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CORRELATES OF PROTECTION AND HOST-RELATED MODIFIERS OF THE IMMUNOGENICITY OF INFLUENZA VACCINES: EVIDENCE MAPS AND EVIDENCE GAPS

Academic Discipline: MED/42

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ABSTRACT

Seasonal influenza is the leading infectious disease in terms of its health and socioeconomic impact. Annual immunization is the most efficient way to reduce this burden. To be clinically effective, influenza vaccines must be immunogenic, and several immunological assays to test their immunogenicity have been developed.

The overall aim of this PhD thesis is to provide the principal stakeholders (including scientists, healthcare professionals, policy-makers, pharmaceutical industry, etc.) with state-of-the-art knowledge and practices related to influenza vaccine-induced immunogenicity. To achieve this aim, we developed a novel empirical approach that incorporated some modern techniques, including, for example, evidence mapping.

Basically, this thesis is composed of three main domains. In the introductory part, we will briefly cover the topics of influenza disease, influenza vaccination, the immunogenicity measurements of influenza vaccines and their correlates of protection.

The second part, which is the core of the present project, is composed of two original case studies. The first study aimed to describe the patterns of use of the various immunological assays available to measure the influenza vaccine-induced adaptive immune response and to determine its correlates of protection. For this purpose, we analyzed 1,164 phase I–IV studies that enrolled a total of 754,935 subjects. Of the studies included in our analysis, 76.5% measured only the humoral immune response. Among these, the hemagglutination-inhibition assay was by far the most widely used. Other, less common, humoral immune response assays were: virus neutralization (21.7%), enzyme-linked immunosorbent (10.1%), single radial hemolysis (4.6%) and assays able to quantify anti-neuraminidase antibodies (1.7%). By contrast, cell-mediated immunity was quantified in only 23.5% of studies. Several variables were significantly ($P < .05$) associated with the use of single assays. Specifically, some influenza vaccine types (e.g. adjuvanted, live attenuated and cell culture-derived or recombinant), study phase and study sponsorship pattern were usually found to be statistically significant predictors.

In the second study, we went further by systematically analyzing host-related factors able to modify influenza vaccine-induced immunogenicity. To this end, a total of 28 systematic reviews/meta-analyses (with thousands of participants) were analyzed. These covered the following domains: intravenous drug use, psychological stress, acute and chronic physical exercise, genetic polymorphisms, use of pre-/pro-/symbiotics, previous Bacillus Calmette–Guérin vaccination, diabetes mellitus, vitamin D supplementation/deficiency, latent cytomegalovirus infection and various forms of immunosuppression. In order to present effect sizes on the same scale, all meta-analyses were re-performed, whenever possible, and cumulative evidence synthesis ranking was carried out. Meta-analysis was conducted separately on each health condition category and virus (sub)type. A total of 295 meta-analyses were re-performed/performed *ex novo*; of these, 97 pooled estimates were used in order to construct an evidence-based stakeholder-friendly map.

Finally, we discussed the principal findings, made some suggestions from the point of view of the various stakeholders and proposed a novel immunogenicity pathway.

RIASSUNTO

Influenza stagionale è la principale malattia infettiva per il suo impatto sia sanitario che socioeconomico. La vaccinazione annuale rappresenta il mezzo più efficace per ridurre questo impatto. Per essere clinicamente efficaci, i vaccini antinfluenzali devono essere immunogeni; a tal fine diversi saggi immunologici sono stati sviluppati.

L'obiettivo principale della presente tesi di dottorato è di fornire le più attuali conoscenze e le pratiche cliniche relative all'immunogenicità dei vaccini antinfluenzali ai principali portatori di interesse (e.g. scienziati, operatori sanitari, decisori politici, industria farmaceutica, ecc.). Questo obiettivo è stato perseguito tramite lo sviluppo di un innovativo approccio empirico che ha inglobato alcune metodologie moderne (e.g. *evidence mapping*).

Schematicamente, la presente tesi può essere suddivisa in tre parti principali. Nella prima parte introduttiva saranno brevemente descritte le tematiche relative alla malattia influenzale, alla vaccinazione antinfluenzale, all'immunogenicità dei vaccini disponibili e ai correlati di protezione.

La seconda parte invece rappresenta il fulcro del presente progetto ed include due studi innovativi. Il primo studio aveva l'obiettivo di descrivere i metodi di utilizzo dei diversi test immunologici che misurano la risposta immune in seguito alla vaccinazione antinfluenzale e per determinare i relativi correlati di protezione. A questo scopo abbiamo analizzato 1.164 studi clinici di fase I–IV che avevano arruolato un totale di 754,935 soggetti. Il 76,5% degli studi inclusi ha misurato soltanto la risposta umorale. Tra i test utilizzati, è stato usato nella maggior parte dei casi il test dell'inibizione dell'emoagglutinazione. Altri test meno utilizzati erano: test di neutralizzazione (21,7%), test immuno-assorbente legato ad un enzima (ELISA) (10,1%), emolisi singola radiale (4,6%) e test che quantificano l'attività antineuraminidasica (1,7%). Contrariamente, l'immunità cellulo-mediata è stata quantificata soltanto nel 23,5% degli studi. Diverse variabili sono state significativamente ($P < ,05$) associate con l'utilizzo dei singoli test. È stato rilevato in particolare che alcuni tipi dei vaccini antinfluenzali (e.g. adiuvati, vivi attenuati, propagati nella coltura cellulare o ricombinanti), la fase dello studio e le caratteristiche relative allo sponsor dello studio sono stati i principali correlati.

Il secondo studio ha invece approfondito il primo: abbiamo analizzato sistematicamente i fattori relativi all'ospite che possono modificare l'immunogenicità dei vaccini antinfluenzali. A questo scopo un totale di 28 rassegne sistematiche e/o meta-analisi (che includevano migliaia di soggetti) sono state analizzate. Le seguenti aree di interesse sono state esaminate: l'uso di droga somministrata per via endovenosa, stress psicologico, esercizio fisico abituale ed eccentrico, polimorfismi genetici, l'uso di pre-/pro-/simbiotici, previa vaccinazione antitubercolare, diabete mellito, l'utilizzo degli integratori della vitamina D/carenza della vitamina D, infezione cronica da citomegalovirus e diverse forme di immunosoppressione. Al fine di rappresentare sulla stessa scala gli effetti osservati, abbiamo ricalcolato tutte le possibile stime meta-analitiche. Ogni meta-analisi condotta è stata specifica sia per la condizione di salute che il (sotto)tipo virale. Un totale di 295 meta-analisi sono stati (ri)eseguite e 97 stime meta-analitiche sono state utilizzate al fine di costruire una mappa *evidence-based* e *stakeholder-friendly*.

Infine, abbiamo discusso i principali risultati, mettendo in luce alcuni suggerimenti che possono essere utili ai diversi portatori di interesse e abbiamo sviluppato un innovativo modello relativo all'immunogenicità dei vaccini antinfluenzali "precisi".

