounding by indication. If this occurs, differences between VE estimates may be (partially) attributable to underlying population characteristics rather than true differences between vaccine types. Understanding whether this type of confounding is present is especially important when directly comparing different types (or brands) of vaccine or when pooling data from countries where these associations may differ. Four studies comparing characteristics of population in the same country receiving different vaccine types were found (Kahn et al. 2015; Talbot et al. 2015; Mannino et al. 2010; Zimmerman et al. 2016 [1-4]).

Live attenuated influenza vaccine (LAIV) vs. inactivated influenza vaccine (IIV) in children in the United States

Data from the United States National Immunisation Survey-Flu for influenza seasons 2011/12 to 2013/14 show that younger children were more likely to received LAIV than older children (13-17 years) with an adjusted prevalence ratio (APR) ranging from 1.37 to 1.50, as were white (compared to black) children (APR 1.22) and children from households with a higher annual income (APR 1.16-1.42 for (> $75,000 vs. at/below poverty; and 1.06-1.33 for ≤ $75,000 vs. at/below poverty) [1].

Split-virion influenza vaccine vs. subunit influenza vaccine in older adults in the United States

There were no differences in baseline characteristics between recipients of split-virion compared to subunit influenza vaccine in older adults participating in a TND study in the United States [2].

Adjuvanted trivalent influenza vaccine vs. non-adjuvanted trivalent vaccine in those aged 65 years and above in Italy

In Italy, adjuvanted vaccine is preferentially recommended for elderly and more frail subjects. In a cohort study examining VE of aTIV and TIV, subjects who received aTIV were indeed found to be older and have more functional impairment and comorbidities [3].

Quadrivalent LAIV vs. quadrivalent IIV vs. trivalent IIV vs. high-dose IIV in the United States

In the Unites States at the FLU VE Network, characteristics of persons receiving quadrivalent LAIV, quadrivalent IIV, trivalent IIV and high-dose IIV are presented. Persons aged 65 years or above did not

receive LAIV vaccine (it is not indicated for this age group), and those receiving high-dose trivalent inactivated vaccine had more high-risk conditions and a poorer general health status than those receiving other vaccines [4].

The administration of different vaccine types is highly context-specific and subject to local vaccine recommendations. It is recommended local vaccine recommendations are consulted to better understand potential differences between groups of vaccine recipients.

References

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6.2. Summary of data for covariates acting as confounders and/or effect modifiers

In recent years, many IVE studies have been conducted according to a similar design in terms of data collection and data analysis. Two important sources are available which have been referenced by many authors over the past few years.

Since 2007, the I-MOVE network exists in Europe with a task to monitor seasonal and pandemic influenza vaccine effectiveness. This network conducted a literature review and a survey on methods used in the European Union (EU)/ European Economic Area (EEA) was conducted to identify the main confounding variables at that time [1].

The WHO has published 'a guide to the design and interpretation of observational studies' for the purpose of evaluation of IVE. Apart from, for example, guidance on study design and reporting of the data, the WHO also provides guidance on the measurement of covariates and which statistical considerations to take into account when assessing potential confounders [2]. These have also been considered in the DRIVE Deliverable 4.1 (Framework for the analysis of influence vaccine effectiveness studies).

During the inclusion and exclusion selection based on title/abstract of the full list of publications identified from the search, titles were also allocated to categories related to identified covariates (Table 3). During full text review additional titles were added to certain categories or titles were excluded based on full text review and the covariates described below are the results of a literature search as described in chapter 6.

Table 3: Selection Procedures for confounders and effect modifiers during the full text screening phase

Confounders and effect modifiers	Identified	Included	Excluded	Reasons for exclusion
Repeated vaccination	45	40	5	Do not discuss repeated vaccination, n=5
Underlying medical condition	8	5	3	Do not discuss underlying medical conditions, n=1 Pandemic vaccine only, n=1 Studies burden of disease, n=1
Obesity	4	0	4	Studied the relation between obesity and the risk of obtaining influenza (no IVE), n=2 No full text available, n=2
Concomitant administration of vaccines	4	0	4	Studied virus interference, n=1 Do not discuss concomitant administration, n=2 Wrong outcome (pneumococcal infection), n=1
Use of statins and/or antivirals	3	2	1	Full text not found, n=1
Vaccine strain Match/mismatch	27	12	15	No discussion on how to deal with mismatch, n=6 Does not discuss mismatch, n=4 Does not discuss VE in relation to mismatch, n=1 Do not discuss Vaccine strain, n=2 Duplication of information with more recent paper, n=1 Measures VE against other resp. illnesses, n=1
Full vs Partial vaccination	7	5	2	Do not discuss full/partial vaccination, n=2
Waning immunity	13	7	6	Waning immunity was the primary outcome measure, not IVE, n=1 No discussion of IVE (n=2) No discussion of waning effect (n=2) Clinical trial (n=1)
Sex	3	3	0	-

References

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6.2.1. Repeated vaccination

Of 45 eligible papers dealing with repeated vaccination in the context of seasonal IVE assessment, 40 were ultimately included as relevant to inform the discussion of confounding in such context.

Influenza vaccination is now widely recommended for individuals at increased risk of mortality/morbidity such as elderly people and people with underlying medical conditions. At risk subjects are recommended for annual vaccination and the impact of repeated vaccination has gained significant interest. Studies from the 1970s and 1980s found inconsistent results regarding the impact of repeated vaccination [1;2].

In 1999, a systematic review and meta-analysis of field studies, trials, and serologic studies found no evidence of negative impacts of repeated vaccination [3]. More recently, some studies have found VE to be reduced in those who received repeated prior influenza vaccinations [4-6]. Beyond the inconsistency between studies, some authors have proposed an explanation for this variability linked to the *antigenic distance* hypothesis [7].

The assumption is that variation in repeated vaccine efficacy would be due to differences in antigenic distances among vaccine strains and between the vaccine strains and the epidemic strain in each outbreak [7]. This impact could vary depending on influenza infection history, interactions between successive years' vaccine components, and between the vaccine components and circulating influenza strains [7].

More recently, a systematic review and meta-analysis [8] identified 27 eligible studies for the qualitative synthesis and 20 for the meta-analysis. The 27 included studies captured influenza seasons between 2004–2005 and 2014–2015, with most reporting estimates for the 2010–2011 to 2014–2015 seasons. One study was from the southern hemisphere [9], one was restricted to pregnant women [10], and two were in pediatric populations [11;12]. All but four studies [13-16] used TND designs [5;6;9-12;17-33]. Among the studies reporting estimates, there were 16 analyses for influenza H1N1, 17 for H3N2, and 14 for B that compared VE among those vaccinated in the index season and one prior season, to those vaccinated in the prior season only.

Compared to no vaccination for either season, Ramsay et al., [8] reported that individuals who received the current season's vaccine had greater protection against H1N1 (Δ VE = 62%; 95% CI 51%, 70%), H3N2 (Δ VE = 45%; 95% CI 35%, 53%), and B (Δ VE = 64%; 95% CI 57%, 71%). We observed no differences in VE between vaccination in both seasons and in the current season only for H1N1 (Δ VE = 3%; 95% CI – 8%, 13%), but less protection against influenza H3N2 (Δ VE = − 20%; 95% CI – 36%, − %), and B (Δ VE = − 11%; 95% CI – 20%, − 2%). The authors concluded that overall the results supported vaccination in the current season regardless of prior season vaccination. However, they also showed that the VE was lower against B and H3N2 for individuals vaccinated in both seasons compared with the current season only.

Those findings are not consistent with results previously reported by Beyer et al., in 1999 [3] who reported no significant difference between the single and multiple vaccination groups for all influenza subtypes combined. The differences might be attributable to the study characteristics that included

more recent laboratory testing methods, influenza subtype-specific analysis, and study designs with consistent vaccination comparison groups. However, those results are aligned with a recently published meta-analysis using similar vaccination groups (prior only, current only, both seasons) [34]. Similar to Ramsay et al, [8], Belongia et al [34] reported VE to be consistently lowest among those vaccinated during the prior season only.

Another more recently published systematic review and meta-analysis from Bartoszko et al, [35] encompassing five RCTs and 28 observational studies. They reported no significant reduction in VE from the RCTs when individuals vaccinated in two consecutive seasons (VE 71%, 95% CI 62–78%) were compared to those vaccinated in the current season (VE 58%, 95% CI 48–66%) (odds ratio [OR] 0.88, 95% CI 0.62–1.26, p = 0.49, I2 = 39%). A similar finding was shown from the observational studies (VE for two consecutive seasons 41%, 95% CI 30–51% compared to VE for current season 47%, 95% CI 39–54%; OR 1.14, 95% CI 0.98–1.32, p = 0.09, I2 = 63%). They concluded that available evidence does not support a negative impact of prior vaccination on the effectiveness of the vaccine in the current season.

More recently, several individual studies reported findings on the potential impact of repeated vaccination and showed inconsistent results. Using a retrospective case-control study, Amer, et al. [36] reported that yearly vaccination with TIV might negatively affect the immune response against the novel pandemic H1N1 strain. Similar findings were reported by Saito et al. [37] who studied schoolchildren over 3 consecutive seasons and reported that repeated previous vaccinations over multiple seasons had significant dose-dependent negative impacts on VE against medically attended influenza A and B.

Gherasim, et al. [38] did not find any interference between the previous and current influenza vaccines against A(H1N1)pdm09 and B viruses, but a possible negative interference against A (H3N2) virus. Cheng et al. [39] showed that vaccination in both the current and previous seasons was associated with a higher VE against hospitalization with influenza than vaccination in either single season, supporting that prior vaccination would not impact the VE in the current seasons among hospitalized patients. Skowronski, et al [40] assessed the IVE against medically attended, laboratory-confirmed influenza A(H3N2) during three A(H3N2) epidemics (2010–2011, 2012–2013, 2014–2015) in Canada and reported that substantial variability associated with prior vaccination effects varied significantly by season in alignment with the antigenic distance hypothesis. They showed that negative effects of prior vaccination were pronounced and statistically significant in 2014–2015 when the antigenic distance between current and prior season was high.

Nichols et al, [41] investigated the potential negative impact of prior season vaccination on VE in the current season, over four consecutive influenza seasons (2011/2012-2014/2015) in Canada. They reported trends of non-significant decreased VE among patients repeatedly vaccinated in both the prior and current season relative to the current season only were observed in the A/H3N2 dominant seasons of 2012/2013 and 2014/2015. Conversely, being vaccinated in both seasons tended to result in a high VE in the current season against the dominant circulating subtype, in 2011/2012, during which B viruses circulated, and in 2013/2014, when A/H1N1 were predominantly circulating. They emphasized that even in circumstances where we observed a trend of negative impact, being repeatedly vaccinated was still more effective than not receiving the current season's vaccine which according to them favours continuation of annual influenza vaccination recommendations, particularly in older adults.

In this chapter, we summarised the controversy surrounding the potential impact of repeated vaccination and highlighted the complexity to appropriately tackle the question which is of major public health interest. The variation of the impact - sometimes negative, sometimes positive or sometimes neutral - have been substantially differing although the observed reductions in VE have been primarily associated with outbreaks of A(H3N2) infection. The antigenic distances hypothesis between past and current vaccine antigens and the viruses that circulate has been proposed by Smith et al. to explain part

of those variations [7]. VE has consistently been lower against A(H3N2), and antigenic changes in circulating viruses have occurred more rapidly, leading to the need for reformulation of strains included in the vaccines [42].

In a recent commentary, Petrie and Monto [43] emphasized the challenge to untangle the contributing factors that lead to an observed reduced VE among previously vaccinated individuals in seasons when A(H3N2) predominates, considering extensive histories of prior exposure to various strains through vaccination and natural infection. Longitudinal studies with reliable information on previous exposure via natural infection or through vaccination are an option, although costly.

Modeling is an alternative approach. Using an age-structured influenza equation-based transmission model, Shim et al. [44] showed that despite the presence of vaccine interference, revaccination reduces the influenza attack rate and provides individual benefits. They added that the negative impact of vaccine interference may be offset by increased vaccine coverage levels. This finding underscores the complexity to adequately evaluate the prior season vaccination impact, phenomenon that may be interconnected to other important factors. In that respect, Skowronski et al. [45] suggested that in addition to repeated vaccination, influenza VE findings may require consideration of viral genomic variation, repeat vaccination, birth (immunological) cohort effects, and potential within-season waning of vaccine protection.

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6.2.2. Underlying medical conditions

Our search yielded seven publications related to the confounding effect of underlying medical conditions. One paper on IVE in diabetic patients was added later [1]. Two papers did not provide relevant information as they described methods of flu surveillance unrelated to vaccination. One paper only discussed the 2009 pandemic vaccination campaign. Even though one paper reported on IVE against ILI (and not lab-confirmed influenza) it did provide some relevant information related to confounding through underlying medical conditions [2].

In most countries, influenza vaccination is recommended for 'at-risk' groups, which covers a number of (chronic) conditions that are known to be exacerbated by influenza or that may increase the patient's risk of catching (severe) influenza. On the other hand, influenza vaccination might not have the same effect on these patients as it does in healthy subjects.

Remschmidt et al. [1] conducted a literature review looking specifically at IVE in patients with diabetes. The authors concluded that stratifying based on a specific underlying disease will probably provide an underestimation of VE due to confounding by indication; meaning that subjects with existing diabetes disease are more likely to be vaccinated. This was of particular concern for this review since none of the studies looked at IVE against lab-confirmed influenza but against nonspecific outcomes [1]. It can be concluded that confounding is a clear concern for underlying medical conditions.

Which (chronic) conditions are included as covariates differs substantially between IVE studies. Most commonly used are pulmonary diseases, heart diseases, diabetes, allergies and cancer [1, 3-5]. However, also the clustering of diseases is very different between the various approaches. Some studies

look at a substantial list of specific diseases. As an example, Hellfritzsch, et al. used the Charlson Comorbidty Index, which includes nineteen major conditions [3]. Others cluster all chronic diseases together [5].

The first step is the collection of data on these underlying medical conditions. This can be done through reviewing medical records [3] or through interviews with the subjects or their physicians. Conditions can be identified through hospitalization, outpatient encounters or drug prescriptions [2].

Next the underlying medical conditions need to be scored for inclusion in a regression model. Some authors use binary approach on whether the condition is present or not; this can be done for each condition separately [4] or as one binary score stating the presence or absence of any underlying disease [5]. Other methods involve including the severity of the disease in the score that is used in the statistical analysis [3] and again this can be done by combining the presence and severity of multiple disease into one score (as is done with the Charlson Comorbidity Index for example) [3] or for each disease separately (with a 0-score for a disease that is not present).

There are several general approaches for inclusion of covariates in a statistical model, and the same goes for underlying medical conditions. Some authors (quoted here above) choose to define the included covariates a priori, based on biological plausibility or using a causal diagram [2,4], while others use statistical significance as factor to determine relevance for inclusion in the model, such as through minimal change in the OR due to a specific covariate [5]. Even though this can be applied to all covariates that are considered for inclusion in a model, the biological plausibility is often not as debatable as with underlying medical conditions. The list can be extremely long and the clustering of the various conditions can be handled in numerous different ways. Therefore, it is of particular importance to this specific covariate on how inclusion in the model is handled.

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6.2.3. Obesity

Obesity is often mentioned as one of the confounding variables in the group of risk factors or pre-existing conditions. Sullivan et al. point out that obesity is one of a long list of conditions that can increase a person's risk of influenza infection and the likelihood of vaccination or eligibility for free vaccination [1]. In addition, the I-MOVE consortium has included obesity as a co-variable that was collected across multiple sites and various seasons (although not consistently) [2].

Our literature search yielded 4 publications that discussed obesity in the title or abstract and these were therefore allocated for full text review with regards to obesity as a confounder. Unfortunately, for two out of four titles no full text was available and the other two discussed the link between obesity and the severity of influenza infection, without regard to influenza vaccination and were therefore excluded from data extraction.

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6.2.4. Concomitant administration of vaccines

Several vaccines could potentially be co-administered with the influenza vaccine, which could influence the IVE estimate if the vaccines interact with each other on the immune response. Next to that though, the likelihood of receiving an influenza vaccination is likely higher among recipients of other vaccines (due to behavioural aspects and the patients' opinion concerning vaccines and access to health care). This is particularly of interest with younger children, who will receive their recommended childhood vaccinations alongside potentially the influenza vaccine and in elderly, where the recommendation for pneumococcal vaccine and herpes zoster vaccine exists in certain countries.

The publications found during our systematic search that address co-administration of vaccines solely focused on the co-administration of pneumococcal vaccine and influenza vaccine in elderly. However, none of the studies looked at the effect of these pneumococcal vaccinations on the causal relationship between influenza vaccination and the odds of being infected with the influenza virus (as confirmed through a laboratory test) and therefore none were considered for data extraction.

6.2.5. Use of anti-virals and statins – effect modification

Antivirals like the neuraminidase inhibitors oseltamivir and zanamivir and the M2 inhibitors amantadine and rimantadine can be used for the prevention of influenza (eg as prophylaxis for individual exposed to influenza virus when admitted to hospital) and for the treatment of influenza in order to mitigate the associated complications [1].

Guy et al. describe the potential bias introduced by use of antivirals during an outbreak of influenza. This is due to reducing the transmission and severity of infection independent of vaccination [2]. VE estimates can be higher if the antiviral therapy is used late during an outbreak and the vaccination coverage is high. If the vast majority of unvaccinated subjects already developed influenza, the antiviral therapy being introduced in the population at that time could potentially reduce the risk for vaccinated subjects from developing influenza later in the flu season. However, VE estimates may also be lowered in outbreak investigations where antivirals are used for both vaccinated and unvaccinated individuals earlier in the course of the outbreak [2].

Since Guy et al. describe the use of antivirals as only influencing the outcome and not the vaccination itself, they do not suggest a confounding effect and thus no adjustment at time of data analysis. However, they do provide a solution on how to deal with this bias by means of stratifying the data based on time. They suggest that to avoid the potential bias introduced by use of antivirals in an outbreak setting, one option is to include cases only up until the time that antiviral therapy is implemented. In the course of an outbreak where antivirals are not used, it can be expected that the VE changes over time (during the course of the season) and the final VE estimate represents an average VE. By calculating a time-specific VE when an outbreak still has some way to run, the VE may have some

way to fall to meet the final outbreak VE estimate. However, if antivirals are used at some point in time while the outbreak is ongoing, that could limit the change in time-specific VE between early and late season and with that, the average season VE [2].

Though only antivirals appeared in our review focusing on published IVE studies, it should be noted that other medications may act as confounding factors or effect modifiers in influenza vaccine studies. Statin use forms a well-known example of a covariate included in some IVE studies. However, the various studies have found varying results in the relation between statin use and the effect of influenza vaccination. Very recently (October 2018 - post our initial literature search) a large study looking at statins as a covariate in influenza vaccine effectiveness estimates was published [3], describing that due to the anti-inflammatory and immunomodulatory side effects of statins, it is biologically plausible that an effect in IVE occurs. The authors quote several recent studies that did find a decrease in effectiveness among statin-users, albeit with some remarks (i.e. against non-lab-confirmed influenza; only an effect found in A/H3N2 infections; only an effect found among synthetic statins).

Havers et al. conducted a post-hoc analysis on the US Flu VE Network data from 2011-2015 using the existing assessments of vaccination and lab-confirmed influenza infection from the annual IVE studies. They added statin use as an additional covariate and stratified the group into statin users and statin nonusers. For both all strains combined as well as separated out per strain, they found similar VE's between the two stratified groups. As a separate analysis, they looked at the link between statin use and odds of influenza infection (sometimes suggested as a causal effect) but did not find a significant association. Haver et al. clearly treated the covariate of statin use as a potential effect modifier in this analysis, but they also discussed the possibility for the covariate to be acting as a confounder. They note that there could be selective use of statins in people with reduced responsiveness to vaccination due to comorbidities or other factors. On the other hand, it is also suggested that (long-term) statin use may show healthier behaviour and other characteristics associated with improved outcomes (the healthy user effect) [3].

In conclusion, several medications can have an effect on the IVE estimate, which means it would be a sensible approach to stratify between the groups of specific medication users and medication nonusers. Nonetheless, some thought needs to be placed on whether the medication use could also be associated with the odds of receiving an influenza vaccine or not and thus a confounding effect.

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6.2.6. Vaccine Strain match/mismatch – effect modification

Our search yielded 27 papers that discussed the level of match between the vaccine and circulating strains in the title or abstract. The full text review showed that while the level of match was addressed in many IVE studies, a limited number of papers discussed the root cause or the consequences for the estimates or provided any insights in how to best deal with the issue. 12 papers are included in the discussion below and 15 were excluded for data extraction.

The efficacy and effectiveness of influenza vaccines in a given year depend on many factors, including the degree of vaccine circulating virus match [1]. Influenza viruses undergo high mutation rates and frequent genetic reassortment (combination and rearrangement of genetic material). This leads to

variability in the HA and NA antigens. Minor changes in the protein structure in influenza A strains ("antigenic drift") occur frequently, enabling the virus to cause repeated epidemics by evading immune recognition. Twice a year, the WHO gives a recommendation on which strains to include in the seasonal vaccine (once for the Northern Hemisphere and once for the Southern Hemisphere).

There are several months between the recommendation and the start of the season and on top of that, the flu season itself can last for several months. During this time the influenza viruses may mutate and cause an antigenic mismatch between the vaccine virus and the virus circulating in the community [2]. Other reasons have been identified over the past years that could cause antigenic mismatch (e.g. antigenic mismatch due to egg-adaptation in vaccine manufacturing [3]), but these will not be discussed here.

The degree of antigenic mismatch is a covariate that is often considered and/or assessed when conducting IVE studies, but apart from stating the facts on the degree of mismatch (e.g. in categories such as mild/moderate/severe or in percentages of the tested samples) often no additional analyses or adjustment methods are suggested [4]. Belongia et al. in 2008 conclude that the height of the VE estimate is consistent with the degree of antigenic match of the vaccine with the circulating strains when assessing IVE across several seasons [4]. A meta-analysis and systematic review by Darvishian et al. in 2017 came to the qualitative conclusion that IVE estimates were generally stronger when the vaccine matched the circulating viruses. However, when the data in this study was assessed stratified by matched as yes/no, no significant difference was found (which was suggested to be due to lack of power) [5].

Another systematic review by Heikkinen et al. in 2011 concluded antigenic match to be one of the key drivers of the clinical effectiveness of influenza vaccination. The authors plotted the IVE point estimates against the vaccine match of several studies in children specifically [6]. This consistent correlation is not always seen though. Sullivan et al. in 2014 concluded that vaccine mismatch does not consistently correlate with vaccine effectiveness in either observational studies or in efficacy studies [7]. A US study conducted for the 2007-2008 Northern Hemisphere season found a reasonable IVE estimate despite antigenic mismatch in both the circulating A/H3N2 strain as well as in the influenza B virus. The authors conclude that, in any given season, assessment of the clinical effectiveness of influenza vaccines cannot be determined solely by laboratory evaluation of the degree of antigenic match between vaccine and circulating strain [8].

For the B-lineages it is also occasionally observed that even though the predominantly circulating B-lineage is different from the lineage included in the trivalent influenza vaccine, there is still substantial IVE measured [9]. There have also been reports that the IVE estimate against the B-lineage included in the trivalent vaccine is not significantly higher than the IVE estimate against the B-lineage not included. Several underlying reasons are being considered for this observation, including that potentially the wrong clade of that lineage was included in the vaccine [10].

There are different methods described in the literature on how the assessment for match or mismatch is made. As mentioned earlier; the variable can be described as binary (yes/no), categorical (mild/moderate/severe) or continuous (proportion of samples that match). The HI assay testing is a common approach and used by the WHO [5]. Antigenic similarity can be defined as an x-fold (i.e 2-fold, 4-fold or 8-fold) reductions in HI titers, determined using antisera (often derived from ferrets) [4,8]. Skowronski et al. address the need in their 2017 paper though, to update these models to more directly link genomic, immunological and epidemiological information [9]. Darvishian et al. also used another potential criterion for defining match (apart from the antigenic similarity based on HI titers), being that all the separate vaccine components were antigenically similar to the reference viruses [5].

Given that a mismatched vaccine only affects the outcome measure and not the individual vaccination itself, it is expected that mismatch is modifying the effect of the relation between vaccination and

occurrence of influenza infection. The most common method to assess the level of effect modification due to mismatch is by stratifying the data based on subjects infected by a vaccine matched strain and subjects infected by a vaccine mismatched strain. This is also applied in RCTs looking at efficacy and both estimates are reported [11].

Redberger-Fritz et al. though, applied a different method, by stratifying by calendar week (within the flu season). In their observational study looking at annual influenza effectiveness in Austria they stratified the flu season in two periods and found both the overall and strain-specific IVE estimates to significantly differ between these two-time periods. In addition to stratification, the IVE was also estimated adjusted by multivariable logistic regression for calendar week of influenza virus infection. The highest VE estimates were obtained after full adjustment for all covariates, whereby the calendar week of infection was the covariate exerting the highest influence on adjusted VE estimates [12].

This method of stratification by calendar week is based on the assumption that the antigenic mismatch is larger towards the end of the season as compared to the beginning of the season, which suggests the virus is drifting during the season. Also, Sullivan et al. in 2014 found that vaccinated cases were observed to present more often later in the surveillance period, leading to higher estimates earlier in the season and higher estimates among those presenting sooner after vaccination [7]. In this particular study, it was found that the peak of the flu season was actually fairly early on and therefore it was suggested that this was due to some degree of mismatch. However, it was hypothesised that if this early peak would not have been observed, the observation could well have supported the notion of waning vaccine-induced immunity as the influenza season and time since vaccination progresses [7;13]. This alternative root cause of finding intra-seasonal differences in IVE estimates is often discussed.

Cross-protection is also being discussed as influencing the correlation between antigenic match (of vaccine virus and circulating strains) and IVE estimates found. The substantial cross-lineage VE reported by the Skowronski et al. for predominantly B/Yamagata vaccine (trivalent) against predominantly B/Victoria epidemic viruses suggests immunological interactions across antigenically distinct viruses – a currently poorly understood phenomenon [9]. A few years earlier it was also suggested by Skowronski that protection may extend beyond a single season with unchanged vaccine components [14]. This might be an explanation when the vaccine components are updated compared to the previous season but turn out to be mismatched and still a fairly high IVE is reported. Moreover, some cross-protection could exist between different clades of the virus.

Trebbien et al. in 2017 found a general low IVE against A/H3N2. Several genetic drifted viruses were observed that raised concern regarding the match with the vaccine A/H3N2 strain. IVE was calculated in a stratified manner for each of the clusters of genetically drifted A/H3N2 viruses. The results showed that protection against a genetically different clade can still exist to some extent and varies per cluster of genetic drift [13].

In summary, even though antigenic mismatch between the vaccine virus and the circulating strain is clearly responsible for modifying the effect size of the VE estimate, it involves a true effect and is mostly only qualitatively addressed in IVE studies. Potential solutions to get a better insight in how it affects the estimate is by stratification on strain-level or by calendar time within the season.

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6.2.7. Full vs. partial vaccination – effect modification

For children under a certain age, it is sometimes recommended to receive two doses of influenza vaccine; particularly when the child has not been previously vaccinated against influenza. Given this recommendation, children that should receive two doses within the same season according to the recommendations, but only were vaccinated with one dose, are often referred to as partially vaccinated. For IVE studies in this age group, there is often consideration of the subject's vaccination status being full or partial and the influence of that on the effectiveness of vaccination. Our literature search yielded seven publications of which two only addressed IVE against non-specific outcomes and were therefore excluded. The remaining 5 papers are discussed below.

Eisenberg 2008 describes the effect of full and partial vaccination against no vaccination in children aged 6-59 months across 2 influenza seasons. The authors describe both a model of stratification

between full and partial vaccination as well as inclusion of the vaccination status (full, partial, no vaccination) in a conditional logistic regression model. Findings were not consistent between the two seasons, but in general the data showed that partial vaccination caused a decline in the IVE to a point where it was no longer significant in reducing influenza infection compared to no vaccination [1].

Yang et al. performed a case-control study (where cases had lab-confirmed influenza and controls had a confirmation of no ILI) among 6-59 months old children in Guangzhou, China. The controls were randomly selected. Data was collected on whether subjects where fully, partially or not vaccinated and the VE was estimated for separate strata (full vs partial). Results showed that for both seasons analysed, partial vaccination gave a much lower and non-significant VE estimate as compared to full vaccination [2].

Thompson et al. conducted a TND study (as part of the Flu VE network) to estimate IVE against lab-confirmed influenza among children 6 months to 8 years. Data was collected on full or partial vaccination and analysis was stratified based on this covariate. The results did not show a conclusive answer on whether partial vaccination was still effective, due to low numbers of partially vaccinated children. Results varied between seasons, unadjusted vs. adjusted IVE estimates and stratification based on the influenza strain that caused the infection. Overall, they did not observe higher VE for children fully vs. partially vaccinated with IIV3 as defined by ACIP in any of the models. The authors findings suggest that reports of VE for children should estimate VE for specific combinations of current and prior season(s)' vaccine exposure [3].

Staat et al. conducted a prospective, population-based, case–control design based on active surveillance in which they compared the influenza vaccination status of children with laboratory-confirmed influenza to laboratory-confirmed influenza-negative matched controls. Children included in the study were classified as fully, partially or not vaccinated. A stratified analysis was done for fully or partially vaccinated children. Over the combined two seasons the point estimates for all age groups were similar for fully vaccinated and partially vaccinated children, however, for the partially vaccinated group none of the estimates were significant. Slightly different results were found when each season was assessed separately [4].

Shuler et al. conducted a matched case-control study to estimate IVE against lab-confirmed influenza. Data was stratified for full vs partial vaccination during analysis. However, the investigators also included vaccination status as a covariate in a multivariate conditional logistic regression model, since they found vaccination status to be independently associated with lab-confirmed influenza [5]. No further assessment was provided on the fit of this model and the results from the regression analysis as compared to a stratified analysis.

For most of the studies discussed above it can be noted that the subjects where stratified as full or partially vaccinated. This can be explained as a partial vaccination modifies the effect of the vaccination (by not being complete) and therefore influences the IVE estimate. This is a true modification of the effect size and thus stratification makes sense. It is also noted that, apart from one study being inconclusive, in general the effect of flu vaccination on the odds of being infected with the influenza virus is modified towards the null in case of only partial vaccination. Some authors do describe adjustment of the IVE estimate by including a covariate related to full or partial vaccination in a logistic regression model, but none of the papers make clear what the effect of that adjustment is and what the true rationale for this is.

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6.2.8. Intra-seasonal waning effectiveness

Intra-seasonal waning effectiveness is often reported in IVE studies. Several root causes are discussed to explain this observation.

During the initial classification of the included titles/abstracts, 12 publications where selected for full text screening on 'waning immunity' or 'time since vaccination'. After full text screening, 6 publications remained for data extraction.

Waning effectiveness is a covariate of interest in all the most common IVE study designs; it has been discussed for TND, cohort studies, nested case-control studies; both prospective and retrospective designs.

There are a few main methods to measure waning effectiveness as a covariate. Two types of variables are commonly used when investigating waning effectiveness; firstly, time since vaccination (i.e. the time passed between vaccination and symptom onset for each individual subject) [1-5]; secondly, comparison of IVE in early season vs. late season, which is done by dividing the season in two [1-6].

Stratification is the most commonly used technique to investigate the potential effects of waning immunity and both time since vaccination as well as early vs. late season estimates are treated categorically. When looking at IVE for early or late vaccinees, stratification is mostly done for just two large groups, the first half of the season and the second half of the season. The cut-off can be either a specific epidemiological week of flu circulation [2;3] or by using the end of a calendar month [1;4;5]. When looking at time since vaccination, there have been studies where the variable is binary (e.g. before or after 93 days post vaccination) [1;3;4] or more categories [2;5]. This is useful when one wishes to stratify the data, but some studies also adjust for time since vaccination in their logistical regression model and in that case the data is mostly included as a continuous variable [3;4]. One study looked at time since vaccination using splines [7].

Most studies find that the IVE is higher early in the season or with less time passed between vaccination and onset of symptoms. The decline in IVE found varies greatly though and is not always statistically significant. Several explanations are provided to explain the decline in IVE over the course of the season, some also related to lack of power or residual confounding. However, in terms of a true effect, most importantly antigenic drift has been mentioned, where the circulating virus mutates and drifts further away from the types included in the vaccine, which as a result offers less protection (see section 6.2.6).

Another explanation, particularly in elderly people, is waning immunity due to immunosenescence. Reduction in antibody titres in these elderly populations has been demonstrated before. Pebody et al. suggest this as a biological explanation for their observed reduction in vaccine effectiveness over a

season that had a late peak and where the median time from vaccination to disease onset was approximately three months [4]. To disentangle the possible effects of waning immunity and antigenic drift, Kissling et al. looked at a combination of time since vaccination and early vs. late season influenza. In the early influenza phase, IVE was higher among persons vaccinated less than 93 days before symptom onset compared to persons vaccinated 93 days or more before symptom onset. Since this was not the case in the late influenza phase, where they expected a greater effect of antigenic drift on the IVE estimates, it provided them with the strong suggestion that the waning immunity hypothesis for that season may be plausible [3].

There is some controversy though on which method is best suitable for measuring intra-seasonal waning effectiveness. Even though most of the studies estimate the risk of confirmed influenza using the number of days elapsed between vaccination and symptom onset or defining different periods during the season., some authors have reported conflicting results using the early or late season approach, whereas others did not observe a waning effect when using days between vaccination and symptom onset. Puig-Barbera et al. assert that results should be interpreted with caution when the explanatory variable is defined as the number of days between vaccination to outcome as the explanatory variable is related to the dependent [6].

It can be concluded that intra-seasonal waning effectiveness is often observed in IVE studies and the effect can be quantified by time since vaccination or using early vs. late season IVE estimates. Most commonly a stratified analysis is done, which seems a logical choice given that the waning effect of vaccination is an effect modifier. To better understand the root cause of the waning effectiveness over the season (e.g. waning immunity or antigenic drift) it is suggested to combine both measurements to see which effect is larger.

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6.2.9. Other key covariates

A handful of studies investigated the potential effect modifier of sex for influenza associated outcomes, though those were related to nonspecific outcomes. Nichol et al. showed evidence of an interaction between vaccination and sex for all-cause mortality, with lower effectiveness found in males (P = .03) [1]. Vila-Corcoles et al. found a lower risk for all-cause mortality in females compared with males across all age groups in community-dwelling elderly adults in Spain from 2002–2005, but with the difference in mortality risk between females and males that declined while age increased [2].

More recently, a research group investigated whether sex should be considered as an effect modifier [3]. Using the historical databases of the Canadian Sentinel Practitioner Surveillance Network (SPSN) from 2010– 2011 to 2016–2017, they reported that overall adjusted VE was higher among females than males, although this varied by influenza subtype/lineage, age group, and season. Overall for any influenza, adjusted VE was 49% (95% confidence interval [CI], 43% to 55%) for females vs 38% (95% CI, 28% to 46%) for males (absolute difference [AD], 11%; P value for interaction term = .03). They also shown the greater differences between males and females in the older adults ≥50 years, but not the younger age groups (20-49 years).

Among adults ≥50 years, the adjusted VE was 48% (95% CI, 38% to 57%) vs 29% (95% CI, 10% to 44%) in females and males, respectively (AD, 19%, P = .03), whereas the VE was 49% (95% CI, 31% to 62%) vs 45% (95% CI, 24% to 59%; AD, 4%; P = .74) in those age <20 years and 47% (95% CI, 37% to 56%) vs 48% (95% CI, 33% to 60%; AD, –1%; P = .90). As far as subtype/lineage was concerned, larger absolute differences were seen among children and adolescents age <20 years for A(H1N1) pdm09 and B(Victoria) and older adults age ≥50 years for A(H3N2), A(H1N1)pdm09, and B(Victoria).