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LIST OF ABBREVIATIONS

aTIV	Adjuvanted trivalent influenza vaccine
αTNF	Tumor necrosis factor α
AMSTAR	A measurement tool for assessing systematic reviews
ANOVA	Analysis of variance
aOR	Adjusted odds ratio
ARR	Absolute risk reduction
BCG	Bacillus Calmette–Guérin
BIC	Bayesian information criterion
CBER	Center for Biologics Evaluation and Research
CCT	Controlled clinical trial
CD	Cluster of differentiation
CDC	Centers for Disease Control and Prevention
CES	Cumulative evidence synthesis
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CMI	Cell-mediated immunity
CMV	Cytomegalovirus
CoP	Correlate of protection
DALY	Disability-adjusted life year
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent assay
ELLA	Enzyme-linked lectin assay
EMA	European Medicines Agency
ES	Effect size
EU	European Union
FDA	Food and Drug Administration
FE	Fixed-effects model
GISRS	Global Influenza Surveillance and Response System
GMT	Geometric mean titer
H/HA	Hemagglutinin
HAI	Hemagglutination-inhibition

hdTIV	High-dose trivalent influenza vaccine
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HTA	Health technology assessment
IBD	Inflammatory bowel disease
IgA	Immunoglobulin G
IgM	Immunoglobulin M
IgG	Immunoglobulin G
ILI	Influenza-like illness
IS	Immunosuppression
IV	Influenza vaccine/vaccination
LAIV	Live attenuated influenza vaccine
LS	Largest study
M1	Matrix protein 1
M2	Matrix protein 2
MA	Meta-analysis
MD	Mean difference
MDCK	Madin-Darby canine kidney
MF	Microfluidized emulsion
MIV	Monovalent influenza vaccine
N/NA	Neuraminidase or non-available (depending on the context)
NCT	National clinical trial
NK	Natural killer
NP	Nucleoprotein
ns	Non-significant
NS	Non-structural protein
Obs	Observational study
OR	Odds ratio
PA	Polymerase A
PB	Polymerase B
PI	Prediction interval
PICO	Population, intervention, comparator, outcome framework
QIV	Quadrivalent influenza vaccine
RA	Rheumatoid arthritis

RCT	Randomized controlled trial
RD	Rheumatic disease
RE	Random-effects model
RNA	Ribonucleic acid
RNP	Ribonucleotide nucleoprotein
RoW	Rest of the world
RR	Risk ratio
RT-PCR	Reverse transcription-polymerase chain reaction
SARI	Severe acute respiratory infection
SC	Seroconversion rate
SD	Standard deviation
SE	Standard error
SLE	Systemic lupus erythematosus
SP	Seroprotection rate
SR	Systematic review
SRH	Single radial hemolysis
sRIR	Summary relative illness ratio
SRMA	Systematic review and meta-analysis
SSE	Small-study effect
TIV	Trivalent influenza vaccine
TIVe	Egg-derived trivalent influenza vaccine
UK	United Kingdom
UN	United Nations
US	United States
VIF	Variance inflation factor
VN	Virus neutralization assay
WHO	World Health Organization

CHAPTER 1. INTRODUCTION

Influenza and its Burden

Influenza is a common, typically seasonal disease (although with pandemic potential) caused by an enveloped single-stranded ribonucleic acid (RNA) virus belonging to the family of *Orthomyxoviridae*. The viral RNA is composed of seven or eight segments, each of which forms a ribonucleotide nucleoprotein (RNP) by encapsidating the nucleoprotein (NP). In turn, each segment codes for the following functional proteins: polymerase B1 (PB1); polymerase B2 (PB2); polymerase A (PA); hemagglutinin (HA; in the description of a virus subtype referred to as H); neuraminidase (NA; in the description of a virus subtype referred to as N); NP; matrix proteins 1 (M1) and 2 (M2); and non-structural (NS) proteins [Wright et al. 2001; Cox et al. 2004; Bouvier and Palese 2008; Wang 2016].

The two outer-layer glycoproteins of HA and NA play a fundamental role in the pathogenesis of disease [Wright et al. 2001; Wang 2016; Petrova and Russell 2018]. Specifically, HA enables the virus to enter the host cell by binding sialic acid on the surface of sialylated cells, thereby causing membrane fusion, while NA enables the viral progeny to be released from infected cells by cleaving the bonds between HA and sialic acid and by facilitating movement of the virus through mucus [Petrova and Russell 2018].

On the basis of their NP antigenic specificity, influenza viruses are classified in types; to date, four types have been identified, namely A, B, C and D [Wright et al. 2001; Su et al. 2017]. Types A, B and C can cause human disease, while the role of the recently identified type D in causing human pathology is unclear [Wright et al. 2001; Su et al. 2017; Trombetta et al. 2020]. The disease caused by virus type C is usually described as mild and cold-like; it is therefore considered to be of limited public health importance [Wright et al. 2001].

Conversely, virus types A and B are the main protagonists of annual epidemics and evolve continuously through the phenomena of antigenic drift (i.e. minor mutations in the surface glycoproteins of HA and NA able to change the antigenicity of the virus) and shift (a major change associated with the appearance of a novel virus subtype) [Wright et al. 2001; Petrova and Russell 2018].

On the basis of HA and NA, virus type A, which originates mainly from birds and swine, is further classified in subtypes. To date, at least 18 HA and 11 NA subtypes have been identified; however, subtypes H1N1 and H3N2 have predominated on the world epidemiological scene for several decades [Petrova and Russell 2018; Tong et al. 2013]. Virus

type B, by contrast, has evolved into two antigenically distinct lineages, Victoria and Yamagata, which have been co-circulating for about 40 years [Rota et al. 1990; Biere et al. 2010].

In summary, the phenomenon of antigenic drift is common to both A and B types and causes seasonal influenza epidemics. By contrast, antigenic shift is pathognomonic to virus type A and may cause a pandemic [Wright et al. 2001; Paules and Subbarao 2017].

Influenza is the most common infectious disease in the world. Indeed, the World Health Organization (WHO) estimates [WHO 2012] that 5–10% of adults and 20–30% of children get influenza each year. In Europe, seasonal influenza ranks first in terms of both incidence and mortality rates, causing on average a burden of 81.8/100,000 disability-adjusted life years (DALYs) [Cassini et al. 2018]. In Italy alone, seasonal influenza may cause up to 25,000 excess deaths [Rosano et al. 2019] and have an annual economic impact of nearly € 1,356,000,000 on average [Lai et al. 2011].

Influenza Vaccination

Annual active influenza immunization is the single public health intervention most able to reduce the burden of disease [WHO 2012]. Several target groups for annual influenza vaccination (IV) have been identified; these, however, display marked between-country variability in terms of both recommendation and reimbursement policies. In Italy, for instance, IV is recommended, and its cost reimbursed, for the following population strata: (i) persons aged 60/65 years or more; (ii) subjects aged 6 months to 64 years who have certain, predefined health conditions (e.g. chronic respiratory and cardiovascular pathologies, some forms of immunodeficiency, etc.); (iii) professionals employed in public services of primary interest (e.g. healthcare professionals, police, firefighters); (iv) professionals who come into close contact with animals that may be the sources of non-human influenza viruses (e.g. farmers, butchers, veterinarians, etc.); (v) some other risk categories (e.g. blood donors, institutionalized individuals) [Italian Ministry of Health 2020]. The Italian recommendations are similar to those issued by many other countries of the European Union (EU) [European Centre for Disease Prevention and Control (ECDC) 2018]. In the United States (US), by contrast, the recommendation is universal (for subjects aged ≥ 6 months) [Grohskopf et al. 2018].

Several types of IVs are currently available. IVs can be broadly classified on the basis of: (i) inactivation issues; (ii) valence; (iii) production platform; (iv) formulation and (v) mode of administration. First of all, IVs may be either live attenuated (LAIV) or inactivated;

this latter category may be further subdivided into whole-virion, split and subunit. Regarding the valence (i.e. the number of virus subtypes/lineages included in the vaccine), currently or formerly available IVs may be mono- (MIV), bi-, tri- (TIV) or quadrivalent (QIV). MIVs usually constitute pandemic or pre-pandemic vaccines, while the currently available seasonal IVs are TIVs [containing the 2009 pandemic H1N1 (H1N1pdm09), H3N2 and either B lineage strain) and QIVs (containing H1N1pdm09, H3N2, B/Victoria and B/Yamagata). Most available IVs are prepared in the traditional way, i.e. through propagation in embryonated hen eggs. However, in recent years, some alternative platforms have become available; these basically include mammalian cell cultures or recombinant platforms. The IV formulation may depend on the amount of antigen, the presence of adjuvants and some other characteristics. IVs may be administered intranasally (mostly LAIVs), intramuscularly/subcutaneously (most available IVs), intradermally or orally (IV candidates). Furthermore, tens of IV candidates at different stages of preclinical and clinical development are being studied [WHO 2012; Grohskopf et al. 2018; Barr et al. 2018; Manini et al. 2015; Chen et al. 2020].