Although the biological mechanisms underlying these potential sex differences are not straightforward, some have hypothesized that females have been shown to have stronger innate and adaptive immune responses, including more pronounced antibody response to influenza vaccine, in association with higher rates of local and systemic adverse events following Immunization [4-6]. Some have attributed these differences to sex steroids that alter the function of immune cells by binding to specific receptors and influencing cell signalling pathways [5;6]. The observed modest effect of sex, notably combining with age effect on IVE deserves further investigation and some authors have proposed sex-based design for influenza vaccination strategies [6].

While age is commonly adjusted for in the identified studies assessing influenza vaccine effectiveness, we did not identify any paper explicitly dealing with this covariate as confounder or effect modifier and discussing its impact on the ultimate IVE assessment. According to the WHO [7], age is a well-recognized confounding factor, as both vaccine coverage and risk of influenza virus infection vary by age. Age is also considered by some research group as an important stratification factor for VE estimates, as VE is expected to differ in different age groups. Stratification of VE estimates allows researchers to assess the presence of confounding by age or of true difference in VE by age (i.e. effect modification).

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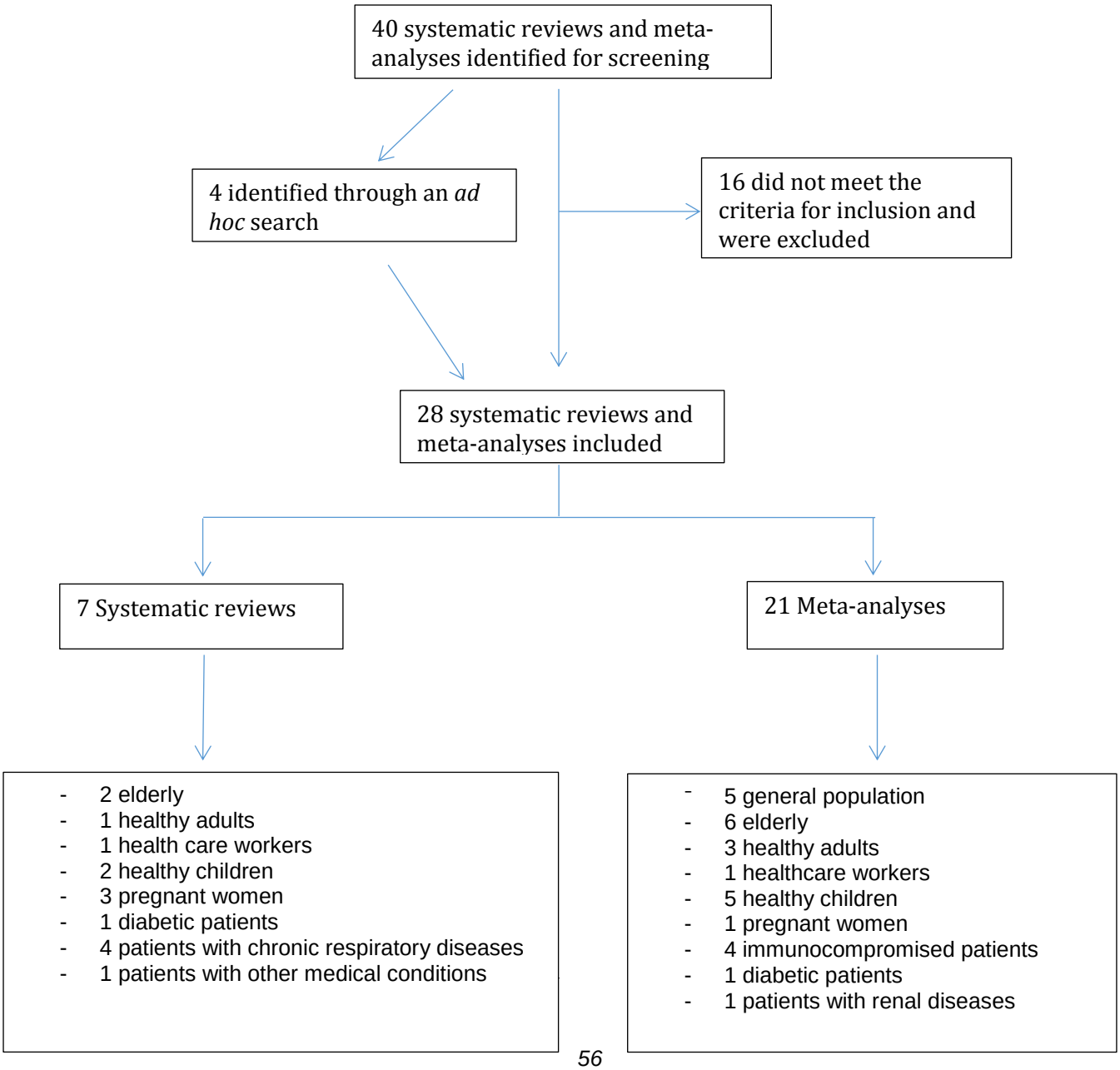
6.3. Summary of data from meta analyses/systematic reviews on IVE

6.3.1. Vaccine effectiveness – Overview of published estimates by population of interest

We took advantage of the search strategy on bias, confounders and effect modifiers to identify and present the outcome of systematic reviews or meta-analyses reporting influenza vaccine effectiveness. In total, thirty-nine systematic reviews and/or meta-analyses that focused on the effectiveness of influenza vaccines by target groups were retrieved; 15 did not meet the criteria for inclusion and were excluded (excluded studies and reasons for exclusion are reported in Section 10, Annex 1), four were identified through an *ad hoc* search: these were studies published in 2018, after the original search was performed [1-4]. In total, 26 systematic reviews and meta-analyses were included in our analysis: 20 meta-analyses and 6 systematic reviews. The selection process of studies is shown in Figure 2.

Figure 2: Study selection process. Two systematic reviews [5,6] and three meta-analyses [2,7,8] considered more than one population group.



The aim of the present chapter is to provide an overview of the potential variability of vaccine effectiveness depending on the population considered for inclusion. The topic of IVE variability was not exhaustively treated, being many the possible causes, including the study design, the health care setting in which studies were conducted etc.

Studies included in the current chapter are reported:

- - by age group (children and/or adolescents, adults, elderly), *OR*
- - by high-risk status: pregnancy, immunodeficiency (primary, secondary or iatrogenic),
- - by pre-existing medical conditions: diabetes, renal diseases, chronic respiratory diseases (i.e. asthma or chronic obstructive pulmonary disease) and other disorders (e.g. coronary or liver diseases).

Only laboratory-confirmed influenza results have been reported below. However, when other non-specific outcomes were also assessed and presented together with laboratory confirmed influenza in the systematic review/meta-analysis, these were also reported in the tables in Annex 2.

When the systematic reviews/meta-analyses qualitatively assessed the studies included, the given assessment is reported in this chapter as well.

In addition, Health Evidence (McMaster University, Hamilton, Ontario, Canada: https://www.healthevidence.org/search.aspx) is a Canadian website developed to provide quality assessment on scientific evidence for public health decision makers and to evaluate the evidence of systematic reviews. Its mission is to help the public health workforce and policy makers search for, interpret, and apply research evidence to their local context. When available, the assessment of retrieved systematic reviews on a scale from 0 (minimum score) to 10 (maximum score) by Health Evidence (HE) was reported. For the quality assessment, HE considers whether the following points are addressed in the review, and the score 1, in case of a "yes" response, or the score 0, in case the answer is "no", is assigned:

1. Did the authors have a clearly focused question [population, intervention (strategy), and outcome(s)]?
2. Were appropriate inclusion criteria used to select primary studies?
3. Did the authors describe a search strategy that was comprehensive?
4. Did search strategy cover an adequate number of years?
5. Did the authors describe the level of evidence in the primary studies included in the review?
6. Did the review assess the methodological quality of the primary studies (including research design, source of bias such as confounders and respondent bias, data collection [measurement of independent/dependent variables], follow-up/attrition rates, and data analysis)?
7. Are the results of the review transparent?
8. Was it appropriate to combine the findings of results across studies?
9. Were appropriate methods used for combining or comparing results across studies?
10. Does the data support the author's interpretation?

6.3.2. General population

Four meta-analyses reported on IVE in the general population (Section 11 Annex 2, Table 7). In the general population seasonal influenza vaccines impact ranged from no effectiveness in case of mismatch to moderate effect on the reduction of laboratory-confirmed pandemic 2009 A (H1N1), influenza infection to 71% when participants were vaccinated in 2 consecutive seasons in a RCT study. Strain specific IVE was estimated by some authors and ranges from no effectiveness to 52% for H1N1, it was 37% for H3N2 and 38% for influenza B according to the meta-analysis by Rondy et al. [7]. Bartoszko [3] and Li [9] found a variability in the calculated estimates due to study design (RCT, cohort or case control studies).

Bartoszko et al. 2018 [3] assessed whether consecutive influenza vaccination reduces VE compared to current season influenza vaccination. 5 RCTs (11,987 participants) did not show a significant reduction in VE when participants vaccinated in two consecutive seasons (VE 71%, 95% CI 62–78%) were compared to those vaccinated in the current season (VE 58%, 95% CI 48–66%) (odds ratio [OR] 0.88, 95% CI 0.62–1.26, p = 0.49, I^2 = 39%); 28 observational studies involving 28,627 participants also did not show a reduction (VE for two consecutive seasons 41%, 95% CI 30–51% compared to VE for current season 47%, 95% CI 39–54%; OR 1.14, 95% CI 0.98–1.32, p = 0.09, I2 = 63%). Results from subgroup analyses by influenza type/subtype, vaccine type, age, vaccine match and co-morbidity support these findings; however, dose–response results were inconsistent. Certainty in the evidence was assessed to be very low due to unexplained heterogeneity and imprecision. Authors concluded that available evidence does not support a reduction in VE with consecutive influenza vaccination, but the possibility of reduced effectiveness cannot be ruled out due to very low certainty in this evidence.

Rondy et al. 2017 [7] performed a systematic review and meta-analysis of test-negative design case-control studies to evaluate the effectiveness of seasonal trivalent and of pandemic monovalent vaccines in preventing severe influenza illness among all adults and stratified to two different age groups (18–64 and 65 years and above), between seasons 2010–11 and 2014–15. Studies published in English, French, Spanish or Portuguese were considered. Among all adults, pooled seasonal IVE against laboratory-confirmed influenza-associated hospitalizations was 41% (95%CI 34; 48) across the abovementioned five seasons (pooled season-specific seasonal IVE estimates against any influenza viruses in all adults ranged between 31% in 2011–12 and 2014–15 and 53% in 2013–14).

Specifically, IVE against laboratory-confirmed influenza-associated hospitalization was 48% (95% CI 37;59) for influenza A(H1N1)pdm09; 37% (95% CI 24;50) for influenza A(H3N2), and 38% (95%CI 23;53) for influenza B. Authors did not assess the risk of bias of the included studies since in their opinion there is no risk-of-bias tool suitable to TND studies. The quality rating of this systematic review and meta-analysis by Health Evidence was 6/10 (moderate). In summary, influenza vaccines may prevent nearly half of influenza lab-confirmed hospitalizations. Lower IVE among persons 65 years and older compared to adults aged 18–64 years was observed (specific results by age groups are reported in respective sub-chapters below).

Li et al. 2015 [9], investigated the partial protection against 2009 pandemic influenza A (H1N1) of seasonal influenza vaccination across seasons 2007-08 to 2009-10. The effects on laboratory-confirmed A (H1N1) influenza, assessed through serological method or Real Time Polymerase Chain Reaction (RT-PCR), derived from 4 RCTs, 2 cohort and 16 case-control studies. RCTs, which were judged to be high-quality according to the Jadad scale [10], and with no underlying risk of bias, showed a non-significant relative risk or risk ratio (RR) increase of 27% (RR = 1.27; 95% CI: 0.46, 3.53; P = 0.64) (non-significant heterogeneity was observed (I2= 19%; P = 0.30) for lab-confirmed influenza. From the cohort studies, insignificant results were reported. In 16 case-control studies, a slight risk reduction in risk for lab-confirmed influenza was observed (OR: 0.80; 95% CI: 0.61, 1.05; P = 0.11), however a significant heterogeneity was found across these studies (I2 = 93%; P <0.00001). In conclusion, authors found a moderate effect of the seasonal influenza vaccine on the reduction of laboratory-confirmed pandemic

2009 A (H1N1) influenza infection. According to the authors, the different results observed among cohort and case-control studies may be caused by the differences in the study designs and potential effects from bias and confounding.

Yin et al. 2012 [11] determined the effect on preventing pandemic influenza A H1N1 2009 infection of seasonal trivalent influenza vaccines (cross-protection) and of pandemic influenza A H1N1 2009 vaccines. RCTs, cohort studies and case-control studies of TIV in any population for the seasons 2007-2008, 2008 - 2009, or 2009 - 2010 in the northern hemisphere, and 2008 - 2009 in the southern hemisphere, were eligible for inclusion. Controls for RCTs and cohort studies had to be participants who did not receive the vaccine that year or in the previous year. Controls for case-control studies were participants who were influenza A H1N1 2009 laboratory test negative. The age of participants ranged from zero to 84 years. The study locations included the USA, UK, Australia, Mexico, Canada, China, and Europe.

Only results for seasonal vaccines (cross-protection) are reported in the present chapter. A meta-analysis from 13 case-control studies showed a cross-protection for confirmed illness was 19% (95% CI = 13–42%), notable heterogeneity was observed. In a sensitivity analysis where 5 studies with moderate/high risk of bias were excluded, a higher cross-protection (34%, 95% CI 9–52%) was observed (8 case-control studies included). Further exclusion of studies 3 that recruited early in the pandemic (when non-recipients of TIV were more likely to have had non-pandemic influenza infection that may have been cross-protective) dramatically reduced heterogeneity (I^2=0) and resulted in a 51% cross-protection. One RCT reported cross-protection of 38% (19–53%) for confirmed illness.

In summary, TIVs provided moderate cross-protection against laboratory-confirmed A(H1N1)pdm09 illness (based on eight case–control studies with low risk of bias and one RCT). A finding of increased risk from seasonal vaccine was limited to cases recruited early in the pandemic. Although cross-protection was less than the direct effect of strain-specific vaccination against A(H1N1)pdm09, TIV was generally beneficial before A(H1N1)pdm09 vaccine was available. The quality of RCTs was assessed using the Cochrane Collaboration's tool, version 5.1.0, which appraised selection, performance, attrition, detection, and reporting biases. The quality of case-control and cohort studies was assessed using the Newcastle-Ottawa scale, which appraised selection, comparability and exposure. The quality of the evidence was mixed, with approximately half of the studies deemed at moderate or high risk of bias. The quality rating of this systematic review and meta-analysis by Health Evidence was 9/10 (strong).

6.3.3. Elderly

Six meta-analyses and two systematic reviews reported IVE among the elderly. One meta-analyses only reported results on unspecific outcomes, those are reported only in Annex 2, Table 8.

In the elderly seasonal influenza vaccines impact against lab-confirmed flu ranged from 28% to 63%. Strain specific IVE was estimated by Rondy et al. [7] and was 54% against influenza A(H1N1)pdm09 viruses, 33% against H3N2 and 31% against influenza B. These estimates were lower than those observed for healthy adults. Dominich et al. 2016 [12], highlighted that the greater protection offered by MF59-adjuvanted vaccines is against hospitalizations due to influenza complications. Estimates by Hirve et al. 2016 [5] in tropical and subtropical countries were slightly lower compared to those observed in developed countries (50-77%), according to authors underlying nutrition deficit or infections (e.g. TBC, malaria) may explain lower IVE in this countries. Michiels, et al. [6] observed inconsistent results among institutionalised elderly, and among elderly with co-morbidities. In their opinion these inconsistencies can only be explained by bias of unknown origin.

A recent study published in the Cochrane Library, 2018 [13] showed an IVE of 58% (RR 0.42, 95% confidence interval, CI 0.27 to 0.66) among the elderly (individuals aged 56 year old or older) against

laboratory-confirmed influenza diagnosed through viral isolation (no details on the diagnosing methods were available in primary studies). However, such evidence, which derives from three randomized-controlled trials (RCTs), was judged to be of low quality by the authors of the reviews, who, in particular, highlighted the limited applicability of findings to laboratory-confirmed influenza, given the lack of detail regarding the diagnosis of influenza. Health Evidence Review Quality rating was strong (10/10).

Other meta-analyses, systematic reviews, not included in the Cochrane review are the following:

A systematic review and meta-analysis by Dominich et al. 2016 [12], took into consideration the effect of MF59-adjuvanted trivalent vaccine (MF59-TIV) on patients ≥60/65 years. Pooling results from two studies, one prospective case-control study in five hospitals (826 patients) and one prospective community-based case-control study enrolling 282 eligible participants, conducted, respectively, in the 2010-2011 and in the 2011-12 influenza seasons, showed an IVE against laboratory confirmed influenza diagnosed by either RT-PCR or culture of 60.1%, although the 95% CI passed through zero (-1.3 to 84.3%). The included studies were judged to be at medium risk of bias. No heterogeneity was found. Authors conclusion were that MF59-TIV is effective in reducing several influenza-related outcomes among the elderly, the greater protection offered being that against hospitalizations due to influenza-related complications. The Health Evidence's Review Quality Rating was 9/10 (strong).

Hirve et al. 2016 [5], reviewed policy, availability, use and effectiveness of seasonal influenza vaccine in tropical and subtropical countries. Thirty-eight countries used the Northern Hemisphere and 21 countries the Southern Hemisphere formulation. Forty-six countries targeted children and 57 targeted the elderly. Authors found that vaccine protection against laboratory-confirmed influenza in the tropics ranged from 43% to 58% in the elderly (no specification on vaccine type/brand used, or on the diagnostic test, was provided) in 21 studies (four RCTs, two non-randomized controlled trials, seven cohort studies, four case–controls and four ecological studies) published between 1993-2014. Such estimates are slightly lower compared to those observed in developed countries (50-77%). Authors suggest the underlying nutrition or infections (e.g. TBC, malaria) may explain lower IVE and stressed that the majority of observational studies included in their review were prone to selection or ascertainment bias.

Chan et al. 2014 [14] systematically reviewed and performed a meta-analysis of 11 observational studies (6 retrospective and 5 prospective studies, 11,262 total subjects included) conducted up to 2013 on institutionalized elderly aged ≥60 years. No information on the vaccine type/brand was provided by authors. Meta-analysis for laboratory-confirmed influenza was not performed because of paucity of studies (only two) (Annex 2, Table 8).