Unlike the case of several other vaccine-preventable diseases, the efficacy of IVs is often judged suboptimal and varies (even to a huge extent) from year to year. Indeed, three Cochrane reviews [Jefferson et al. 2018; Demicheli et al. 2018a; Demicheli et al. 2018b] conducted in order to establish the efficacy of IVs in children, adults and the elderly seem to confirm this thesis. In these reviews, the pooled absolute efficacy (i.e. versus placebo) of the inactivated IVs was 64% [95% confidence interval (CI): 52–72%], 59% (95% CI: 53–64%) and 58% (95% CI: 34–73%) in healthy children [Jefferson et al. 2018], adults [Demicheli et al. 2018a] and the elderly [Demicheli et al. 2018b], respectively. However, these three Cochrane reviews did not distinguish between different IV types; in this regard, it has been shown, for example, that MF59®-adjuvanted TIV [Domnich et al. 2017] and high-dose TIV (hdTIV) [Lee et al. 2018] formulations may be more effective than standard TIVs in preventing several influenza-related outcomes.

However, it should be borne in mind that influenza is a very common, annually occurring disease; therefore, even in seasons when the observed efficacy of IVs has been low, both the health-related and economic benefits of vaccination have still been significant (although relatively low purchase prices of IVs have been applied) [Gasparini et al. 2002; de Waure et al. 2012]. In this regard, in 2009 the European Council recognized the value of seasonal influenza immunization, and recommended that the EU Member States reach a vaccination coverage rate of at least 75% (ideally 95%) in the elderly and, if possible, other risk categories [EU 2009]. However, despite the documented benefit of annual IV and the

clearly established goals, IV coverage rates are still low/suboptimal and, on average, far below these targets [ECDC 2018].

Influenza Vaccine Immunogenicity and Correlates of Protection of Influenza Vaccines

Immunogenicity can be broadly defined as the ability of an antigen to provoke an immune response in the immunized subject [Leroux-Roels et al. 2011]. In turn, the immune response is usually categorized as innate and adaptive; the adaptive response may be further divided into humoral and cell-mediated immunity (CMI). Briefly, the cells of the innate immune system (e.g. macrophages and neutrophils) constitute the first line of defense against many common pathogens; however, in some situations, they may be unable to recognize, and hence to eliminate, certain microorganisms. A more versatile and evolutionarily newer mechanism of host defense is the adaptive immune response, which is basically implemented by lymphocytes. These cells may provide increased protection against subsequent reinfections by the same microorganisms. Humoral adaptive immunity is realized via antibodies against a specific antigen that are produced by plasmablasts (in turn, differentiated from B lymphocytes and with assistance from T helpers). CMI, by contrast, is mediated by T cells: T helper (CD4+) cells release chemokines that help activated T lymphocytes to bind to the main histocompatibility complex of the infected host cells and differentiate the T cells into cytotoxic T cells (CD8+) with subsequent lysis of the infected host cell [Janeway et al. 2001; Plotkin et al. 2017].

The host–virus interaction involves both mucosal and systemic immunity, and humoral and CMI responses. Locally secreted immunoglobulin A (IgA) and, to some extent, IgM are the main neutralizing antibodies involved in protecting the upper respiratory tract, i.e. they prevent viral entry and subsequent replication. IgG also plays an important role in the first-line defense; IgG, however, is derived as a serum transudate. In sum, nasal secretions contain all three major Ig classes directed against the viral proteins, HA and NA in particular [Cox et al. 2004; Choi et al. 2020].

Serum antibodies produced by B cells are primarily directed against HA, and can be detected within 10–14 days following infection. Of note, IgM and IgG play a crucial role during the primary immune response, while IgA and IgG are mainly involved in secondary immunogenicity. The NA-specific antibodies, by contrast, reduce the release of virions from

infected host cells; at the same time, however, they are probably less effective in preventing infection [Cox et al. 2004; Choi et al. 2020].

Finally, CMI is particularly effective in clearing virus-infected cells and probably in preventing influenza-related complications, though its role in preventing infection seems to be less significant. On the other hand, CMI is an effective mechanism that seems to be essential to both the establishment of long-term immunological memory and heterologous protection [Cox et al. 2004; Gianchecchi et al. 2019].

The clinical protection provided by IVs can be measured though both randomized controlled trails (RCTs) and observational studies. In the former case, we are measuring vaccine efficacy (which could be deemed the "gold standard"), which is manifested as a reduction of disease following vaccination in ideal conditions. Instead, vaccine effectiveness refers to the same reduction when it occurs in real-life conditions, as estimated through observational studies (case–control, cohort and their modifications) [WHO 2017a]. However, RCTs aimed at quantifying IV efficacy face several challenges, such as the probability of low attack rates, high seasonality, multiplicity of circulating strains and their frequent intra-seasonal mutations. As RCTs require a large number of study participants, they are also costly [Dewe et al. 2013]. Moreover, placebo-controlled trials may be ethically unsuitable for those population groups for whom IV is highly recommended (e.g. the elderly) [Domnich et al. 2017]. A relatively simpler, more efficient and less expensive way of measuring IV-induced protection is to conduct observational studies. However, the resulting parameter of vaccine effectiveness (even if the study is well-designed) may be affected by a number of typical biases (selection bias *in primis*) [Jackson et al. 2018]. Furthermore, as observational research can be conducted only in the post-marketing phase of the product's life-cycle, these studies are usually inappropriate for licensing/regulatory issues. In sum, the above-described facts provide some rationale for using immunogenicity endpoints not only in RCTs but also in some observational studies.

To be clinically protective, a vaccine must be immunogenic. On the other hand, the fact that the vaccine of interest is immunogenic does not necessarily mean that it is also protective. In order to address these issues, the concept of "correlates of protection" (CoPs) was introduced several decades ago. According to the classical definition, a CoP is "*an immune response that is responsible for and statistically interrelated with protection*" [Plotkin 2010]. The immunogenicity of IVs can be quantified by several immunological assays that cover a variety of viral targets. The principal assays will be described and discussed in the following thesis sections.

The Overall Rationale and Objective of the PhD program

The nature of the present PhD thesis is intended to be both multidisciplinary and multi-stakeholder. National, regional and local health policies and strategies may be effectively implemented by engaging a variety of stakeholders, who may even come from outside the healthcare sector. According to the WHO [WHO 2020], the principal stakeholders are: governments (which may include not only the Ministry of Health, but also Finance, Treasury, Education, etc.); political parties; local governments; non-governmental organizations and the not-for-profit sector; community groups and civil society organizations; business and the private sector (including the pharmaceutical industry); healthcare payers (e.g. insurance groups); donors and aid agencies, including global health initiatives; United Nations (UN) agencies, including the WHO; healthcare workers' associations; patients and health service users [WHO 2020]. Obviously, this stakeholder heterogeneity may generate some conflicts of interest, since each stakeholder category pursues its own specific aims. However, the ultimate goal of all the above-listed stakeholders is to improve the overall health of the population [Omachonu and Einspruch 2010].

Unlike other vaccines, IVs are somewhat stand-alone products, since they have several particularities. First of all, on account of the above-mentioned evolutionary patterns of the virus, the vaccine composition has to be updated annually. Vaccine strains are selected under the guidance of the WHO, which reviews the Global Influenza Surveillance and Response System (GISRS) data on circulating viral populations throughout the world [Russell et al. 2008]. This review is carried out twice a year: in February for the northern hemisphere and in September for the southern hemisphere. The WHO's recommendations are based on several factors, including: (i) forecasts of which strains will probably cause the next epidemic; (ii) the degree of antigenic similarity between the chosen vaccine strains and the predicted circulating strains; (iii) the immunogenicity of the selected vaccine strains; and (iv) suitability of the selected vaccine strains for large-scale vaccine manufacturing [Tosh et al. 2010; Keshavarz et al. 2019].

Secondly, the efficacy/effectiveness of the currently available IVs may be far below those of other vaccines. For instance, the effectiveness of tick-borne encephalitis vaccines is approximately 99%, with non-significant between-age differences [Heinz et al. 2007], while meta-analytical estimates of the effectiveness of IVs range from 7% to 74% [Belongia et al. 2016]. Moreover, efficacy and effectiveness differ markedly according to the virus (sub)type,

the age-class of vaccinees and the seasonal degree of matching [Tricco et al. 2013; Belongia et al. 2016].