A meta-analysis of 35 test-negative design case-control studies by Darvishian et al. 2014 [15], considered the effects of influenza vaccination (no vaccine type/brand specified) on laboratory-confirmed influenza (confirmed by at least one of the following: culture, rapid antigen testing, fluorescent antibody assays, HI tests or PCR) on community dwelling citizens aged 60 or more in Spain, Germany, Lithuania, Australia, New Zealand, Taiwan, South Africa and Japan (4,975 total subjects, heterogeneity between studies 28.34%). The OR was 69%, 95% CI 0.48-0.99 during sporadic activity, when the vaccine matched; it was OR 42% (95% CI 0·30-0·60) with matching vaccines, and 57% (95% CI 0·41-0·79) in case of mismatch between circulating and vaccine strains. During widespread outbreaks, the IVE was 46% (95%CI 0.46-0.62) in case of match and 28% (95% 0.60-0.85) in case of mismatch. Authors conclusions are that IVE against lab-confirmed flu is moderate in the elderly during epidemic seasons and that more research is needed to investigate factors affecting vaccine protection in such population group (e.g., brand-specific or type-specific vaccine effectiveness and repeated annual vaccination).

The following are two meta-analyses and one systematic review that considered several population

groups, including the elderly.

Rondy et al. 2017 [7] performed a systematic review and meta-analysis of TND case-control studies to evaluate the effectiveness of seasonal trivalent (27 studies) and of pandemic monovalent vaccines (three studies) in preventing severe influenza illness among all adults and stratified to two different age groups (18–64 and 65 years and above), between seasons 2010–11 and 2014–15.

Studies published in English, French, Spanish or Portuguese were considered. Among ≥65 years, between 2010–11 and 2014–15, the pooled seasonal IVE was **37%** (95%CI:30;44) for any influenza. Summary IVE was **54%** (95%CI: 26;82) against influenza A(H1N1)pdm09 viruses, with I2 = 64%; IVE against A(H3N2) was **43%** (95%CI:33;53) in seasons when circulating and vaccine strains were antigenically similar and **14%** (95%CI:-3;30) when A(H3N2) variant viruses predominated; summary IVE against influenza A(H3N2) viruses was **33%**, 95% CI: 21,45). Summary IVE against influenza B was **31%** (95% CI: 11;51).

It is not clear which diagnostic tests were used, however authors state that in sensitivity analyses, whereby data from studies not using clear clinical criteria for patients' inclusion or those not exclusively using RT-PCR for laboratory testing were excluded, resulted in similar summary estimates. The quality rating of this systematic review and meta-analysis by Health Evidence was 6/10 (moderate). In summary, seasonal trivalent vaccines seemed to provide low protection among elderly in seasons where vaccine and circulating A(H3N2) strains were antigenically variant.

Manzoli et al. 2012 [8] found that IVE of parenteral inactivated vaccine against laboratory- confirmed influenza was 41% in one meta-analysis, and 63% in another one. Authors concluded that the efficacy/effectiveness of current seasonal vaccines is generally modest for the elderly.

Michiels et al. 2011 [6], published a review of studies conducted between 2006 and 2011 on the effects of trivalent inactivated vaccines in different target groups. Among elderly subjects (65 years of age. or more) those institutionalized, community-dwelling (healthy and at risk for complications) and mixed were separately analysed. Their results on IVE among the elderly come from previous versions of Jefferson's Cochrane systematic review, the IVE in a mixed population of elderly being 41%.

Authors concluded that inconsistent results are found among institutionalised elderly (65 years or older), and elderly with co-morbidities, which can only be explained by bias of unknown origin. The quality of systematic reviews assessed using AMSTAR; evidence quality was graded using GRADE. Authors found a considerable lack of high-quality evidence for the effect of vaccination on complications and inconsistent results were found in studies of elderly in nursing homes. The review quality assessment by Health Evidence was 5/10 (moderate).

6.3.4. Healthy adults

Three meta-analyses and one systematic review reported IVE among healthy adults aged 18 to 64 years (Annex 2, Table 10). In this population group, seasonal influenza vaccines impact ranged from 49% to 88%, the higher estimates being reported for healthcare workers by Ng, et al. [16]. Strain specific IVE was estimated by Rondy, et al. [7] and was 55% against influenza A(H1N1)pdm09 viruses, 50% against H3N2 and 45% against influenza B. These estimates were significantly higher compared to those observed for elderly (aged 65 years or older). Manzoli, et al. [8] compared parenteral inactivated vaccines with live attenuated and aerosol inactivated vaccines and found higher IVE against lab-confirmed influenza for parenteral inactivated vaccines. Important evidence for decision makers on the effectiveness of influenza vaccination in reducing the incidence of the disease amongst HCW is provided by a recent meta-analysis by Imai, et al. [4].

A recent systematic review and meta-analysis published in the Cochrane Library [17] found an IVE of 59% (RR 0.41, 95% CI 0.36-0.47) against laboratory-confirmed influenza in healthy adults. IVE was

assessed by 25 RCTs that compared inactivated parenteral influenza vaccine against placebo or do-nothing control groups, carried out over single influenza seasons in North America, South America, and Europe between 1969 and 2009. Over 71,200 subjects participated in those studies. Some used viral culture, others used a four-fold antibody increase to confirm influenza cases. Authors found that inactivated parenteral influenza vaccine reduce influenza in healthy adults from 2.3% without vaccination to 0.9%, the RR being 0.41 (95% CI 0.36 to 0.47; 71,221 participants). The quality of the evidence was moderate (GRADE). The Review Quality Rating by Health Evidence was 10/10 (strong).

Rondy et al. 2017 [7] performed a systematic review and meta-analysis of TND case-control studies to evaluate the effectiveness of seasonal trivalent and of pandemic monovalent vaccines in preventing severe influenza illness among adults belonging to different age groups. In adults aged 18 to 64 years, pooled seasonal IVE against laboratory-confirmed influenza-associated hospitalizations was 51% (95%CI 44 to 58) for any influenza.

Summary IVE against influenza A(H1N1)pdm09 viruses was 55% (95%CI 34 to 76), that against influenza A(H3N2) viruses was 50% (95%CI 38 to 62) and the summary IVE against influenza B was 45% (95%CI 8 to 81). Authors concluded that IVE was significantly higher among adults aged 18-65 compared to those ≥ 65 years (51% vs. 37%, respectively).

The meta-analysis of meta-analyses by Manzoli et al. 2012 [8] considered three previously published meta-analyses and found that the overall IVE of influenza vaccination against laboratory confirmed among healthy adults varied between 49 and 61%; the IVE of parenteral inactivated vaccine varied between 59 and 67%, and that of live attenuated vaccines (LAV) varied between 32 and 62%. Authors concluded that in adults and children, the effectiveness of seasonal influenza vaccines is generally high for laboratory-confirmed cases.

After examining one randomized controlled-trial, three cohort and three case–control studies, Hirve et al. 2016 [5] found that IVE against laboratory-confirmed influenza among healthy adults varied between 50% and 59% (no specification on vaccine type/brand was used and the diagnostic test was not provided). Authors pointed out that the protection observed in healthy adults in the tropics and subtropics was comparable to that seen in developed countries, although in general evidence on vaccine effectiveness in the tropics and subtropics is scarce, and thus countries in this region need to strengthen and expand their local and regional evidence-base required for informed decision-making on influenza vaccine introduction and expansion, and how much benefit to expect.

6.3.5. Healthcare workers

Imai et al. 2018 [4] produced a systematic review and meta-analysis to synthesize the latest (1980-2018) evidence of the direct epidemiological and economic effectiveness of seasonal influenza vaccination among healthcare workers (HCW). Pooled analyses of results from 3 observational studies and 1 RTC, for a total of 1464 HCWs enrolled, found a significant effect of seasonal influenza vaccination reducing influenza infection of HCW when defined using laboratory-confirmed cases: RR=0.40 (95% CI; 0.23-0.69) (Annex 2, Table 9).

Serologically-defined cases showed a strong preventative effect against influenza infection while the vaccine effects of cases identified through other diagnostic tests such as rapid influenza diagnostic test (RIDT) and RT-PCR were not as evident. This heterogeneity was seemingly attributed to the methodological differences. There was only one study included for review that made use of RT-POCR and consequently authors could not conclusively evaluate the effect. Authors concluded that their study provides important evidence for decision makers on the effectiveness of influenza vaccination in reducing incidence of influenza (and absenteeism duration) amongst HCW.

In a systematic review, Ng et al. 2011 [16],evaluated the effectiveness of seasonal influenza vaccination

in HCW. Only one study, among those selected by authors, reported IVE against laboratory-confirmed influenza, therefore authors concluded that there was limited evidence that influenza vaccination may reduce incidence of laboratory-confirmed infection in healthcare workers. IVE of any kind of influenza vaccine was 88% (95% CI 59 to 96%). Authors rated such study as a high-quality trial- the review quality rating by Health Evidence was 9/10 (strong).

6.3.6. Healthy children

Five meta-analyses and two systematic reviews reported IVE among healthy children (Annex 2, Table 12). Among healthy children, seasonal influenza vaccines impact against lab-confirmed influenza ranged from no effect to 83%, the higher estimates being reported for live attenuated vaccine in trivalent formulation for the 2013-2014 season by Caspard et al. [18].

A recent Cochrane review [19], found higher IVE against lab-confirmed influenza for live attenuated vaccine compared to parenteral inactivated vaccines, consistently with findings from a meta-analysis published in 2012 by Manzoli, et al. [8]. However, Caspard et al. highlighted that while effectiveness against matched A(H3N2) strains and influenza B was consistently observed, effectiveness of LAIV against A(H1N1)pdm09 strains, both in the case of the trivalent and of the quadrivalent formulation and irrespectively whether children were previously vaccinated or not, has been lower than effectiveness against A(H1N1)pdm09 strains of inactivated vaccine.

Jefferson et al. performed a systematic review and meta-analysis of studies conducted over single influenza seasons in the USA, Western Europe, Russia, and Bangladesh between 1984 and 2013, with the aim to compare live attenuated influenza and inactivated vaccines with placebo or do nothing in children aged <16 years [19]. For live attenuated influenza vaccines, children aged 3 to 16 years had a RR= 0.22, 95%, CI 0.11 to 0.41 (seven RCTs, 7,718 children; moderate-certainty evidence).

For inactivated vaccines, children aged 2 to 16 years had a RR of 0.36, 95% CI 0.28 to 0.48 (five RCTs 1628 children; high-certainty evidence). Authors concluded that in children under the age of 16 years, both influenza vaccines probably reduce influenza, although decision-makers willing to inform local or national policies should be aware of the variable certainty of the evidence.

Caspard et al. 2017 [18] assessed effectiveness of LAIV in seasons 2010-2016. LAIV has not been consistently demonstrated since the 2009 pandemic. LAIV3 was effective in 2011–2012:68%; 95% CI, 48–80; in 2012–2013: 43%; 95% CI, 27–56; in 2013–2014: 83%; 95% CI, 25–96). LAIV4 was effective in 2015–2016: 48%; 95% CI, 29–61.

The LAIV was not shown to be effective as a monovalent formulation in 2009–2010 (79%; 95% CI, –16 to 96), trivalent formulation in 2010-2011 (42%; 95% CI, –1 to 85), or quadrivalent formulation in 2013–2014 (18%; 95% CI, –3 to 34) and 2014–2015 (28%;95% CI, –18 to 56).

Consolidated estimates across seasons show that LAIV was effective as a trivalent formulation (53%; 95% CI,35–66) and a quadrivalent formulation (33%; 95% CI, 17–46) and since the 2009 pandemic irrespective of the formulation (42%; 95% CI, 30–52).

According to authors findings were most clearly driven by suboptimal effectiveness against influenza A(H1N1)pdm09 strains. LAIV was not shown to be effective against A(H1N1)pdm09 strains in 2010–2011, 2012-2013, and 2013–2014, whether it was distributed as a trivalent or quadrivalent formulation. The LAIV4 was effective against A(H1N1)pdm09 strains in 2015–2016 but with significantly reduced effectiveness relative to IIV.

Effectiveness against influenza B strains was consistently observed. Trivalent LAIV was also effective against matched A(H3N2) strains, but there were insufficient data to estimate effectiveness against matched A(H3N2) of the quadrivalent formulation because of limited circulation of these strains in

recent seasons. Similar to IIV, LAIV4 was not effective against mismatched A(H3N2) strains in 2014–2015.

Lukšić et al. 2013 [20] assessed the impact of influenza vaccination in children and adolescents aged ≤18 years against ILI confirmed either clinically or by laboratory techniques. The effectiveness of live vaccines was evaluated by four RCTs and four cohort studies. The effectiveness of inactivated vaccines was evaluated by five RCTs and three cohort studies. The IVE of live vaccines against ILI, using random effects model, was 31.4% (24.8%-39.6%) and 44.3% (42.6%-45.9%) using fixed-effect model. IVE of inactivated vaccines was 32.5% (20.0%-52.9%) using random effects model, and 42.6% (38.3%-47.5%) using fixed-effect model.

The quality of studies was evaluated using GRADE criteria. Observational studies, in general, had lower validity than RCTs, and overall study quality was moderate. Directness of the studies was good, with age groups, interventions used, and definition of outcomes showing the expected directness when compared to the final outcomes. The heterogeneity of ILI definitions weakened directness slightly. Authors concluded that the lower values for effectiveness than for efficacy reflect the differences in outcomes used in the studies, being less specific the outcomes used to assess IVE (ILI). The review quality rating by Health Evidence was 6/10 (moderate).

In a re-analysis of five meta-analyses by Manzoli et al. 2012 [8], the overall IVE of influenza vaccination against lab-confirmed influenza ranged between 51 and 75%; IVE of parenteral inactivated vaccines ranged from 46% to 65%; vaccine efficacy of live-attenuated vaccines ranged from 72% to 83%. Authors concluded that the effectiveness of current seasonal vaccines was generally high for laboratory-confirmed cases, especially for LAV in children aged 2–17 years. For children aged < 2 years the evidence on parenteral inactivated vaccines remains scarce.

The meta-analysis performed by Restivo et al. 2018 [2] demonstrated a clear significant overall effect of 39% (95%CI: 32–46%) for visits and 57% (95%CI: 30–74%) for hospitalization. Authors judged some of the studies included to potentially overestimate the vaccination status, as vaccination status was partially or totally referred without validation technique. Authors concluded highlighted the high VE of influenza vaccination in all population groups considered, children included, often regardless of season, circulating strain, type of vaccination.

In the systematic review by Hirve et al. 2016 [5] in which 12 studies (nine randomized controlled trials, one cohort and two case–control studies) were examined in order to assess the impact of influenza vaccination in children in the tropics and subtropics (excluding Australia), Overall IVE against laboratory-confirmed flu ranged from 20 to 77%; LAIV IVE ranged from 62% to 83% and TIV IVE ranged from 48% to 72%. Vaccinating school children provided 23.3% (66.3–74.9) protection against influenza. Authors concluded that the protection observed in children in the tropics and subtropics was comparable to that seen in developed countries.

6.3.7. Pregnant women

One meta-analyses and three systematic reviews reported on vaccine effectiveness against influenza in either the mother or the newborn (Annex 2, Table 11). In mothers, seasonal influenza vaccines impact against laboratory confirmed influenza was around 50% for trivalent inactivated vaccines [5, 17] and 70% for the adjuvanted A(H1N1)pdm09 [21]. In newborns IVE ranged from 41% to 91%. The evidence gathered so far is however quite scarce, and further studies with appropriate study design may help clarifying the effects of vaccinating women during pregnancy in preventing influenza both among the mothers themselves and their newborns.

A recent Cochrane systematic review and meta-analysis [17] , assessed the effects of vaccination in pregnant women on the prevention of influenza and ILI in both the mothers and their newborns up to

24 weeks of life. The results of one RCT (at low risk of bias) and one clinical controlled trial (at high risk of bias) showed that vaccination with trivalent inactivated vaccine containing pH1N1 was weakly protective against influenza (RCT data only from Demicheli, 2018 [17], 2116 women entered the study: 1062 received the vaccine, 1054 the placebo) in mothers within 24 weeks after delivery (vaccine efficacy or effectiveness) (VE) 50%, 95%CI 14% to 71%), as well as among newborns of vaccinated mothers up to 24 weeks (VE 49%, 95% CI 12%to 70%). Authors concluded that the protection provided to pregnant women and their newborns is very modest. Further RCTs with appropriate study designs are required before promoting universal seasonal influenza vaccination during pregnancy, as current evidence is insufficient.

In the systematic review by Manske et al. 2014 [21], studies included were deemed to be heterogeneous, as they did not measure any uniform outcome, and provided very little effectiveness data based on laboratory-confirmed influenza. Consequently, wide-ranging estimates of VE in pregnant women, from -15 to 70 %, were reported. From two retrospective cohorts (1st:113,331 pregnant women: 59,266 vaccinated and 54,065 non-vaccinated; 2nd: 3,236 mothers who gave birth between May 25, 2009 and April 17, 2010), that evaluated the impact of the adjuvanted A(H1N1)pdm09, IVE adjuvanted A(H1N1)pdm09 was 70% (aHR = 0.30, CI 0.25–0.34) in the first cohort study, and 61% IVE nonadjuvanted A(H1N1)pdm09(CI15.5-82.5%) in the 2nd. Confirmation of influenza infection was based on RT-PCR or on a medical visit during pregnancy with an influenza-related International Classification of Diseases (ICD)-9 diagnosis code. Authors concluded that the foundation for recommending TIV for seasonal influenza in pregnant women is "somewhat weak" given the scarcity of studies conducted, with only one of these showing significant protection.

Seven studies (two retrospective cohorts, one retrospective matched cohort, one RCT, one matched case-control, one prospective cohort and one case-control) examined the potential for maternal vaccination to protect infants. These involved 94,119 infants over ten influenza seasons. According to reviews' authors, combining these studies in an attempt to provide conclusions regarding IVE of maternal immunization in preventing newborn disease was problematic, as they measured different outcomes of disease, used different means of determining infection rates, and all but one combined data across multiple influenza seasons, often without reporting how well the vaccine matched the circulating influenza strain. Four of these studies applied some form of laboratory confirmation, with VE ranging from 41 to 91%. Authors concluded that the evidence for newborn protection through maternal vaccination is encouraging. The review quality rating by Health Evidence was 6/10 (moderate).