Thirdly, the current market of the available IVs is probably the most diversified [Centers for Disease Control and Prevention (CDC) 2019; Italian Ministry of Health 2020]. Indeed, the availability of single products differs substantially from one country or jurisdiction to another, the US being the largest market. Table 1.1 reports the principal IV types available in Europe during the 2019/20 season [ECDC 2019].

Table 1.1. Seasonal influenza vaccine types available during the 2019/20 influenza season in Europe.

Type/valence	Inactivated/Live attenuated	Production platform	Adjuvant
TIVe	Inactivated	Egg	–
QIVe	Inactivated	Egg	–
QIVc	Inactivated	Cell culture	–
QLAIV	Live attenuated	Egg	–
aTIV	Inactivated	Egg	MF59®
hdTIV	Inactivated	Egg	–

aTIV: adjuvanted trivalent influenza vaccine; hdTIV: high-dose trivalent trivalent influenza vaccine; MF59: microfluidized emulsion; QIVc: cell culture-derived quadrivalent influenza vaccine; QIVe: egg-derived quadrivalent influenza vaccine; QLAIV: quadrivalent live attenuated influenza vaccine; TIVe: egg-derived trivalent influenza vaccine.

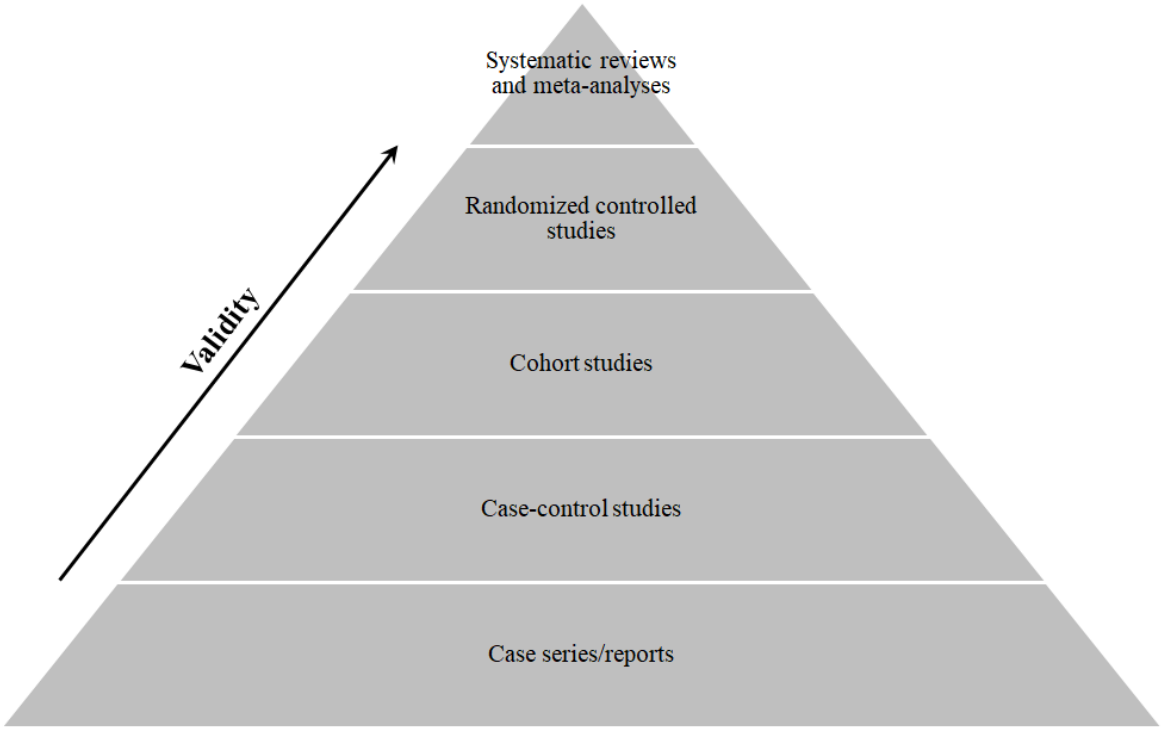
Fourthly, as mentioned above, some CoPs of IV-induced immunogenicity were established several decades ago. Consequently, they have been unable to keep up with novel technologies and currently circulating influenza virus populations [Trombetta et al. 2014].

In this thesis, we will try to incorporate the above-described features of influenza viruses into a novel *ad hoc* empirical approach that focuses on identifying, characterizing, systemizing and mapping modifiers of IV-induced immunogenicity from the multi-stakeholder point of view. Methodologically, the empirical approach adopted is essentially based on four high-grade evidence-based techniques, namely: (i) systematic synthesis; (ii) umbrella review; (iii) meta-analysis and/or meta-regression; and (iv) evidence-based mapping.

In the hierarchy of evidence-based medicine, systematic reviews (SRs) and meta-analyses (MAs) or both (SRMAs) stand at the top of the so-called "evidence-based medicine pyramid", as they have the highest level of internal validity and, therefore, the lowest risk of bias (Figure 1.1) [Murad et al. 2016]. On the other hand, despite their methodological rigor and minimization of the risk of bias SRMAs, which are usually performed by scientists whose

final product is an academic paper, are rarely consulted by policymakers and practitioners, who may need to think strategically and require a more user-friendly output (e.g. a summary written in a plain language). In this regard, a "knowledge-brokering platform pyramid" was recently constructed by White (2018) in order to describe evidence-based medicine platforms that adopt systematic approaches to drawing on bodies of rigorous evidence. Indeed, in the pyramid depicted in Figure 1.2, SRs are no longer located at the top [White 2018].

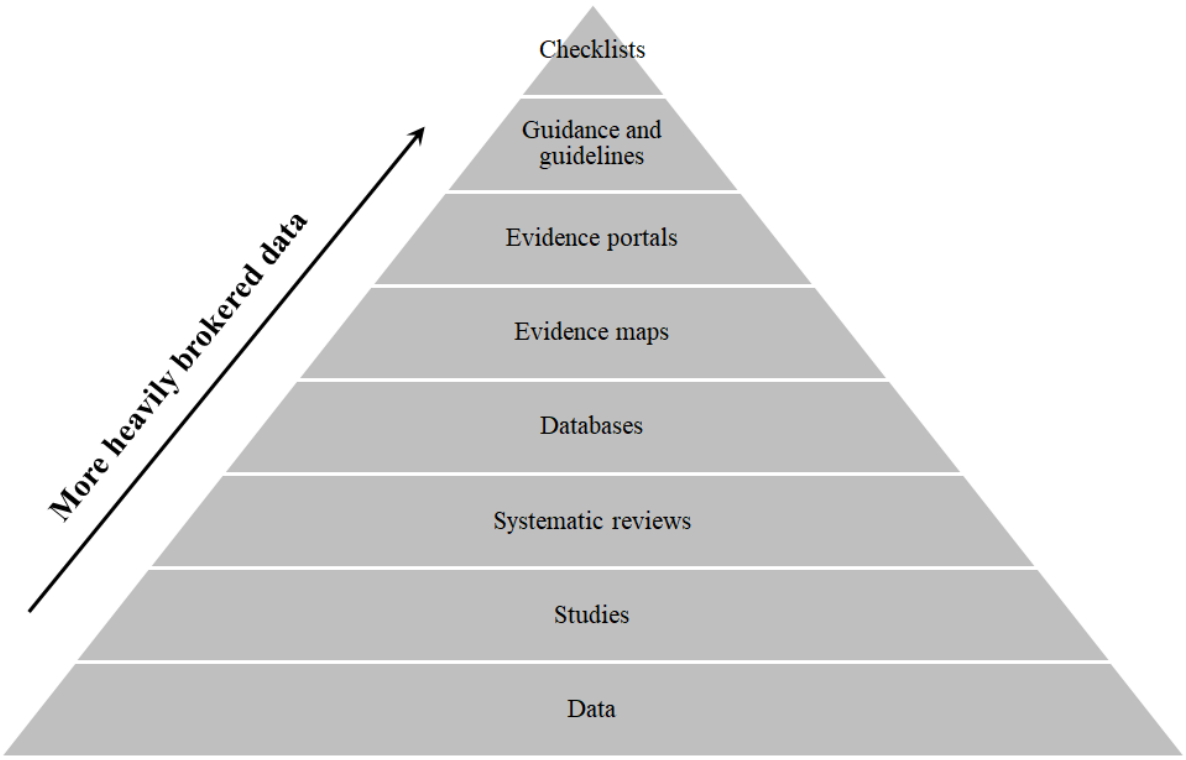
Figure 1.1. The traditional evidence-based medicine pyramid [adapted from Murad et al. 2016].



In the context of knowledge brokering, evidence mapping is given a prominent place. This novel evidence-based technique is characterized by five key components: (i) identification of gaps or needs; (ii) audience engagement/user-friendly products; (iii) broad field; (iv) systematic process; and (v) visual depiction [Miake-Lye et al. 2016]. Evidence maps may be constructed by using a variety of systematic methods, one of the most efficient of which is the umbrella review approach. This involves making a systematic synthesis of the existing SRMAs; as they preserve all the major strengths of SRMAs, umbrella reviews can be located at a higher level of evidence in the "classical" evidence-based medicine pyramid

depicted in Figure 1.1 [Fusar-Poli and Radua 2018]. The main advantage of umbrella reviews is that they are ideally suited to revealing whether the evidence surrounding the topic of interest yields similar conclusions or discrepant findings. Any discrepancies may then be explored in order to identify potential reasons for them [Aromataris and Munn 2020].

Figure 1.2. The knowledge-brokering platform pyramid [adapted from White 2018].



CHAPTER 2. STUDY 1: IMMUNOGENICITY MEASURES OF INFLUENZA VACCINES. A MAPPING STUDY OF REGISTERED CLINICAL TRIALS

Declarations

This chapter is a slightly modified version of the manuscript entitled "Immunogenicity Measures of Influenza Vaccines: A Study of 1,164 Registered Clinical Trials" by Alexander Domnich, Ilaria Manini, Donatella Panatto, Giovanna Elisa Calabrò and Emanuele Montomoli (© 2020 by the authors) published in Vaccines [Domnich et al. 2020]. The article is published in open access modality and distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/), which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Background and Rationale

Influenza is the leading infectious disease worldwide from the point of view of both attack rate and socioeconomic burden [Cassini et al. 2018]. Together with some general preventive strategies (e.g. frequent hand-washing, social distancing, etc.), IV is a cornerstone public health intervention and can substantially reduce the burden of disease [WHO 2012].

To be clinically effective, vaccines must, first of all, be immunogenic. Almost all currently available vaccines, including IVs, work through serum or mucosal antibodies that can block infection or bacteremia/viremia; the antibody level therefore predicts protection [Plotkin 2010]. In these situations, a precise, widely recognized threshold of the magnitude of antibody levels (as determined through a well-standardized immunological assay) may constitute the so-called CoP. According to a definition by Plotkin (2010), a CoP is "*an immune response that is responsible for and statistically interrelated with protection*".

The immune response to an IV can be measured by means of a variety of immunological assays, the most common being the hemagglutination-inhibition (HAI) assay. The almost ubiquitous use of the HAI assay is probably due to several factors. First of all, this assay is simple, rapid and inexpensive [Trombetta et al. 2018]. Second, it is/was required by

the principal regulatory agencies, such as the US Food and Drug Administration (FDA) (2007) and the European Medicines Agency (EMA) (2016), in order to register and/or update annual IV formulations. Third, unlike many other immunological assays, the HAI assay has a universally accepted CoP threshold (≥ 1:40) that is assumed to indicate a 50% reduction in the risk of acquiring laboratory-confirmed influenza [Trombetta et al. 2018; FDA 2007; EMA 2016]. This estimate comes from an old challenge study conducted in the early '70s [Hobson et al. 1972]. More recent studies have tried to verify the previously established ≥ 1:40 threshold of the HAI titer as a CoP. Some investigations have mostly corroborated this threshold [de Jong et al. 2003; Coudeville et al. 2010], while others have underlined the age-dependence of this cut-off [Black et al. 2011]. Indeed, it seems that children need a higher HAI titer (at least 1:110) in order to be protected.

Single radial hemolysis (SRH) is another immunological assay used to determine the immunogenicity of IVs. An SRH output of ≥ 25 mm^2 lysis zone roughly corresponds to the HAI cut-off titer of ≥ 1:40 [Delem and Jovanovic 1978]. Of note, the SRH assay has been officially recognized as a CoP by the EMA (2016) but not by the FDA (2007).

Apart from HAI and SRH, other tests are commonly used and may provide some additional and useful immunogenicity information. Specifically, virus neutralization (VN) assays quantify neutralizing antibodies, are among the only tests that measure the so-called "functional" antibodies directly [Trombetta et al. 2018], and may therefore predict vaccine effectiveness better [Cheng et al. 2012]. VN assays, however, are more laborious and time-consuming and less standardized than HAI; moreover, although HAI and VN titers usually show a high correlation, agreement in terms of nominal titers has been found to be relatively low [Sicca et al. 2020]. No universally recognized VN titer for 50% protection has been established so far [Dunning et al. 2016].

The enzyme-linked immunosorbent assay (ELISA), by contrast, is able to measure several class-specific antibodies (including IgM, IgA and IgG) in both serum and oropharyngeal/nasal wash samples [Trombetta et al. 2018]. ELISA correlates well with the output of HAI, SRH and VN [Trombetta et al. 2018]. However, it has the advantages of: (i) quantifying HA stalk-specific antibodies and (ii) assessing the immunogenicity of novel universal IV candidates. This is why ELISA is particularly suitable for large studies, as it can yield relatively unbiased results in little time, being fully automatic [Trombetta et al. 2018].

The quantification of anti-NA antibodies is becoming more common. After HA, NA is the second most abundant surface glycoprotein and allows the virus, in general terms, to spread the viral progeny from the infected host cell to uninfected cells [Eichelberger and Wan

2015]. Nevertheless, the NA antigen has been somewhat neglected in recent decades [Eichelberger and Monto 2019]. However, IVs containing optimal quantities of NA may be particularly useful in order to counteract the phenomena of viral drift and shift, given that NA immunity can offer broader protection [Eichelberger and Monto 2019].

Finally, only the humoral adaptive immunity assays have so far been discussed. However, CMI plays an important role in the host immune response in protecting against virus-related illness and in establishing long-term immunological memory. Although correlates of protection are not currently available for CMI, it would be advisable to investigate this kind of immunological response with a view to evaluating next-generation vaccines [Gianchecchi et al. 2019].

The objectives of this study were to summarize current patterns of the use of the various available immunological assays for measuring the IV-induced immune response and to identify the determinants of their use.

Materials and Methods

Search Strategy, Eligibility Criteria and Data Extraction

In the present study, clinical trials of interest were sought from the freely available US-based prospective registry of studies ClinicalTrials.gov [US National Library of Medicine 2020], which is the largest in the world.

In order to ensure the maximum number of potentially eligible items, we searched only for the term “Influenza” in the field “Condition or disease”. Adding the more colloquial word “Flu” did not increase the search output. No filters were used.

To be included in the study, clinical trials had both to be composed of vaccinees in at least one study arm and to use at least one immunological assay to quantify IV-induced immunogenicity. Studies were excluded if the actual enrollment was zero, as these trials probably never started. No other restrictions were applied.

The search output (all available columns) was downloaded in a comma-separated values file on 23 April 2020. Data of interest were then extracted in an *ad hoc* spreadsheet. If any information was unclear and/or missing, we consulted (whenever possible) publications of the results in peer-reviewed journals that are automatically linked to that particular study by ClinicalTrials.gov unique identifier.

Study Variables

As per our main objective, the study outcome was the relative distribution of immunological assays used to quantify IV-induced immunogenicity. On the basis of our previous experience, the following outcomes were set *a priori*: HAI, VN, ELISA, SRH, anti-NA and any CMI assays.

The independent variables of interest were categorized in three domains, namely: spatiotemporal, design-related and IV-related. The first included the start year and location of the study. The study location was categorized in macro-areas as follows: (i) Europe; (ii) US & Canada; (iii) Asia & Pacific; (iv) rest of the world (RoW) and (v) "Multicontinental". This last category included multicenter trials conducted in different parts of the world.