Michiels et al. 2011 [6] found that trivalent inactivated vaccines had an IVE of 36% (95%CI: 4–57%). in preventing respiratory illness with fever in mothers (result from one RCT), and an IVE of 29% (95%CI: 7–46%) in preventing all respiratory tract infections with fever in newborns. Authors concluded that the vaccinating pregnant women might be beneficial for their newborns. The review quality assessment by Health Evidence was 5/10 (moderate).

In the systematic review by Hirve et al. 2016 [5] in which 2 RCTs (340 and 2116 pregnant women, respectively) were examined in order to assess the impact of influenza vaccination in the tropics and subtropics (excluding Australia). Vaccinating pregnant women against seasonal influenza prevented laboratory-confirmed (diagnosis made using RT-PCR) influenza in both mothers (50%) and their infants <6 months (49-63%). Authors pointed out that the evidence on IVE in the tropics and subtropics was scarce.

6.3.8. Specific populations

6.3.8.1. Immunodeficient patients

Immunodeficiency can be classified as primary (i.e. genetically determined), acquired (not traceable to a genetic basis, but resulting from infections or malignancies, particularly hematopoietic and lymphoid

cancer) or iatrogenic (immunosuppression resulting from the use of therapies modulating the immune system).

Three meta-analyses and one systematic review reported IVE among immunocompromised patients, suffering from acquired immunodeficiency, i.e. in HIV-infected patients and patients with malignancies, particularly hematopoietic and lymphoid cancers (Annex 2, Table 12). No study focusing on primary or iatrogenic immunodeficiency was retrieved. In patients with immunodeficiencies, seasonal influenza vaccines impact against laboratory-confirmed influenza was positive, with most studies reporting IVE estimates ranging from 71 to 85%. However, no effect in children under 5 years of age could be demonstrated, although this finding comes from only one RCT.

A recent Cochrane review by Bitterman, et al. [22] found a variability in the calculated estimates due to study design: confirmed influenza rates were lower with vaccination in one RCT and the three observational studies, the difference in IVE between vaccinated and non-vaccinated patients with cancer reaching statistical significance only in one retrospective case-control study, that however was judged to be at high risk of bias. Although scarce, current evidence suggests a benefit for influenza vaccination amongst immunodeficient patients.

Bitterman, et al. [22] updated in 2018 a Cochrane systematic review and meta-analysis published in 2013 on the effects of influenza vaccination in immunosuppressed cancer patients. Laboratory-confirmed influenza rates were lower with vaccination in one RCT and the three observational studies, the difference reaching statistical significance in one retrospective case-control study conducted in Brazil among patients with haematologic malignancies and haematopoietic stem cell transplantation (HSCT). In a study influenza diagnosis was based on immunofluorescence assay; VE was 80%. The study was however, judged to be at high risk of selection bias, attrition bias and reporting bias. IVE estimates were not significant in one RCT, also conducted in Brazil among HSCT patients, more than 7 days before allogeneic transplantation, in one retrospective, observational cohort study conducted in Boston, USA, in which participants were stage 4 colorectal adenocarcinoma patients with active chemotherapy treatment and in one prospective observational cohort study conducted in Israel, targeting patients with solid malignancies with active chemotherapy and haematological patients with active disease. Authors concluded that, although weak, current evidence suggests a benefit for influenza vaccination amongst adults with cancer. The review quality rating by Health Evidence was 9/10 (strong).

Beck et al. 2011 [23], found an IVE of 85% (OR 0.15; 95% CI 0.03-0.63) p=0.01 against laboratory-confirmed influenza in immunodeficient patients (primary or secondary immunodeficiency, age not specified). This finding comes from pooling estimates from two studies (study design not specified), with moderate statistical heterogeneity (I2 = 50.4%; p = not significant). Authors concluded that influenza vaccines probably confer a similar level of clinical protection against influenza and that the rate symptomatic disease is comparable to that observed in vaccinated healthy controls.

Regarding *HIV-infected adults*, the systematic review and meta-analysis by Remschmidt et al. 2014 [24], assessed to be of moderate quality of evidence by Health Evidence (rating was 7/10), found an IVE of 71% (95% CI, 44–85%) of TIV against laboratory-confirmed influenza. This finding comes from one cohort study conducted in Japan in which 262 HIV-infected patients received TIV, and 66 did not, judged to be at high risk of sampling bias (according to GRADE criteria).

The same review also assessed the effect on *HIV-infected children:* results from one RCT conducted in South Africa, in which 206 vaccinated and 204 unvaccinated patients were recruited, reported an IVE of 11% (-70 to 54%) of TIV against laboratory-confirmed influenza (moderate quality of the evidence). Authors concluded that influenza vaccination with TIV prevents laboratory-confirmed influenza in adults with HIV, but not in children < 6years of age.

For *patients with haematological malignancies*, the systematic review by La Torre et al. 2016 [25], considered several nonspecific outcomes, which are reported in Annex 2, Table 12.

6.3.8.2. Other at-risk patients

The impact of influenza vaccines among patients at risk of serious influenza complications because of underlying medical conditions such as diabetes, renal diseases, chronic respiratory diseases (i.e. asthma and chronic obstructive pulmonary diseases, COPD) was also investigated. Results in terms of IVE against laboratory-confirmed influenza were available only with regard to patients suffering from asthma or COPD, and it was 45% and 70%, respectively, according to the results of one meta-analysis [26] and one systematic review [5] (Annex 2, Table 13).

6.3.8.2.1. Diabetic patients

One meta-analysis and one systematic review reported on IVE in diabetic patients.

In the meta-analysis by Remschmidt et al. 2015 [27], adults patients and elderly diabetic patients were considered.

Regarding adult diabetic patients (18-64 years), IVE against all-cause hospitalization was 58% (95% CI, 6–81%) from three case-control and one cohort study (N=93,472) (very low quality of the evidence according to GRADE criteria, I^2 = 77%. IVE against hospitalization due to influenza or pneumonia was 43% (95% CI, 28–54%); results deriving from one case-control study (N=91,605, low quality of the evidence). No statistically significant protective effects against ILI (one case-control study of low quality of the evidence) nor against all-cause mortality were observed (one case-control study of very low quality of the evidence).

In the elderly diabetic patients (65 years or older), VE against ILI was 13% (95% CI, 10–16%) in one case-control study (very low quality of the evidence). VE against all-cause hospitalization was 23% (95% CI, 1–40%) (results from two case-control studies of very low quality of the evidence, I2 = 94%). VE against hospitalization due to influenza or pneumonia was 45% (95% CI, 34–53%). VE against all-cause mortality was 38% (95% CI, 32–64%), from two cohort studies, whereas VE was 56% (95% CI, 47–64%), in two case control studies (heterogeneity of cohort studies: I2 = 0%; the evidence of case-control studies was judged to be of low quality; I2 = 0%). The review quality rating by Health Evidence was 8/10 (strong).

Dos Santos et al. 2018 [1] assessed immunogenicity, safety, and effectiveness of seasonal influenza vaccination in patients with diabetes mellitus. The systematic review identified 15 studies published between January 2000-March 2017 in PubMed, Embase and Cochrane Library. The search also considered non-specific outcome such as hospitalization or death and concluded that seasonal influenza vaccination reduced the risk of hospitalization and mortality in diabetic patients, particularly those aged ≥65 years (Annex 2, Table 13).

6.3.8.2.2. Patients with renal diseases

Remschmidt et al. 2014 [28] conducted a systematic review and meta-analysis to assess the IVE in patients with end-stage renal disease. Five observational studies and no randomized-controlled trial were identified. The body of evidence was considered very low for all outcomes according to GRADE. No study reported on laboratory-confirmed influenza virus infections. Results on other clinical outcomes considered are reported in Annex 2, Table 13.

6.3.8.2.3. Chronic respiratory diseases and other medical conditions

Hirve et al. 2016 [5] reported an IVE =70% against laboratory-confirmed influenza in COPD patients.

Vasileiou et al. 2017 [26], performed a systematic review and meta-analysis assessed pooled VE in 1,825 persons with asthma from two TND case-control studies [26]: IVE of all licensed vaccine (IIV3, and LAIV3) was 45% (95% CI 31%-56%) for laboratory-confirmed influenza (confirmation by RT-PCR). The quality of evidence was judged low, using the GRADE system. Based on their findings, authors concluded that influenza vaccination prevents influenza and other clinically important health outcomes in persons with asthma.

In the systematic-review by Bekkat-Berkani, et al. 2017 [29], the effectiveness of seasonal influenza vaccination in patients with COPD. Seventeen articles describing 13 different studies were found to be pertinent to this review. Results of four RCTs and one observational study demonstrate that seasonal influenza vaccination is immunogenic in patients with COPD. Two studies assessed the occurrence of COPD exacerbations 14 days after influenza vaccination and found no evidence of an increased risk of exacerbation. Three RCTs showed no significant difference in the occurrence of systemic effects between groups receiving influenza vaccine or placebo. Six out of seven studies on vaccine efficacy or effectiveness indicated long-term benefits of seasonal influenza vaccination, such as reduced number of exacerbations, reduced hospitalisations and outpatient visits, and decreased all-cause and respiratory mortality. According to authors, the evidence supports a positive benefit-risk ratio for seasonal influenza vaccination in patients with COPD and supports current vaccination recommendations in this population group (Annex 2, Table 13).

Michiels et al. 2011 [6] conducted a systematic review to assess the evidence regarding the efficacy, effectiveness and risks of the use of inactivated influenza vaccines in children, healthy adults, elderly individuals and individuals with co-morbidities such as diabetes, chronic lung disease, cardiovascular disease, kidney or liver disease and immune suppression. They showed that there is strikingly limited good-quality evidence (all GRADE B, C or not existing) of the effectiveness of influenza vaccination on complications such as pneumonia, hospitalisation and influenza-specific and overall mortality. They also revealed inconsistent results in studies among children younger than 6 years, individuals with COPD, institutionalized elderly (65 years or older), elderly with co-morbidities and healthcare workers in elderly homes, which can only be explained by bias of unknown origin. Results from the review are reported in Annex 2, Table 13. The review quality assessment by Health Evidence was 5/10 (moderate).

In summary, the overview on seasonal influenza vaccine effectiveness against laboratory-confirmed influenza revealed heterogeneous findings. While seasonal influenza vaccine has been reported as effective in preventing laboratory-confirmed influenza among healthy adults (16-65 years) and children (≥6 years), there is limited good-quality evidence of the effectiveness of influenza vaccination on specific populations such as immunocompromised or immunosuppressed patients. In addition, inconsistent results were found in individuals with COPD, institutionalized elderly (65 years or older), elderly with co-morbidities and healthcare workers in elderly homes, probably due to residual confounding or bias.

Researches focusing on the vaccination of pregnant women and or potential benefit transferred to their newborns constitute an area that deserves future investigation.

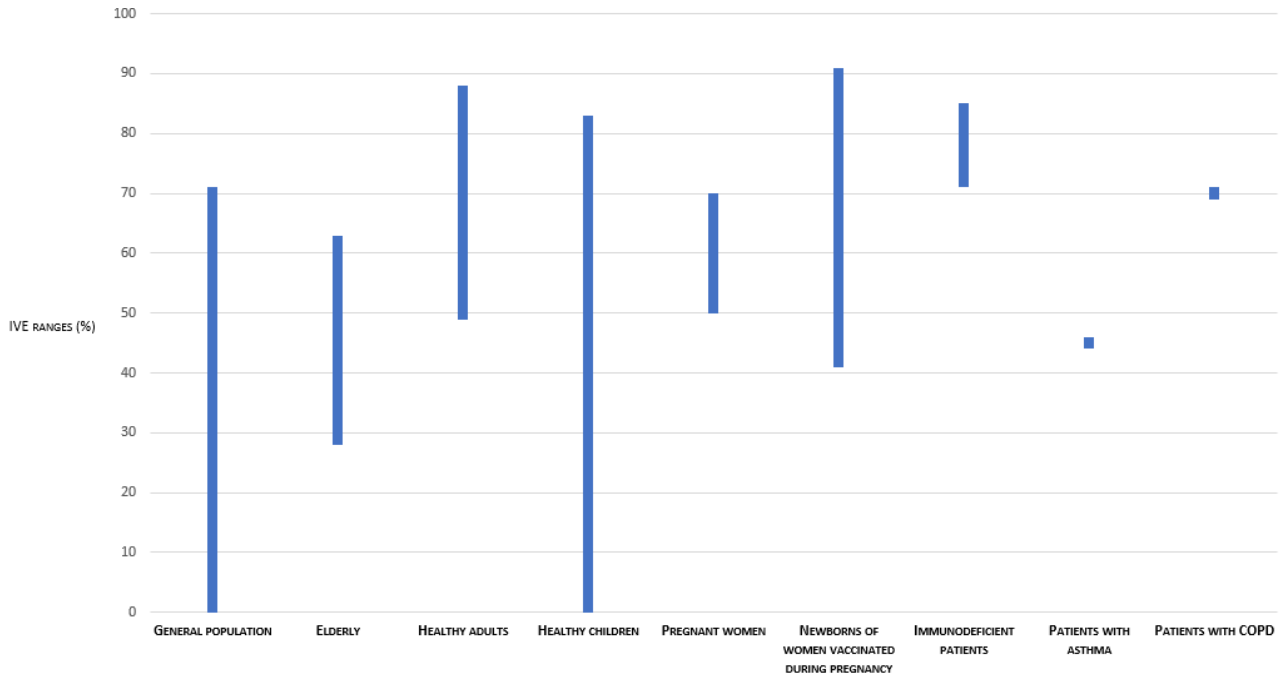
IVE against laboratory-confirmed influenza in the general population ranged from no impact to 71% (Figure 3). In the elderly it ranged from 28% (in case of mismatch) to 63% when the vaccine strains were similar to circulating viruses. The overall IVE of influenza vaccination against laboratory-confirmed among healthy adults varied between 49% and 59% (to 88% when considering health care workers). As for healthy children, IVE against lab-confirmed influenza ranged from no impact% to 83%; IVE of parenteral inactivated vaccines ranged from 46% to 72%; that of live attenuated vaccines from

no effect in certain seasons, due to reduced effectiveness against A(H1N1)pdm09 strains, to 83%.

Vaccinating pregnant women resulted in an IVE ranging from 50% to 70% in the mother and from 41% to 91% in the newborns.

As for patients with pre-existing medical conditions, IVE in immunocompromised patients varied from 71% to 85% in adults, whereas it appears to have no effect in children under 5 years of age, however this finding comes from only one RCT. IVE was 45% in asthma patients (only two TND studies investigated IVE in such population group), 70% in patients with COPD Figure 3).

Figure 3: IVE ranges per population group



The RT-PCR is considered the gold standard test for laboratory confirmation of influenza virus infection during acute illness, because of its higher sensitivity and specificity compared to other techniques. However, especially in observational studies, the diagnosis of confirmed influenza is often made through other test types, such as viral culture (another direct diagnostic test), or through serological method (an indirect diagnostic test). The use of methods with imperfect sensitivity and/or specificity may cause misclassification of the disease status, thus it may lead to biased VE estimates.

Several studies did not critically appraise the methodological quality of included studies. The set of criteria on the basis of whom the critical appraisal is conducted should be standardized, as they might have a significant effect on the risk of bias in the results reported and conclusions drawn.

No study assessed IVE against laboratory confirmed influenza in patients with cardiovascular diseases, diabetes, or kidney diseases. Further studies should be carried out with the aim to assess IVE in such population groups.

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7. RECOMMENDATIONS

This section contains the deliverable authors' recommendations based on the findings of the systematic review. The complexity of accurately and robustly evaluating influenza vaccine effectiveness has been illustrated with the multiple biases, confounders and effect modifiers that could impact the true estimate and thus ending with risk of erroneous interpretations which supports the importance of accurately consider those elements.

General framework

While not specific to influenza vaccination, the vaccine effectiveness research framework developed by Crowcroft et al. [1] is very informative in the context of IVE assessment (Table 4). They mentioned the importance to the following dimensions/criteria when performing vaccine effectiveness studies: characteristics of the vaccine recipient, vaccine features, timing, environment and pathogen, the outcomes of interest and the study design. These criteria are very much aligned with the findings from the systematic review and provide an additional pragmatic tool/framework to better define the study at the time of the design, implementation and analysis.

Table 4: Vaccine effectiveness framework adapted from [1]

Dimension/Criteria	Description
1. Characteristics of vaccine recipient	Vaccination status: Priming type of vaccine, boosting vaccine type, total number of previous doses, date of administration, time-period between doses _ Age at receipt, age at priming, age at boosting, age at time of study _ Co-administered vaccines and drugs _ Age at time of study, comorbidity, immune disorders, nutritional status, immune hypo-responsiveness, time of day of vaccination _ Previous history of infection _ For immunization in pregnancy, maternal characteristics and infant characteristics (e.g. prematurity) _ Socio-demographic characteristics – e.g., contact with young children, healthcare provider, social determinants of health
2. Vaccine	_ Each type of vaccine and formulation used for all priming and boosting doses _ Manufacturer for each vaccine dose, vaccine manufacturer lot numbers _ Mechanism of protection _ Natural history model of vaccine failure and protection _ Site, route, dose of vaccination
3. Time	_ When was the study conducted? Epidemic or non-epidemic period? Other secular trends? _ Time between doses _ Time since vaccination (waning) _ How are age and time-varying covariates being assessed?
4. Environment and exposure to pathogen	_ Indirect/herd effects _ Intensity of exposure to the pathogen, setting of exposure

	_ Comparability of likely exposure to infectious pathogen in cases and controls _ Boosting effects of circulating pathogens
5. Pathogen	_ What infection is being prevented? o Strain match _ Laboratory diagnostic methods used to identify cases (culture, PCR) _ Vaccine-driven evolution e.g., vaccine strain _ Natural history of infection and protection/immunity _ Co-infection
6. Outcomes of interest	_ What are the goals and objectives of the program? _ What is the target group – whole population or individual protection of high risk groups? _ Which outcomes are being studied? o Asymptomatic o Typical disease, atypical disease, reinfection, mild infection, severe infection, deaths o Direct impact in the vaccinated individual or indirect effects? o Impact on infectiousness and transmission o Protection of newborn (maternal immunization) _ What is included in the case definition(s)?
7. Study Design considerations	_ Choosing appropriate controls. _ Study design and analysis should also consider a wide range of elements including case ascertainment, sensitivity and specificity of case definition, potential bias and confounding including health care seeking behaviour, social determinants, exposure to prophylaxis, diagnostic method (culture, PCR), infectiousness, and risk of misclassification

Additionally, Simonsen et al. (Table 5) provided a framework that can be useful to detect residual bias. They proposed some recommendations to account for those biases in the context of influenza VE assessment [2]. The authors consider that if any of the listed expectations are not met, unadjusted bias should be strongly suspected.

Table 5: Framework to detect residual bias in cohort studies of elderly people. Adapted from [2]

Framework criteria	Setting of greater expected RR reduction	Setting of lower expected RR reduction	Expectations when applying framework criteria
Seasonality	Influenza period	Pre-influenza periods	Expect no difference in risk (RR=1·0) during pre-influenza periods between the vaccinated and unvaccinated groups
Vaccine match	Well-matched seasons	Mismatched seasons	Expect the measured RR reduction to be least pronounced for seasons when the vaccine components were severely mismatched relative to circulating strains, and to be most pronounced for well-matched seasons
Severity	Severe seasons	Mild seasons	Expect the measured RR reduction to be least pronounced for seasons with low national excess mortality and most pronounced for severe seasons with high excess mortality
Age	Younger people	Older people	Expect the RR reduction measured in the oldest groups of elderly people to be less pronounced than that of younger age-groups, because of immune senescence
Endpoints specificity	High-specificity endpoints	Low-specificity endpoints	Expect the measured RR to be most pronounced for clinical endpoints with higher specificity, and less pronounced for low-specificity outcomes
In the absence of selection bias, for each framework criterion there are defined settings in which the reduction in risk ratio (RR) is expected to be than in other settings.			

Simonsen et al., [2] concluded that, at a minimum, observational studies should make every effort to use the most specific endpoints available, and to identify the epidemic period for each season by use of virus surveillance data, rather than a standard 4-month period in winter. Beyond that, they state a commonly agreed set of standards for carrying out and reporting observational studies of influenza vaccine effectiveness would be very helpful.

Besides these useful overviews, other recommendations arising from the reviewed literature are presented below.

Misclassification

To avoid misclassification bias, complete and reliable sources of vaccination information should be used to determine the vaccination status of study subjects. Medical records, prescribing information and vaccination registries are preferable to self-reporting. Laboratory confirmation of influenza should ideally be carried out by RT-PCR or viral culture and be performed by trained staff. It is also important to consider the time between the symptom onset and influenza testing.

A tool developed by De Smedt et al. [3] may help researchers to evaluate, during study design phase, the magnitude and direction of the bias when estimating VE based on potentially misclassified data and

thus account for the caveats during the analysis using existing methods in pharmacoepidemiology [4]. The application by De Smedt et al. can be found at http://www.advance-vaccines.eu/ or at http://apps.p-95.com/VEMisclassification/. This model allowed to test different scenarios and showed that decreased exposure specificity (poorer identification of non-vaccinees) had greatest impact for influenza VE estimation and noted that exposure misclassification had a larger impact compared to disease misclassification. Additional methods can be also considered under specific assumptions, such as others probabilistic bias analyses [5], Bayesian bias analyses [6], modified maximum likelihood methods [7] and imputation-like methods [8;9].

Frailty bias, healthy vaccine bias and confounding by indication

Frailty bias and healthy vaccinee bias or confounding by indication have shown to substantially impact the accuracy of IVE in either direction if not fully accounted for and accurately measured. Recommendations have been made to preferentially use specific outcomes and perform analysis per calendar time using the virus surveillance data to identify the epidemic period for each season. Several works emphasized the importance of accounting for frailty using standardized tools. For instance, the Frailty Index, which measures the degree to which a person is frail, relates to the accumulation of deficits in all aspects of health and functional status [10], and predicts mortality risk as well as the risk of health care use and changes in health status [11;12]. Calculation of the Frailty Index incorporates the presence and severity of the multiple chronic conditions and functional status and has been shown to be a better predictor of overall health status compared to the type or number of chronic diseases, or self-report of fatigue or balance problems; frailty measures are thus beginning to be incorporated into vaccination studies [13]. Frailty accelerates immunosenescence although the impact of frailty on immune response specific to influenza vaccine among older adults varies [14]. In this context, using the Frailty Index to capture reliably the health condition of participants should be favoured over individual characteristics such as comorbidities, or dependency in ADLs as a proxy for frailty, considered as incomplete [15].

Useful frameworks to capture confounding by indication or healthy vaccinee bias in the context of influenza VE assessment have been identified during the search [16], summarized in Table 6.

Table 6: Conceptual framework: Indicators and conclusions for presence of confounding by indication and healthy vaccinee bias in influenza vaccine effectiveness [16]

Indicator	Conclusion	References
Vaccinated study participants have a higher proportion of comorbidities than unvaccinated study participants, as indicated by baseline characteristics	High risk of confounding by indication in the unadjusted data set	[17;18]
Vaccinated study participants have a lower proportion of comorbidities than unvaccinated study participants, as indicated by baseline characteristics	High risk of healthy vaccinee bias in the unadjusted data set	[19;20]
Inclusion of comorbidities in the regression model increases vaccine effectiveness	Confounding by indication has led to underestimation of vaccine effectiveness in the unadjusted data set	[21]
Inclusion of comorbidities in the regression model decreases vaccine effectiveness	Healthy vaccinee bias has led to overestimation of vaccine effectiveness in the unadjusted data set	[21]
Significant effects of influenza vaccination appear outside the influenza season ("off-season estimates"), despite adjustment for comorbidities	Residual confounding by healthy vaccinee bias	[20;22;23]

Hak and collaborators [17] studied confounding by indication in observational studies in the context of prevention of influenza complications. They summarised methods to reduce confounding by indication and highlighted 3 statistical methods that are usually used for adjustments:

1. Statistical control of confounding factors in multivariable regression model [24;25]

2. Sub-classification of patients on levels of the propensity score [26-28]

3. Pseudo-randomisation on levels of instrumental variables [29].

Covariates: confounders and effect modifiers

We have listed covariates reported as confounders or effect modifiers based on the literature review. While some of them may act either as confounders or effect modifiers, the classification has been based on the more frequently reported findings. Ethnicity/race has been inconsistently reported as potential confounder, depending on the geographic location of the study/population of interest and appeared more frequently adjusted for in IVE studies conducted in North America.

Age is commonly considered as a confounder and adjusted for in the univariate or multivariate models. In some studies, this has been handled as an effect modifier, especially when the population considered included broad age ranges. Considering that the immune system of children is evolving and not fully mature in younger age groups, and that older adults experience increase frailty and immunosenescence [30], it is favoured to considered stratifying the results by age groups to better account for the vaccine recipients' features. The vaccine formulation including the volume of antigen, the number of doses administered or the presence or absence of adjuvant, potential co-administrations are also important elements to obtain in order to more confidently interpret the findings.

Other critical aspects to account encompass the time dimension, the environment, virus circulation, and recruitment of study subjects

It is acknowledged that IVE varies with time and the two important time-varying components, calendar time and time since vaccination, need to be handled with caution [31]. Influenza virus circulation are dynamic and might impact the likelihood of being exposed to the virus. To safeguard that cases and controls have the same probability of being in contact with the virus (i.e., whether or not it was an outbreak period), analyses should control for calendar time. In addition, another critical element relates to time since vaccination reflecting the duration of protection [32;33]; The current evidence does not allow to determine confidently the potential impact of repeated vaccination or confounding by natural infection. In addition, it is uncertain whether sex/gender should be systematically considered as an effect modifier because of differences in biological mechanisms that mitigate infection, or because of variability in behaviour that lead to different propensities to seek care and adhere to vaccination programs.

We concur with the importance to further investigate those aspects and thus more formally investigate such effects in IVE studies. In order to better understand how vaccines perform, it also favoured to accurately capture and investigate several types of outcome of interest (i.e., medically attended influenza, asymptomatic infection, severe influenza, influenza-related death) and adjust the design and settings accordingly. In subject recruitment, systematic or randomized sampling is preferred to routine clinical testing which may introduce bias.

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8. DISCUSSION & CONCLUSIONS

The importance of influenza vaccines has been recognized by numerous health authorities worldwide, especially in specific populations at high risk of influenza complications; nearly all national immunization programmes prioritize older adults [1;2]. In this context, estimates of influenza vaccine efficacy established through randomized, placebo-controlled trials are no longer considered ethical in light of these near-universal recommendations as the standard of care for older adults. Few placebo-controlled RCTs of influenza vaccine efficacy in elderly people have been done, and none have been powered to study severe outcomes, including mortality [1-3]. The scarcity of gold-standard RCT data places greater weight on evidence from cohort and other observational studies and supports the importance of identifying and controlling as efficiently as possible for confounding and bias.

Studies conducted in the 1990s provided estimates of vaccine effectiveness of 30%–40% for all-cause hospitalizations and all-cause mortality in community-dwelling older adults but were heavily criticized because influenza could not be responsible for a high proportion of hospitalizations and deaths [4;5], with some studies even questioning the benefit of influenza vaccination in older adults [6;7].

Methodologic differences in selecting the control period for these observational studies, the lack of specificity of the case definition, and the adjustment for functional status and life expectancy led to very heterogenous findings ranging from no mortality benefit [6-11] to varying degrees of benefit related to the complications of influenza disease [12-15]. The "healthy vaccinee" bias [5] was considered to be a major factor for inflating the benefit of vaccination in older adults, wherein those who were at highest risk of death during the winter were least likely to be vaccinated [6].

In accounting appropriately for bias and confounding in observational studies there are critical challenges that necessitate careful attention. The objective of this work was to screen the literature and extract the relevant information from multiple sources with the ultimate intent to inform the design and implementation of influenza vaccine effectiveness studies and limit/preclude as much as possible the possibility to generate biased estimates that would ultimately lead to erroneous conclusions.

Selection bias is among the most frequently reported bias. Even if vaccination does not always prevent the disease, it may reduce the disease severity to the point that the individual chooses not to seek medical care. Consequently, VE against medically-attended influenza vs. any symptomatic influenza may not be the same which needs to be accounted for when interpreting the results. There may be inherent differences between vaccinated and non-vaccinated people in that they are not equally likely to seek medical care when faced with a respiratory infection. In TND studies, it is important that sampling is done according to a systematic protocol and not at clinician's discretion, as the latter could result in differential diagnostic testing. Frailty bias and healthy vaccinee bias or confounding by indication have shown to substantially impact the accuracy of IVE in either direction, if not fully accounted for and accurately measured. Recommendations have been made to preferentially use specific outcomes and perform analysis per calendar time using the virus surveillance data to identify the epidemic period for each season. Useful framework to detect residual bias [16] or to capture confounding by indication [17] in the context of influenza VE assessment have been identified during the search.

The effect of repeated vaccination shows conflicting results with variation of the impact of VE; observed reductions in VE have been primarily associated with outbreaks of A(H3N2) infection. The antigenic distances hypothesis between past and current vaccine antigens and the viruses that circulate has been proposed by Smith et al. to explain part of those variations [18].

Misclassification bias has been reported as an important source likely contributing to biased IVE estimates. This bias is expected to be reduced by using accurate and complete sources of vaccination information, highly specific and sensitive outcomes (e.g. influenza infection confirmed by RT-PCR) and

by accounting for the seasonality of influenza. Study protocols should ensure that the vaccinated and unvaccinated groups are treated equally in the study process. These measures should be implemented in all studies where applicable. Although the findings were somewhat conflicting, the choice of study design does not appear to play a major role in the misclassification bias.

Additional findings showed that the study design could also impact the true IVE. Several publications discuss the bias of VE estimates from TND studies; however, they do not provide numerical evaluations of the magnitude and direction of the bias under realistic conditions. The choice of controls in TND studies has been reported as having limited impact on the VE estimate. Comparing different study designs demonstrated the importance of well designing the different studies and well defining the objective to use the appropriate setting.

Confounders and effect modifiers are important factors to account for in the context of IVE to generate accurate and robust estimates. The approach to include such covariates varies depending on studies. Some authors choose to define the included covariates a priori, based on biological plausibility or using a causal diagram while others use statistical significance as factor to determine relevance for inclusion in the model, such as through minimal change in the OR due to a specific covariate.

It is difficult to specifically determine whether a factor should be considered as a confounder, an effect modifier, or both at the same time in a specific study. Effect modification has been explicitly reported by a handful of studies, although several mentioned the need to stratify on several identified factors. The level of match between the vaccine component and the circulating strains, the full vs. partial vaccination effect, or intra-seasonal waning effectiveness are potential factors acting as effect modifiers.

Even though antigenic mismatch between the vaccine virus and the circulating strain is clearly responsible for modifying the effect size of the VE estimate, it involves a true effect and is mostly only qualitatively addressed in IVE studies. Potential solutions to get a better insight in how it affects the estimate is by stratification on strain-level or by calendar time within the season. There are different methods described in the literature on how the assessment for match or mismatch is made. The variable can be described as binary (yes/no), categorical (mild/moderate/severe) or continuous (proportion of samples that match) or as a score aiming to account for the degree of mismatch.

Regarding waning immunity, it can be concluded that intra-seasonal waning effectiveness is often observed in IVE studies and the effect can be quantified by time since vaccination or using early vs. late season IVE estimates. To better understand the root cause of the waning effectiveness over the season (e.g. waning immunity or antigenic drift) it is suggested to combine both measurements to see which effect is larger.

The present work has identified biases, confounders and effect modifiers relevant in the context of IVE assessment. We considered also relevant to provide an overview of the variability of IVE depending on the population of interest, to underline the need to carefully define the objective of the research and accounting for the population's characteristics.

The overview on seasonal influenza vaccine effectiveness against laboratory-confirmed influenza revealed substantial variability. While seasonal influenza vaccine has been reported as effective in preventing laboratory-confirmed influenza among healthy adults (16-65 years) and children (≥6 years), there is limited high-quality evidence of the effectiveness of influenza vaccination on specific populations such as immunocompromised or immunosuppressed patients. In addition, inconsistent results were found in individuals with COPD, institutionalized elderly (65 years or older), elderly with co-morbidities and healthcare workers in elderly homes, probably due to residual confounding or bias. Researches focusing on the vaccination of pregnant women and or potential benefit transferred to their newborns constitute an area that deserves future investigation.

IVE against laboratory-confirmed influenza in the general population ranged from no impact to 71%. In

the elderly it ranged from 28% (in case of mismatch) to 63% when the vaccine strains were similar to circulating viruses. The overall IVE of influenza vaccination against laboratory-confirmed among healthy adults varied between 49% and 59% (to 88% when considering health care workers). As for healthy children, IVE against lab-confirmed influenza ranged from no impact to 83%; IVE of parenteral inactivated vaccines ranged from 46% to 72%; that of live attenuated vaccines from no effect in certain seasons, due to reduced effectiveness against A(H1N1)pdm09 strains, to 83%. Vaccinating pregnant women resulted in an IVE ranging from 50% to 70% in the mother and from 41% to 91% in the newborns. As for patients with pre-existing medical conditions, IVE in immunocompromised patients varied from 71% to 85% in adults, whereas it appears to have no effect in children under 5 years of age, however this finding comes from only one RCT. IVE was 45% in asthma patients (only two TND studies investigated IVE in such population group), and 70% in patients with COPD.

The work presents several limitations. Due to the qualitative nature of the systematic review, no formal quality assessment of the included studies was performed. This review focused on IVE in first instance; it cannot be ruled out that some references, especially those not specific to influenza but potentially transferable to IVE or which did not explicitly report bias, confounders or effect modifiers have been disregarded.

Nevertheless, this work has several strengths. It provides an extensive picture of relevant factors that can potentially impact the accuracy/robustness of the IVE assessment with pragmatic insights to account for them. As such it provides a useful tool to inform the design and implementation of IVE studies and complements existing guidelines and technical reports. Pragmatic recommendations have been provided to detect biases such as frailty, healthy vaccinee bias with some insights to further verify whether residual confounding was still present in the final analyses. Furthermore, the present work provided an overview of the potential variability of vaccine effectiveness depending on the population considered for inclusion, with regards to age, pre-existing medical conditions and high-risk status.

In conclusion, it is acknowledged that accurate assessment of IVE is a complex endeavour considering the plethora of factors that could bias the results or complicate their interpretation. The substantial variability exemplified by the summary of IVE studies by study population supports the necessity to carefully account for the population of interest to detect potential sources of error, with a specific attention to the age of vaccine recipients, the health status of the population considered, including an assessment using standardized tools (e.g., frailty index); considering also that the approach to conduct the study will depend on the pre-defined question(s) to be addressed.

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10. ANNEX 1: STUDIES EXCLUDED FROM SUMMARY OF DATA FROM META ANALYSES/SYSTEMATIC REVIEWS ON IVE, WITH REASONS FOR EXCLUSION

1. M. Goeijenbier *et al.* "Benefits of flu vaccination for persons with diabetes mellitus: A review," *Vaccine*, vol. 35, no. 38, pp. 5095–5101, Sep. 2017.

   - Results included in C. Remschmidt, O. Wichmann, and T. Harder, "Vaccines for the prevention of seasonal influenza in patients with diabetes: systematic review and meta-analysis," BMC Med., vol. 13, no. 1, p. 53, Dec. 2015 [27] ;

2. Bozat Emre et al. "Effectiveness of seasonal influenza vaccines against influenza A (H1N1) infection in post-pandemic seasons: A systematic review". Pharmacoepidemiology and Drug Safety - Volume 24, Issue 0, pp. 178-179, Jan 2015

   - Only the abstract was available

3. Cates CJ, Rowe BH. Vaccines for preventing influenza in people with asthma. Cochrane Database Syst Rev. 2013 Feb 28;(2):CD000364.