Attributes regarding the study design included: (i) age-class of the study population; (ii) study type; (iii) study phase; (iv) sponsorship characteristics and (v) sample size. As per ClinicalTrials.gov, three principle age-groups were defined: children (< 18 years), adults (18–64 years) and the elderly (≥ 65 years). Study type was either interventional or observational, while the study phase was I to IV. Regarding the latter variable, observational research was attributed to phase IV. Studies of phase I/II and II/III were classified as phase II and phase III, respectively. Regarding study sponsorship characteristics, we dichotomized this variable into "industry-sponsored" if a for-profit organization was the study (co)-sponsor, and "non-industry-sponsored" otherwise. The study sample size was the size on enrollment and was readily available in the downloaded file.

The IVs used were classified according to their valence [MIVs (usually pre-pandemic/pandemic), TIVs or QIVs], production platform (traditional egg-based or cell culture-derived/recombinant), presence of adjuvants, mode of administration (e.g. intramuscular/subcutaneous, intradermal, intranasal) and inactivation-related issues (inactivated and LAIV). The inactivated IVs included whole-virion, split or subunit formulations. Moreover, a dummy variable of the universal/supra-seasonal vaccine candidates (e.g. peptide-based, DNA-based) was also created. Virosomal and virus-like particle vaccines were included in the category of adjuvanted IVs [Kang et al. 2012]. As almost all intranasally administered IVs were LAIV, these two attributes were analyzed as the single dummy variable "live/intranasal" IVs. Of note, single studies could use different IV types.

Data Analysis

For descriptive purposes, the study outcome of the use of immunological assays was expressed as proportions with 95% CIs.

In order to identify potential predictors of the use of single immunological assays, we implemented a set of multivariable logistic regression models in which the outcome was a binary variable concerning the use of a given immunological assay (0 = No, 1 = Yes). The potential predictors were the variables described in the above subsection. We implemented both the fully-adjusted models and models selected on the basis of the Bayesian information criterion (BIC) minimization approach. The regression outputs were expressed as adjusted odds ratios (aORs) with their corresponding 95% CIs.

In our models, almost all independent variables were dichotomous. The only exception was the study sample size, which was continuous. This latter variable was highly right-skewed (skewness coefficient: 12.7) with an average of 649 [standard deviation (SD): 1,855] and a median of 180 (interquartile range: 78–471). For modeling purposes, we split the median. Indeed, the use of the continuous variable worsened the model fits.

P-values of < .050 were deemed statistically significant. McKelvey–Zavoina's pseudo-R^2 was used to quantify the explained variability. The Hosmer–Lemeshov test was performed to test the goodness-of-fit. Other model diagnostics included a formal check for multicollinearity; indeed, some potential predictors (e.g. study phase and sample size) could be highly correlated. Multicollinearity was tested by verifying the variance inflation factor (VIF).

All analyses were done in R stats packages, version 4.0.0 [R Core Team 2020].

Results

Selection of Clinical Trials and Immunological Assays Used

In total, 2,294 search items were available on the date of retrieval. Of these, 1,186 met the inclusion criteria. Another 22 studies reported zero enrollment and were excluded. Thus, 1,164 (50.7%) trials were analyzed (Annex A).

As expected, the HAI assay was used in the majority (80.6%) of studies, and about half of the studies used only this test. Other, less commonly used, humoral immunity assays were distributed as follows: VN (21.7%), ELISA (10.1%) and SRH (4.6%). Anti-NA antibodies were quantified only in 20 trials (1.7%). CMI was measured in 273 [23.5% (95%

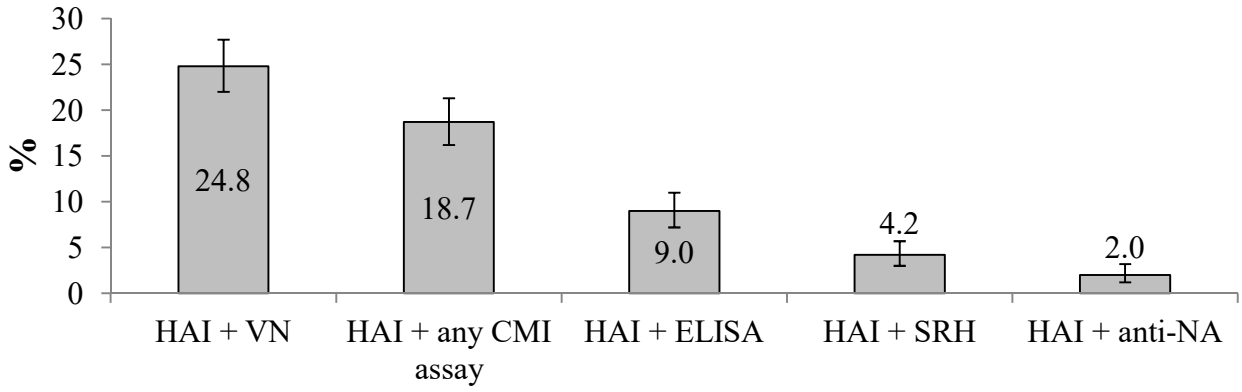
CI: 21.1–26.0%)] trials. About 60% of the studies included employed a single assay. Table 2.1 reports the descriptive statistics on the immunological assays used across the trials. The most common single co-occurrences of the use of HAI with other assays are instead reported in Figure 2.1.

Table 2.1. Frequency of the immunological assays used in the clinical trials included (*N* = 1,164).

Parameter	% (*N*)	95% CI
Humoral response only	76.5 (891)	74.0–79.0
Cell-mediated response only	3.0 (35)	2.1–4.2
One assay only	61.3 (714)	58.5–64.2
Two assays	26.8 (312)	24.3–29.4
Three assays or more	11.9 (138)	10.1–13.9
HAI assay	80.6 (938)	78.2–82.8
HAI assay only	47.8 (556)	44.9–50.7
VN assays	21.7 (253)	19.4–24.2
VN assays only	1.0 (12)	0.5–1.8
ELISA	10.1 (117)	8.4–11.9
ELISA only	0.9 (10)	0.4–1.6
SRH assay	4.6 (54)	3.5–6.0
SRH assay only	1.2 (14)	0.7–2.0
Anti-NA response	1.7 (20)	1.1–2.6
Anti-NA response only	0 (0)	0.0–0.3
Humoral response assay unclear	11.4 (133)	9.7–13.4

ELISA: enzyme-linked immunosorbent assay; HAI: hemagglutination-inhibition; NA: neuraminidase; SRH: single radial hemolysis; VN: virus neutralization.

Figure 2.1. Relative frequency of the single co-usage of hemagglutination-inhibition assay (HAI) and other tests of interest.



CMI: cell-mediated immunity; ELISA: enzyme-linked immunosorbent assay; HAI: hemagglutination-inhibition; NA: neuraminidase; SRH: single radial hemolysis; VN: virus neutralization.

Determinants of the Immunological Assays Used

As HAI was used in most of the studies included, it was deemed useless to establish its correlates.

The results of the multivariable models predicting the assessment of neutralizing antibodies are reported in Table 2.2. Compared with the fully-adjusted model, the best-subset model was associated with a significant (-6.5%) BIC reduction; however, the model fit of the latter was poor (Hosmer-Lemeshov test: $P = .043$). By contrast, the fully adjusted model fitted well (Hosmer-Lemeshov test: $P = .90$); we therefore retained this latter model for interpretation. From the point of view of the vaccine characteristics, monovalent, adjuvanted and cell culture-derived/recombinant IV formulations were significantly associated with a higher use of VN. There was also some increase in VN use as age increased; however, the effect was not significant in the mixed age-classes. Each additional year was associated with about a 7% increase ($P = .016$) in the odds of performing a VN. By contrast, studies (co)-sponsored by an industry and those conducted in the RoW were associated with lower odds of using VN assays. Moreover, the later phases of clinical development (phases III and IV) correlated negatively with the use of VN. The model explained 35.3% of variance.