   - Findings included in E. Vasileiou, A. Sheikh, C. Butler, K. El Ferkh, B. von Wissmann, J. McMenamin, L. Ritchie, J. Schwarze, N. G. Papadopoulos, S. L. Johnston, L. Tian, and C. R. Simpson, "Effectiveness of Influenza Vaccines in Asthma: A Systematic Review and Meta-Analysis," Clin. Infect. Dis., vol. 65, no. 8, pp. 1388–1395, 2017.

4. E. Hak *et al.* "Clinical effectiveness of influenza vaccination in persons younger than 65 years with high-risk medical conditions: the PRISMA study.," *Arch. Intern. Med.*, vol. 165, no. 3, pp. 274–80, Feb. 2005.

   - Wrong publication type (case-control study)

5. T. Jefferson, C. Di Pietrantonj, L. A. Al-Ansary, E. Ferroni, S. Thorning, and R. E. Thomas, "Vaccines for preventing influenza in the elderly.," *Cochrane database Syst. Rev.*, no. 2, p. CD004876, Feb. 2010.

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6. Jefferson, S. Smith, V. Demicheli, A. Harnden, A. Rivetti, and C. Di Pietrantonj, "Assessment of the efficacy and effectiveness of influenza vaccines in healthy children: systematic review.," *Lancet (London, England)*, vol. 365, no. 9461, pp. 773–80

   - Updated in T. Jefferson, A. Rivetti, C. Di Pietrantonj, and V. Demicheli, "Vaccines for preventing influenza in healthy children," *Cochrane Database Syst. Rev.*, Feb. 2018. [19]

7. Demicheli V, Jefferson T, Al-Ansary LA, Ferroni E, Rivetti A, Di Pietrantonj C. Vaccines for preventing influenza in healthy adults. Cochrane Database Syst Rev. 2014 Mar 13;(3):CD001269. doi: 10.1002/14651858.CD001269.pub5.

   - Updated in: Demicheli V, Jefferson T, Ferroni E, Rivetti A, Di Pietrantonj C. Vaccines for preventing influenza in healthy adults. Cochrane Database Syst Rev. 2018 Feb 1;2:CD001269

8. McMillan et al. 2014 "Influenza vaccination during pregnancy: A systematic review of effectiveness and safety"

- The full-text could not be retrieved

9. E. Negri, C. Colombo, L. Giordano, N. Groth, G. Apolone, and C. La Vecchia, "Influenza vaccine in healthy children: a meta-analysis," *Vaccine*, vol. 23, no. 22, pp. 2851–2861, Apr. 2005.

   - Results included in L. Manzoli, J. P. A. Ioannidis, M. E. Flacco, C. De Vito, and P. Villari, "Effectiveness and harms of seasonal and pandemic influenza vaccines in children, adults and elderly," Hum. Vaccin. Immunother., vol. 8, no. 7, pp. 851–862, Jul. 2012. [8];

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11. L. C. Ramsay, S. A. Buchan, R. G. Stirling, B. J. Cowling, S. Feng, J. C. Kwong, and B. F. Warshawsky, "The impact of repeated vaccination on influenza vaccine effectiveness: a systematic review and meta-analysis," BMC Med., vol. 15, no. 1, p. 159, Dec. 2017.

    - The authors have retracted this article; retraction note published in BMC Med. 2018 Aug 12;16(1):133. doi: 10.1186/s12916-018-1137-0.

12. Vasileiou et al. "Safety and effectiveness of influenza vaccines in people with asthma: A systematic review and meta-analysis"

    - Wrong publication type (poster)

13. Vasileiou et al. "Effectiveness of influenza vaccines in Asthma: A systematic review and meta-analysis": wrong publication type

    - Wrong publication type (protocol)

14. Vasileiou et al. "Seasonal influenza vaccine effectiveness in people with asthma: A systematic review"

    - Updated in 2017 in [26];

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16. K. Wilkinson et al. "Efficacy and safety of high-dose influenza vaccine in elderly adults: A systematic review and meta-analysis," Vaccine, vol. 35, no. 21, pp. 2775–2780, May 2017

    - It compared the effects of high-dose (Fluzone High-Dose vaccine, Sanofi Pasteur) VS standard-dose.

11. ANNEX 2 TABULATED SUMMARY OF DATA FROM META ANALYSES/SYSTEMATIC REVIEWS ON IVE

Table 7: Main findings from selected systematic reviews and meta-analysis on IVE in the general population

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
Bartoszko 2018 (MA)	World-wide	All ages	1946-2017	IIV, LAIV or other (e.g. high-dose vaccine)	Lab-confirmed flu, tested by PCR or viral culture as the primary outcome	RCT showed VE 71% (95% CI 62–78%) when participants were vaccinated in 2 consecutive seasons VS VE=58%, 95% CI 48-66% for those vaccinated in current season (OR: 0.88, 95% CI 0.62-1.26, p=0.49, I^2=39%) Observ. studies: VE for 2 consecutive seasons 41%, 95% CI 30–51% ; VE for current season 47%, 95% CI 39–54%; (OR 1.14, 95% CI 0.98–1.32, p = 0.09, I2 = 63%).	5 RCTs involving 11,987 participants; 28 observational studies involving 28,627 participants
Rondy 2017 (MA)	Europe (11 studies), North America (6 studies), Oceania (10 studies), Asia (3 studies)	Adults of all ages (≥ 18 years)	seasons 2009–10 through 2015–16	Seasonal trivalent vaccine (27 studies) Pandemic monovalent (3 studies)	Pooled seasonal IVE against lab-confirmed influenza-associated hospitalizations	IVE 41% (95%CI:34;48) for any influenza (pooled season-specific seasonal IVE estimates against any influenza viruses in all adults ranged between 31% in 2011–12 and 2014–15 and 53% in 2013–14. Summary monovalent pandemic IVE against influenza A(H1N1)pdm09 hospitalization in 2009–10 was 72% (95%CI: 22;100)	24 TND studies through 6 seasons
		Adults of all ages			Lab-confirmed influenza-associated hospitalization - influenza	48% (95%CI:37;59)	7 TND studies through four seasons

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
					A(H1N1)pdm09		
		Adults of all ages			Lab-confirmed influenza-associated hospitalization - influenza A(H3N2)	37% (95%CI:24;50)	9 TND studies through four seasons
		Adults of all ages			Lab-confirmed influenza-associated hospitalization - influenza B	38% (95%CI:23;53)	5 TND studies through four seasons
Li 2015 (MA)	Worldwide	Any age	2007-08 to 2009-10	seasonal influenza vaccination	lab-confirmed A (H1N1) influenza *	**RCTs** showed an insignificant RR increase of 27% (RR = 1.27; 95% CI: 0.46, 3.53; P = 0.64). From **cohort studies** non-significant results were reported. In the **case-control studies,** a slight risk reduction in risk was observed (OR: 0.80; 95% CI: 0.61, 1.05; P = 0.11).	4 RCTs; 2 cohort; 16 case-control studies
					ILI	From **RCTs** results, a significant 9% risk reduction (RR = 0.91; 95% CI: 0.84, 0.99; P=D 0.02) was detected. From **cohort** studies nonsignificant results were reported	

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
Yin 2012 (MA)	USA, UK, Australia, Mexico, Canada, China, and Europe	Any age	Seasons 2007 to 2008, 2008 to 2009, or 2009 to 2010 in the northern hemisphere, and 2008 or 2009 in the southern hemi-sphere	trivalent influenza vaccines	Laboratory-confirmed infection**, influenza-like illness, sickness absence, and acute respiratory illness	Seasonal influenza vaccination was not associated with statistically less confirmed influenza A H1N1 2009 illness (OR 0.81, 95% CI 0.58 to 1.13; I^2=94%; 13 case-control studies). Sensitivity analysis excluding high- and moderate-risk studies made the results significant in favour of vaccine (OR 0.66, 95% CI 0.48 to 0.91; I^2=91%; eight case-control studies). Sensitivity analysis further excluding studies with recruitment early in the pandemic was also statistically significant in favour of vaccine (OR 0.49, 95% CI 0.43 to 0.55; I^2=0; five case-control studies). Results in individual RCTs and cohort studies showed mixed effects.	See IVE
				Pandemic vaccine		Compared with no vaccination, pandemic influenza vaccination was associated with statistically less confirmed influenza A H1N1 2009 illness (OR 0.14, 95% CI 0.07 to 0.27; I^2=81%; 11 case-control studies). Sensitivity analysis excluding high- and moderate-risk studies was significantly in favour of vaccine (OR 0.15, 95% CI 0.08 to 0.28; I^2=45%; six case-control studies).	See IVE

MA= meta-analysis; SR= systematic review; * diagnosed through serological method or RT-PCR; ** diagnosed through RT-PCR and/or culture; ç Not clear which tests were used to make diagnosis of influenza infection

Table 8: Main findings from selected systematic reviews and meta-analysis on IVE among the elderly

Authors/ Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
Demicheli 2018(MA) /Worldwide	≥ 65	1966 to 31/12/2016	Any influenza vaccine given independently, in any dose, preparation, or time schedule, compared with placebo or with no intervention	Lab confirmed flu *	IVE 58% (RR 0.42, 95% CI 0.27 to 0.66)	3 RCTs
				ILI	RR0.59 (0.47-0.73) Overall IVE: 23% (6%to 36%; RR 0.77, 95%CI 0.64 to 0.94) when vaccine matching was good and not significantly different from no vaccination (RR 0.80, 95%CI 0.60 to 1.05) when matching was poor /unknown.	4RCTs (6894 participants)
				All deaths	RR 1.02 (0.11-9.72)	1 RCT (699 participants)
				Fever	RR 1.57 (0.92-2.71)	3 RCTs (2519)
				Nausea	RR 1.75 (0.74-4.12)	1 RCT (672)
Rondy 2017 (MA)/ Europe (11 studies), North America (6 studies), Oceania (10 studies), Asia (3 studies)	≥65 years	seasons 2009–10 through 2015–16	Seasonal trivalent vaccine (27 studies) Pandemic monovalent (3 studies)	Pooled IVE	37% (95%CI:30;44)	
				Summary IVE against influenza A(H1N1)pdm09 viruses	54% (95%CI: 26;82)	
				Summary IVE against influenza A(H3N2) viruses	33% (95%CI: 21;45)	
				Summary IVE against influenza B	31% (95%CI: 11;51)	

Authors/ Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
Domnich 2016 (MA)/ Worldwide	≥60/65	01/01/1990 - 26/04/2016	MF59-TIV	Hospitalization due to pneumonia/influenza	IVE 51% (95% CI: 39–61%) among community-dwelling seniors	4 case-control studies
			MF59-TIV	Lab confirmed^	IVE 60.1% (95% CI 1.3 to 84.3%)	1 prospective case-control study in five hospitals, in Valencia, Spain (826 patients) and 1 prospective community-based case-control enrolling 282 eligible participants (84 cases)
			MF59-TIV	ILI	Unadjusted IVE 94% (95% CI 47–100%] among institutionalized elderly.	1 Prospective study conducted in long-term care facilities in Italy (N=3173)

Authors/ Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
Hirve 2016 (SR)/ Tropics and subtropics (excluding Australia)		1993-2014	N. A.	Laboratory-confirmed flu	IVE ranged from 43% to 58%	4 RCT, 2 non randomized controlled trials, 7 cohort studies, 4 case–controls and 4 ecological
Chan 2014(MA)/ Worldwide	≥ 60	1946-June 2013	N. A.	Lab confirmed	Not performed	11 observational studies (6 retrospective and 5 prospective): 11,262 subjects included
				ILI	OR 0.79, CI 0.61-1.03; p=0.86	
				Pneumonia	IVE:37%, 95% CI 18%-53%, p=0.001	
				Death due to pneumonia or influenza	VE: 34%, 95% CI 10%-53%, p=0.01	
Darvishian 2014 (MA)/ Spain, Germany, Lithuania, Australia, New Zealand, Taiwan, South Africa, Japan	community-dwelling people ≥ 60 years	Up to July 13, 2014	N.A.	Lab confirmed**	IVE during sporadic activity: OR 0.69 (95% CI 0.48-0.99) when the vaccine matched. Vaccination was significantly effective during regional (match: OR 0·42, 95% CI 0·30–0·60; mismatch: OR 0·57, 95% CI 0·41–0·79) and widespread (match: 0·54, 0·46–0·62; mismatch: OR 0·72, 95% CI 0·60–0·85) outbreaks.	35 TND studies (4,975 subjects)

Authors/ Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
Manzoli 2012 (MA)/ Worldwide	Elderly		Parenteral inactivated	Lab- confirmed	IVE 41%-63%	2 meta-analyses (Jefferson, Osterholm)
				Clinically- confirmed	IVE 26%-56%	3 meta-analyses (Jefferson, Gross, Vu)
				Hospitalization for influenza or pneumonia	IVE 27%-48%	3 meta-analyses (Jefferson, Gross, Vu)
				Mortality for any cause	IVE -2%-68%	3 meta-analyses (Jefferson, Gross, Vu)
Michiels 2011(SR)/ Worldwide	≥65 years	2006-2011	Trivalent inactivated vaccines	IVE	41%	Cochrane systematic review by Jefferson et al
				Pneumonia	IVE 41%	cohort studies
				Hospitalisation for influenza or pneumonia	50%	
				hospitalisation for influenza or pneumonia,	26%	
				specific mortality from influenza or pneumonia	8%	
				Overall mortality	61%	
				Overall IVE	24%	
				Pneumonia	47%	
				Hospitalisation for influenza or	49%	

Authors/ Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
				pneumonia		
				Mortality from influenza or pneumonia	54%	
				Overall mortality	60%	

MA= meta-analysis; SR= systematic review; ^RT-PCR or culture; *viral isolation; ** Flu confirmed by at least 1 of the following: culture, rapid antigen testing, fluorescent antibody assays, HI tests or PCR; ç Not clear which tests were used to make diagnosis of influenza infection

Table 9: Main findings from selected systematic reviews and meta-analysis on IVE among healthy adults, including healthcare workers

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
IMAI 2018 (MA)	Worldwide	Healthcare workers	1980-2018	Seasonal influenza vaccination	Lab-confirmed	Pooled effect among observational studies: RR = 0.50, 95% CI; 0.33-0.76 Similarly, in the RCT RR = 0.12 (95% CI; 0.04-0.41). The overall pooled RR across the all four studies was 0.40 (95% CI; 0.23-0.69) (high heterogeneity in the measured effects between the RCT and a group of the observational studies: *I*2 = 79%, p = 0.03; no significant heterogeneity among the observational studies: I2 = 0%, p = 0.45). In an updated subgroup analysis there was no significant difference between two non-serology groups, but when each non-serology group was compared with the serology group they were heterogeneous (vs. RIDT: I2 = 76%, p = 0.04; vs. RT-PCR: I2 = 74.2%, p = 0.05). Serological	3 observational studies and 1 RTC (1,464 HCWs totally enrolled). Self-reported data through surveys commonly used, therefore the risks of bias in ascertainment of exposure and assessment of outcome were assessed as potentially high. The quality of the observational studies was moderate assessed: 3 studies were classified as high quality, 5 studies were moderate, and 2 studies were low

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
						testing showed a much stronger preventative effect of influenza vaccine (pooled RR = 0.20, 95% CI: 0.09-0.44) than RIDT (pooled RR = 0.56, 95% CI: 0.31-0.99) and RT-PCR (RR = 0.59, CI: 0.28-1.24).	quality. In the RCTs, the risk of selection bias was the most uncertain of all biases as there was a lack of information regarding randomization procedures in 2 of the 3 studies. There was 1 study each at high risk for performance and attrition bias. Other biases were considered at highest risk due to the potential of bias from self-reporting and differential recall in 2 studies.
					ILI	Pooled effect among the 5 studies: RR = 1.07, 95%CI; 0.95-1.20. No significant heterogeneities existing within (observational studies; $I2$ = 4%, p = 0.37) and between study designs	4 observational studies and 1 RCT (1578 HCWs totally enrolled). The quality of the

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
						(*I*2 = 0%, p = 0.98).	observational studies was moderate assessed. In the RCT, the risk of selection bias was the most uncertain of all biases
					Incidence of absenteeism due to ILI	Pooled RR = 0.62, 95%CI; 0.45-0.85	5 observational studies and 1 RCT (8,073 HCWs totally enrolled)
Demicheli 2018 (MA)	Worldwide	16 to 65	1966-31/12/2016	Inactivated parenteral	Lab confirmed*	IVE 59%: RR 0.41 (0.36-0.47)	25 RCTs (71,221 subjects)
					ILI	IVE 16%: RR 0.84 (0.75-0.95)	16 RCTs (25,795 subjects)
					Hospitalizations	RR 0.96 (0.85-1.08)	3 RCTs (11,924 subjects)
					Fever	RR 1.55 (1.26-1.91)	13 RCTs (23,850 subjects)

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
					Nausea or vomiting	RR 1.80 (0.65-5.04)	4 RCTs (6,315 subjects)
Rondy 2017 (MA)	Europe (11 studies), North America (6 studies), Oceania (10 studies, Asia 3 studies)	Adults of all ages	seasons 2009–10 through 2015–16	Seasonal trivalent vaccine (27 studies) Pandemic monovalent (3 studies)	pooled seasonal IVE against lab-confirmed influenza-associated hospitalizations ç	IVE 41% (95%CI:34;48) for any influenza (Pooled season-specific seasonal IVE estimates against any influenza viruses in all adults ranged between 31% in 2011–12 and 2014–15 and 53% in 2013–14. Summary monovalent pandemic IVE against influenza A(H1N1)pdm09 hospitalization in 2009–10 was 72% (95%CI: 22;100))	24 TND studies through 6 seasons
		Adults (aged 18–64y)			Pooled IVE	51% (95%CI:44;58)	
		Adults of all ages			IVE against lab-confirmed influenza-associated hospitalization - influenza A(H1N1)pdm09	48% (95%CI:37;59),	7 TND studies through four seasons
		Adults (aged 18–			summary IVE against influenza	55% (95%CI: 34;76)	