Table 2.2. Multivariable logistic regression models to predict the use of immunological assays measuring neutralizing antibodies.

Variable	Level	Best-subset model		Full model	
		aOR (95% CI)	***P***	**aOR (95% CI)**	***P***
Vaccine	Monovalent[a]	5.68 (3.89–8.30)	< .001	3.35 (1.75–6.44)	< .001
	Trivalent[a]	–	–	0.70 (0.41–1.22)	.21
	Quadrivalent[a]	–	–	1.07 (0.57–1.98)	.84
	Adjuvanted[a]	1.48 (1.02–2.15)	.038	1.65 (1.10–2.46)	.015*
	Intradermal[a]	–	–	1.04 (0.45–2.37)	.93
	Live/intranasal[a]	–	–	1.43 (0.81–2.54)	.22
	Cell-derived/recombinant[a]	2.03 (1.37–3.00)	< .001	1.82 (1.18–2.80)	.006
	Universal candidates[a]	–	–	0.55 (0.19–1.63)	.28
Age	Any	Ref	–	Ref	–
	Children only	3.49 (1.22–9.99)	.020	2.92 (0.97–8.83)	.058
	Adults only	3.59 (1.32–9.75)	.012	2.90 (1.01–8.34)	.048
	Elderly only	5.58 (1.85–16.89)	.002	4.71 (1.49–14.88)	.008
	Children and adults	1.83 (0.52–6.42)	.35	1.68 (0.45–6.22)	.44
	Adults and elderly	1.67 (0.60–4.65)	.33	1.59 (0.54–4.66)	.40

Table 2.2 (*continued*). Multivariable logistic regression models to predict the use of immunological assays measuring neutralizing antibodies.

Variable	Level	Best-subset model		Full model	
		aOR (95% CI)	***P***	**aOR (95% CI)**	***P***
Study type	Observational	–	–	Ref	–
	Interventional	–	–	0.63 (0.26–1.49)	.29
Study phase	1	–	–	Ref	–
	2	–	–	0.72 (0.45–1.16)	.17
	3	–	–	0.49 (0.26–0.90)	.022
	4	–	–	0.36 (0.20–0.64)	< .001
Industry sponsored	No	Ref	–	Ref	–
	Yes	0.48 (0.33–0.68)	< .001	0.40 (0.26–0.90)	< .001
Time	Year[b]	1.08 (1.04–1.13)	< .001	1.07 (1.01–1.12)	.016
Sample size	< 180	–	–	Ref	–
	≥ 180	–	–	1.27 (0.87–1.86)	.21
Study location	Multicontinental	–	–	Ref	–
	Europe	–	–	0.67 (0.28–1.58)	.36
	US & Canada	–	–	0.62 (0.27–1.46)	.27
	Asia & Pacific	–	–	0.85 (0.36–2.04)	.72
	Rest of the world	–	–	0.09 (0.02–0.49)	.006
Pseudo-R^2, %		30.0		35.3	
BIC		1008.6		1074.2	

[a]Yes versus No; [b]One-year increase; ·P < .10; aOR: adjusted odds ratio; BIC: bayesian information criterion.

Factors associated with the use of ELISA are described in Table 2.3. Both models showed similar results, fit reasonably well (Hosmer-Lemeshov test: $P \geq .80$) and explained up to 42% of variance. Live attenuated/intranasal (aOR = 8.28) and cell-derived/recombinant (aOR = 2.41) IVs were positively associated with ELISA testing. By contrast, the use of adjuvanted IV formulations was a significant negative predictor. Compared with phase I clinical trials, those of phases II to IV were associated with a 64–90% lower rate of ELISA testing. Analogously, trials involving industry made less use of ELISA.

CMI was among the outcomes in 273 of the trials included [23.5% (95% CI: 21.1–26.0%)]. Results of the adjusted logistic models with the outcome of CMI assays are reported in Table 2.4. On the basis of goodness-of-fit, only the fully adjusted model was retained (Hosmer-Lemeshov test: $P = .61$) since the best-subset model proved to have a poor fit (Hosmer-Lemeshov test: $P = .002$). Trials investigating adjuvanted, live attenuated/intranasal IVs and, especially, universal vaccine candidates displayed significantly higher odds of quantifying CMI. As in the previously described models, industry co-sponsored trials and phase III studies reported a lower use of CMI assays. Moreover, larger studies were also associated with 38% lower odds of the model outcome.

In none of the models reported in Tables 2.2–2.4, did multicollinearity issues emerge: no VIF exceeded the nominal value of 5.

Finally, the outcomes of SRH and anti-NA assay use were relatively infrequent, and thus displayed a low event-per-predictor ratio. We therefore decided against the multivariable regression approach. However, two notable features regarding the SRH assay emerged: (i) all [100% (95% CI: 93.4–100%)] studies were industry sponsored and (ii) two thirds [66.7% (95% CI: 52.5–78.9%)] were conducted in Europe. By contrast, most [65.0% (95% CI: 40.8–84.6%)] anti-NA antibody testing was performed in the US.

Table 2.3. Multivariable logistic regression models to predict the use of enzyme-linked immunosorbent assay (ELISA).

Variable	Level	Best-subset model		Full model	
		aOR (95% CI)	***P***	**aOR (95% CI)**	***P***
Vaccine	Monovalent[a]	–	–	1.04 (0.37–2.96)	.94
	Trivalent[a]	–	–	1.62 (0.65–4.04)	.30
	Quadrivalent[a]	–	–	0.82 (0.34–2.01)	.67
	Adjuvanted[a]	0.34 (0.17–0.67)	.002	0.32 (0.15–0.65)	.002
	Intradermal[a]	–	–	0.30 (0.06–1.37)	.12
	Live/intranasal[a]	8.28 (4.86–14.11)	< .001	8.60 (4.75–15.56)	< .001
	Cell-derived/recombinant[a]	2.41 (1.37–4.24)	.002	2.28 (1.19–4.37)	.013
	Universal candidates[a]	–	–	1.97 (0.57–6.80)	.28
Age	Any	–	–	Ref	–
	Children only	–	–	1.40 (0.33–6.02)	.65
	Adults only	–	–	1.34 (0.34–5.35)	.68
	Elderly only	–	–	2.62 (0.56–12.29)	.22
	Children and adults	–	–	1.26 (0.25–6.33)	.78
	Adults and elderly	–	–	1.06 (0.26–4.35)	.94
Study type	Observational	–	–	Ref	–
	Interventional	–	–	0.57 (0.21–1.55)	.27
Study phase	1	Ref	–	Ref	–
	2	0.36 (0.19–0.66)	.001	0.37 (0.19–0.71)	.003
	3	0.10 (0.04–0.29)	< .001	0.12 (0.04–0.36)	< .001
	4	0.32 (0.18–0.59)	< .001	0.28 (0.14–0.59)	< .001
Industry sponsored	No	Ref	–	Ref	–
	Yes	0.45 (0.27–0.75)	.002	0.46 (0.27–0.81)	.007
Time	Year[b]	–	–	1.06 (0.99–1.14)	.090
Sample size	< 180	–	–	Ref	–
	≥ 180	–	–	0.72 (0.41–1.27)	.26

Table 2.3 (*continued*). Multivariable logistic regression models to predict the use of enzyme-linked immunosorbent assay (ELISA).

Variable	Level	Best-subset model	Full model

		aOR (95% CI)	*P*	aOR (95% CI)	*P*
Study location	Multicontinental	–	–	Ref	–
	Europe	–	–	1.89 (0.22–16.63)	.57
	US & Canada	–	–	1.16 (0.13–10.10)	.90
	Asia & Pacific	–	–	1.02 (0.11–9.36)	.98
	Rest of the world	–	–	0.89 (0.06–12.55)	.93
Pseudo-R^2, %		38.3		42.4	
BIC		571.3		666.9	

[a]Yes versus No; [b]One-year increase; aOR: adjusted odds ratio; BIC: bayesian information criterion.

Table 2.4 Multivariable logistic regression models to predict the use of immunological assays measuring cell-mediated immunity.