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
		64y)			A(H1N1)pdm09 viruses		
		Adults of all ages			IVE against lab-confirmed influenza-associated hospitalization - influenza A(H3N2)	37% (95%CI:24;50)	9 TND studies through four seasons
		Adults (aged 18–64y)			Summary IVE against influenza A(H3N2) viruses	50% (95%CI: 38;62)	
		Adults of all ages			IVE against lab-confirmed influenza-associated hospitalization - influenza B	38% (95%CI:23;53)	5 TND studies through four seasons
		Adults (aged 18–64y)			Summary IVE against influenza B	45% (95%CI: 8;81)	

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
Hirve 2016 (SR)	Tropics and subtropics (excluding Australia)		1993-2014	N. A.	Lab-confirmed flu	IVE ranged from 50 to 59%	1 RCT, and 3 cohorts and 3 case–control studies
Manzoli 2012 (MA)	Worldwide	Adults	1995-2011	Overall VE	Lab- confirmed	IVE 49-61%	3 meta-analyses (Villari, Jefferson, Osterholm)
				parenteral inactivated vaccines		IVE 59-67%	
				LAV		IVE 32-62%	
				Aerosol inactivated (AIV)		N.A.	
				Overall VE	Clinically-confirmed	IVE 19-22%	2 meta-analyses (Villari, Jefferson)
				parenteral inactivated vaccines		IVE 20-23%	
				LAV		IVE 10-15%	
				Aerosol inactivated		IVE 42-55%	

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
				(AIV)			
Michiels 2011 (SR)	Worldwide	Healthy adults	1966-2010	Trivalent inactivated vaccines	Proven influenza/ILI	From no effectiveness in case of bad match to 30% in case of good match	Results from Jefferson's Cochrane SR
Ng 2011 (SR)	Worldwide	Healthcare workers	From the databases launch up to 14 March 2011	Any kind of influenza vaccine	Lab-confirmed flu	VE 88% (95% CI 59 to 96%): risk of infection significantly lower in the vaccination group, RR 0.12 (95% CI 0.04 to 0.41)	1 randomized, prospective, double-blind, controlled trial over 3 consecutive years, from 1992-1993 to 1994-1995.
					ILI	No significant difference between the vaccine and control groups	3 studies: 1 reported incidence of ILI (significant difference between the vaccine and control groups); a 2nd study reported the N of ILI episodes and found no significant difference between the vaccine and control groups. The 3rd study

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE or RR (95% CI)	N of studies included with specification of study design and N of subjects
							reported no significant difference in days with ILI symptoms between the vaccine and control groups.
					Mean N of working days lost	No significant difference	2 studies

MA= meta-analysis; SR= systematic review; *diagnosis through virus isolation from culture or through a four-fold antibody increase; ç Not clear which tests were used to make diagnosis of influenza infection

Table 10: Main findings from selected systematic reviews and meta-analysis on IVE among children and/or adolescents

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
Jefferson 2018 (MA)	Worldwide	<16 y.o.	Up to 31/12/2016	Live attenuated	Lab confirmed	RR 0.22 (0.11-0.41)	7 RCTs (7,718 subjects)
					ILI	RR 0.69 (0.60 - 0.80)	7 RCTs (124,606 subjects)
					Otitis media	RR 0.98 (0.95-1.01)	1 RCT (1784 subjects)
				Inactivated	Lab confirmed	RR 0.36 (0.28-0.48)	5 RCTs (1628 subjects)
					ILI	RR 0.72 (0.65-0.79)	4 RCTs (19,044 subjects)
					Otitis media	RR 1.15 (0.95 - 1.4)	3 RCTs (884 subjects)
Restivo 2018 (MA)	Worldwide	Children	2007-2016	N. A.	Influenza visits	39% (95%CI: 32–46%)	7 studies
				N. A.	Hospitalizations	57% (95%CI: 30–74%)	9 studies

Caspard 2017 (MA)	USA, Canada, Germany, UK; Finland	2-17 years	2010-2016	Live attenuated trivalent or quadrivalent	Laboratory-confirmed flu	LAIV3 was effective in 2011–2012:68%; 95% CI, 48–80, in 2012–2013: 43%; 95% CI, 27–56, in 2013–2014: 83%; 95% CI, 25–96). The LAIV4 was effective in 2015–2016: 48%; 95% CI, 29–61. The LAIV was not shown to be effective as a monovalent formulation in 2009–2010 (79%; 95% CI, –16 to 96), as LAIV3 in 2010-2011 (42%; 95% CI, –1 to 85), or LAIV4 in 2013–2014 (18%; 95% CI, –3 to 34) and 2014–2015 (28%;	29 observational studies (25 of these were TND)

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
						95% CI, –18 to 56). Consolidated estimates across seasons show that LAIV was effective as a trivalent formulation (53%; 95% CI, 35–66) and a quadrivalent formulation (33%; 95% CI, 17–46) and since the 2009 pandemic irrespective of the formulation (42%; 95% CI, 30–52).	
Hirve 2016 (SR)	Tropics and subtropics (excluding Australia)	Not specified	1993-2014	N. A.	Laboratory-confirmed flu	Overall IVE ranged from 20% to 77% LAIV IVE ranged from	

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
						62% to 83% TIV IVE ranged from 48% to 72%	
Lukšić 2013 (MA)	Worldwide	Children ≤18 years	Up to 2011	any preparation of seasonal influenza vaccine, administered via any route: live vaccines or inactivated vaccines	ILI	IVE for live vaccines, using random effects model, was 31.4% (24.8%-39.6%) and using fixed-effect model 44.3% (42.6%-45.9%). IVE for inactivated vaccines, using random effects model, was 32.5% (20.0%-52.9%) and 42.6% (38.3%-47.5%) using fixed-effect model	Effectiveness of live vaccines evaluated by 4 RCTs and 4 cohort studies. Effectiveness of inactivated vaccines evaluated by 5 RCTs and 3 cohort studies

Authors	Region	Age groups	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
Manzoli 2012 (MA)	Worldwide	Children/adolescents	Up to December 1, 2011	Overall VE	Lab- confirmed	IVE 51-75%	5 meta-analyses (Negri, Manzoli, Jefferson, Rhorer, Osterholm)
				parenteral inactivated vaccines		VE ranged from 46% to 65%	
				LAV		vaccine efficacy ranging from 72% to 83%.	
				Overall VE	Clinically-confirmed	IVE 33-38%	3 meta-analyses (Negri, Manzoli, Jefferson)
				parenteral inactivated vaccines		IVE 33-45%	
				LAV		IVE 33-37%	
Michiels 2011 (SR)	Worldwide	Children <16 y.o.	2006-2011	Trivalent inactivated vaccines	ILI	From no significant effect in children <16 y.o. to 36% (95%CI: 24–46) (n = 19,388) compared with placebo or no intervention	

MA= meta-analysis; SR= systematic review

Table 11: Main findings from selected systematic reviews and meta-analysis on IVE of influenza vaccination during pregnancy on the mother and/or the child

Authors	Region	Population group	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
Demicheli 2018 (MA)	Worldwide	Mothers	1966-31/12/2016	TIV	vaccine efficacy or effectiveness	50% (95%CI 14% to 71%)	1 RCT (2116 women entered the study: 1062 received the vaccine, 1054 the placebo)
		Newborns up to 24 weeks of life				VE 49% (95% CI 12%to 70%)	
Hirve 2016 (SR)	Tropics and subtropics (excluding Australia)	Pregnant women		N. A.	Laboratory-confirmed flu****	IVE = 50% in the mothers;	2 RCTs (340 and 2116 pregnant women, respectively)
		Their infants <6 months				IVE ranged from 49 to 63%	
Manske 2014 (SR)	USA	Pregnant women	January 1, 1964 to February 1, 2013		ILI *	Non-significant reduction in ILI incidence (20 vs. 11 %)	1 Retrospective and prospective cohort study (544 pregnant women: 363 immunized, 181 non-immunized)
					ARI	From a non-significant (p=0.24) trend toward lower ARI incidence (18.9 and 22.6 %, respectively) (VE was -20 % for ARI any time during pregnancy (CI -59 to 9 %); and 39 % (CI -56 to 76 %) during the peak of the influenza season	1 retrospective cohort (Pregnant women 252 immunized 826 non-immunized)

Authors	Region	Population group	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
				TIV	Non-specific respiratory illness with fever	Clinical IVE of 35.8 % (CI 3.7–57.2 %) was reported for respiratory illness with any fever, and 43.1 % for fever over 38°C (CI -9.0 to 70.3 %).	1 RCT (340 pregnant women: 172 immunized with TIV 168 immunized with pneumococcal vaccine)
				adjuvanted A(H1N1)pdm09	Influenza lab- or clinically confirmed**	IVE adjuvanted A(H1N1)pdm09: 70 % (aHR = 0.30, CI 0.25–0.34) in the first cohort study, 61% IVE nonadjuvanted A(H1N1)pdm09(CI15.5-82.5%) in the 2nd	2 retrospective cohorts (First:113,331 pregnant women: 59,266 vaccinated and 54,065 non-vaccinated; 2nd: 3,236 mothers who gave birth between May 25, 2009 and April 17, 2010)
					Medical visits for respiratory symptoms	IVE -15% (No difference in medical visits (p = 0.088); aHR = 1.151 (CI 0.979–1.352)	Retrospective cohort (49,585 pregnant women: 3,707 immunized and 45,878 non-immunized
		Infants of women vaccinated during pregnancy				4 of the 7 studies applied some form of lab confirmation, with IVE ranging from 41% to 91%. ***	7 studies: 2 retrospective cohort, 1 retrospective matched cohort, 1 RCT, 1 matched case-control, 1 prospective cohort and 1 case-control
Michiels 2011 (SR)	Worldwide	Pregnant women	2006-2011	TIV	Respiratory illness with fever in mothers	IVE 36%	1 RCT

MA= meta-analysis; SR= systematic review; * RT-PCR or culture-confirmation; ** RT-PCR or medical visit during pregnancy with an influenza-related ICD-9 diagnosis code; ***only one study out of the total 7 used viral culture or RT-PCR to confirm influenza infection, the other used DFA or rapid

tests; **** RT-PCR

Table 12: Main findings from selected systematic reviews and meta-analysis on IVE among immunocompromised patients

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
Bitterman 2018 (MA)	Worldwide	≥ 16 years	Patients with cancer	Up to May 2017	Inactivated influenza vaccine of any type	Lab confirmed *	Confirmed influenza rates were lower with vaccination in one RCT and the three observational studies, the difference reaching statistical significance in one retrospective case-control study (Machado 2005): VE was 80%	1 RCT and 3 observational studies

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
						ILI	Ful text of RCT (Msto 1997) not available; the observational study (Vinograd, 2013), did not reported OR	1 RCT (MM, with active chemotherapy treatment 50 adults: 25 vaccinated 25 unvaccinated), 1 prospective, non-interventional cohort study (806 patients)
						Pneumonia	Pneumonia was observed significantly less frequently with vaccination in one observational study, but no difference was detected in another or in the RCT (*Full-text of RCT (Msto 1997) not*	I RCT (50 patients), 2 observational studies (1 retrospective cohort study, 1225 adults; 1 prospective observational cohort study, 806 patients)

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
							available)	
						Any hospitalisation	Two studies, one RCT (Musto 1997) and one cohort study (Vinograd 2013), reported on hospitalisations. The RCT showed a significantly lower rate of hospitalisations in vaccinated participants, while in the cohort study there was no difference. Two cohort studies reported on	1 RCT (50 patients), 1 cohort study (806 adults)

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
							hospitalisation duration (Earle 2003;, Vinograd 2013), both showing no significant associations, but a mean duration shorter by 0.9 to 1.8 days with vaccination.	
						All-cause mortality	OR 1.25 (95% CI 0.43 to 3.62)	1 RCT study, 78 participants
						All-cause mortality	HR 0.88 (95% CI 0.78 to 1)	1 retrospective, observational cohort study, 1577 participants
						All-cause mortality	OR 0.42 (95%CI 0.24 to 0.75)	1 prospective observational cohort study,

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
								806 participants
La Torre 2016 (SR)	Argentina	Mean age: 51 y	Patients with haematological malignancies	January 2000 to May 2016	N. A.	30-day mortality in all patients with a diagnosis of H1N1 influenza	0% in 19 vaccinated patients, and 27% (12/45) in non-vaccinated patients: all deaths occurred among the non-vaccinated patients	1 CT (47 patients)
	Italy	Children			N. A.	influenza-related morbidity (N of upper and lower respiratory tract infection, days of fever, antibiotic courses, and lost school days)	One of the major benefit: reduction in the N of hospitalizations; IVE in decreasing the number of URTIs and LRTIs, days of fever, antibiotic courses, and lost school days	1 cohort (182 children)

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
							was greater in children who had been off therapy for less than 6 months	
Remschmidt 2014 (MA)	Japan	Adults	HIV-infected patients	December 31, 2005 to January 28, 2014	TIV	laboratory-confirmed flu	IVE 71% (95% CI, 44–85%)	1 cohort study (262 HIV-infected patients received TIV, 66 did not)
	Italy, USA					ILI	IVE 60% (95% CI, −39 to 88)	2 cohort studies (90 vaccinated VS 55 not vaccinated and 42 vaccinated VS 29 not vaccinated)
	Japan, USA					All-cause hospitalization and all-cause	No significant effect observed (values not	2 cohort studies

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
						pneumonia	reported)	
	South Africa	Children aged 6-59 m				laboratory-confirmed flu	IVE 11% (-70 to 54%)	1 RCT (206 vaccinated and 204 unvaccinated children)
					TIV	ILI	23% (95% CI, −26 to 53%) when the 1st episode of ILI was considered, while after including all ILI episodes (36%; 95% CI, 2–58%).	
Beck 2011 (MA)	Worldwide	Any age	Immunocompromised	up to Jan 2011	N. A.	Lab confirmed	IVE 85% (OR 0.15; 95% CI 0.03-0.63) p=0.01	2 studies (study design not specified)
						ILI	OR = 0.23; 95% confidence interval [CI] = 0.16–0.34; p=0.001	7 studies (study design not specified)

MA= meta-analysis; SR= systematic review; *Immunofluorescence assay (in Machado, 2005)

Table 13 Main findings from selected systematic reviews and meta-analysis on IVE among patients with chronic diseases

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
Dos Santos 2018 (SR)	Worldwide	≥65 years	Diabetic patients	January 2000-March 2017		All-cause mortality	OR from 0.35 (95% CI 0.25-0.49) for males and 0.32 (95% CI 0.20-0.50) for females to 0.67 (95% CI 0.47-0.96) in both sexes.	5 studies (1 case-control studies and 4 retrospective cohort studies)
Bekkat-Berkani 2017 (SR)	Australia, India, Spain, Taiwan, Thailand, UK and the US	Any age	COPD	01/01/1990 to 15/09/2015	N. A.	ARI	after 2 doses: IVE: 76% (RR 0.2, 95% CI 0.06–0.7)	1 RCT
						N of hospitalisations or episodes of mechanical ventilation	No statistically significant difference	1 RCT
						All cause mortality	No reduction of risk	1 prospective Spanish cohort study (1298 subjects)
							No reduction of risk	1 retrospective cohort study
							Risk reduced by 41%: RR 0.59, 95% CI 0.57–	1 retrospective study in the UK (almost

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
							0.61	41,000 patients)
						Risk of hospitalization due to exacerbations	IVE: 90.8% (95% CI 83.5–94.8) (OR 0.092 (0.052–0.165)	1 Retrospective cohort study in Spain in 1,323 vaccinated and unvaccinated subjects
Vasileiou 2017 (MA)	USA	>6 months	Asthma patients	1970-2016		Lab confirmed influenza *	IVE 45% (95% CI 31-56)	2 TND (1,825 subjects)
Hirve 2016 (SR)	Tropics and subtropics (excluding Australia)		COPD patients	1993-2014	N. A.	Laboratory-confirmed flu	IVE =70%	Primary study not available
Remschmidt 2015 (MA)	Worldwide	18-65	Diabetic patients	From inception to December 31, 2013		All cause hospitalizations	IVE 58% (95% CI, 6–81%	3 case–control and 1 cohort study (N=93472)
						Hospitalization due to influenza or pneumonia	VE 43%; 95% CI, 28–54%	1 case-control study (N=91,605)
						ILI	no statistically significant protective effects were	1 case-control study

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
							observed	
						All cause mortality	no statistically significant protective effects were observed	1 case-control study
		>65				All cause hospitalizations	VE 23%; 95% CI, 1–40%	2 case–control studies
						Hospitalization due to influenza or pneumonia	VE 45%; 95% CI, 34–53%	1 case-control study
						ILI	VE 13%; 95% CI, 10–16%	1 case- control study
						All-cause mortality	VE 38%, 95% CI, 32–64% from 2 cohort studies; VE 56%, 95% CI, 47–64%, in 2 case control studies I2 = 0%,	2 case–control studies
Remschmidt 2014 (MA)	4 studies were	Not specified	End-stage renal disease	Up to 07 May 2014	N.A.	ILI	VE 12%; 95% CI, 10–14%	5 retrospective cohort studies Total study

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
	conducted in the US and 1 in Taiwan					All-cause mortality	pooled confounder-adjusted IVE 32%; 95% CI, 24–39%	population: n = 174,663 (Since all US studies used the same database, overlapping of the populations cannot be ruled out)
						Cardiac death	IVE 16%; 95% CI, 1–29%	
						Hospitalization due to influenza or pneumonia	VE 14%; 95% CI, 7–20%	
						ICU admission	VE 81%; 95% CI, 63–86%	
Michiels 2011 (SR)	Worldwide	Any age	COPD patients	2006-2011	Trivalent inactivated vaccines	Non-specific respiratory infections and/or exacerbations	No significant effectiveness	2 studies, n = 180
						Hospitalisations	No effect	2 studies, n = 180
						Overall mortality	No effect	
			Asthma			Hospitalizations	No effect	
			Coronary			Cardiovascular	74% (95%CI: 37–89%)	2 studies, n = 858

Authors	Region	Age groups	Population characteristics	Study period	Vaccine type/brand	Outcome evaluated	IVE (95% CI)	N of studies included with specification of study design and N of subjects
			disease			mortality		
			Liver disease			ILI	no significant effectiveness	1 RCT, n=311

* RT-PCR; MA= meta-analysis; SR= systematic review