Variable	Level	Best-subset model		Full model	
		aOR (95% CI)	*P*	aOR (95% CI)	*P*
Vaccine	Monovalent[a]	–	–	1.48 (0.79–2.78)	.22
	Trivalent[a]	–	–	1.56 (0.90–2.70)	.11
	Quadrivalent[a]	–	–	1.76 (0.99–3.14)	.055
	Adjuvanted[a]	1.56 (1.09–2.23)	.014	1.53 (1.03–2.29)	.035
	Intradermal[a]	–	–	1.15 (0.58–2.27)	.69
	Live/intranasal[a]	2.56 (1.65–3.96)	< .001	2.66 (1.65–4.29)	< .001
	Cell-derived/recombinant[a]	–	–	1.35 (0.86–2.12)	.20
	Universal candidates[a]	10.10 (4.17–24.19)	< .001	10.05 (4.17–24.19)	< .001
Age	Any	–	–	Ref	–
	Children only	–	–	0.63 (0.27–1.48)	.29
	Adults only	–	–	1.16 (0.55–2.47)	.70
	Elderly only	–	–	0.92 (0.38–2.26)	.86
	Children and adults	–	–	0.60 (0.22–1.63)	.32
	Adults and elderly	–	–	0.83 (0.40–1.76)	.63
Study type	Observational	–	–	Ref	–
	Interventional	–	–	0.64 (0.35–1.17)	.15
Study phase	1	Ref	–	Ref	–
	2	0.56 (0.37–0.87)	.009	0.72 (0.45–1.14)	.16
	3	0.32 (0.18–0.56)	< .001	0.47 (0.25–0.90)	.022
	4	0.94 (0.62–1.42)	.76	1.17 (0.70–1.95)	.54
Industry sponsored	No	Ref	–	Ref	–
	Yes	0.32 (0.23–0.45)	< .001	0.31 (0.21–0.46)	< .001
Time	Year[b]	–	–	0.99 (0.94–1.03)	.60
Sample size	< 180	–	–	Ref	–
	≥ 180	–	–	0.62 (0.44–0.88)	.007

Table 2.4 (*continued*). Multivariable logistic regression models to predict the use of immunological assays measuring cell-mediated immunity.

Variable	Level	Best-subset model		Full model	
		aOR (95% CI)	*P*	aOR (95% CI)	*P*
Study location	Multicontinental	–	–	Ref	–
	Europe	–	–	3.95 (0.84–18.54)	.08
	US & Canada	–	–	2.28 (0.49–10.68)	.30

Asia & Pacific	–	–	1.68 (0.35–8.14)	.52
Rest of the world	–	–	2.32 (0.41–13.31)	.34
Pseudo-R^2, %	23.4		29.5	
BIC	1144.4		1224.4	

[a]Yes versus No; [b]One-year increase; aOR: adjusted odds ratio; BIC: bayesian information criterion

Discussion

To our knowledge, the present study is the first to describe and analyze the use of the available immunological assays for quantifying the immunogenicity of the currently licensed IVs and vaccine candidates. Several findings emerged from the present analysis. First (and not to our surprise), we found that most of the studies included used only one assay, which in most cases was the HAI. Second, we found that some IV formulations and some study design attributes, such as phase or sponsorship, were associated with the patterns of use of particular immunological assays. We will now discuss our principal findings.

Regarding IV valence characteristics, we did not generally find any meaningful correlation, the only exception being the significantly higher probability of neutralizing antibody quantification in trials involving MIVs. In our study most MIVs were either pandemic A(H1N1)pdm09 or pre-pandemic vaccines against several avian type A subtypes with pandemic potential [e.g. A(H5N1), A(H7N9)]. Indeed, VN has proved to be particularly useful in studying the serology of avian type A strains, and several studies have documented the unsuitability of HAI for the detection of antibodies against these viruses [Rowe et al. 1999; Stephenson et al. 2009; Trombetta et al. 2014]. Moreover, the pattern observed may be somehow linked to antigen-sparing as a strategy for pandemic preparedness promoted by the WHO (2004). Indeed, in the present analysis, many (pre)-/pandemic studies were dose finding. Compared with HAI, VN can detect antibodies at lower titers, distinguish better between small differences (e.g. less than two-fold) in pre- and post-vaccination titers, and requires a lower concentration in order to yield a judgment of protection (though no formally established threshold has been universally recognized) [Trombetta et al. 2014; Sui et al. 2009; Nunes et al. 2018; Hsu et al. 2014; Verschoor et al. 2016].

Adjuvanted IVs (including virosomal and virus-like particle formulations) were associated with a higher use of both VN and CMI assays. Adjuvanted IVs have been systematically shown to induce both stronger and broader humoral immune responses [Nicolay et al. 2019; Banzhoff et al. 2003]. However, widespread use of the standard HAI assay may downplay some important potential advantages of the adjuvanted formulations, such as cross-protection and immunological memory. According to the FDA's Center for

Biologics Evaluation and Research (CBER) guidelines [FDA 2007] despite the public health advantage of adjuvants in terms of dose-sparing, some safety issues may arise; however, the subsequent risk–benefit assessment asserts that "meaningful differences may also include a demonstration of cross-reactivity against drifted strains" [FDA 2007]. In such situations, VN assays may provide an advantage: they identify a wide range of antibodies, including those that neutralize the virus by inhibiting its entry/replication in mammalian cells, while HAI only measures antibodies against HA, which act by preventing the agglutination of red blood cells [Trombetta et al. 2014]. Ansaldi et al. (2010), for example, showed that, in elderly subjects immunized with an adjuvanted TIV, the correlation coefficient *r* between the mean-fold increase in neutralizing antibody titers (from pre- to post-vaccination) and the antigenic distance of several drifted A(H3N2) strains was substantially higher than the *r* between the corresponding mean-fold increase in HAI titers (.701 vs .501). Analogously, the use of CMI, which plays a crucial role in protecting against influenza by establishing the long-term immunological memory [Gianchecchi et al. 2019], may also positively "differentiate" the adjuvanted formulations from their non-adjuvanted counterparts. For instance, Zedda et al. (2015) found that adding an adjuvant to standard IVs induced a larger expansion of vaccine-specific CD4+ cells, and that this advantage was evident with regard to the drifted heterologous strains. In sum, our results suggest that measuring neutralizing antibodies and CMI may constitute the so-called "correlates of adjuvanticity" [Del Giudice et al. 2018]; it is therefore advisable to better standardize protocols for these assays, in order to reduce intra- and inter-laboratory variation, and to revise the current immunogenicity guidelines [Trombetta et al. 2014].

Intranasal IV formulations (mostly LAIV) proved more likely to be tested in ELISA, with a huge effect size (ES) of 8.3. The use of LAIV formulations was also seen to be a positive predictor of CMI assays. The standard HAI assay is often judged poorly suitable for LAIVs [Trombetta et al. 2014] and, unlike the case of inactivated IVs, no CoPs have been established for LAIVs [Weinberg et al. 2016]. For instance, in their challenge study, Wright et al. (2016) showed that some traditional measurements of immune response, such as HAI, did not correlate with protection provided by a LAIV. Indeed, LAIV formulations are believed to induce multifaceted immunogenicity ascribable to both local/mucosal immunoglobulins and T cell responses [Mohn et al. 2018]. In an analysis of three clinical studies, Ambrose et al. (2012) found that nasal wash IgA measured by means of ELISA contributed to the efficacy of LAIV in young children. Nasal wash IgG and IgM may also increase in recipients of LAIV [Ambrose et al. 2012]. With regard to CMI, Forrest et al. (2008) showed that this may have

greater importance in subjects immunized with LAIVs in comparison with inactivated formulations. In their study, it was also estimated that the majority of children with ≥ 100 interferon-γ spot-forming cells per 10^6 peripheral blood mononuclear cells were protected against clinical influenza, suggesting that this level could be a possible target in clinical trials. As discussed by Ambrose et al. (2012), in Plotkin's framework of CoPs [Plotkin 2010], the association between the protection induced by live IV and IgA responses measured by means of ELISA constitutes the so-called co-correlate of protection, which is "*one of two or more factors that correlate with protection in alternative, additive, or synergistic ways*" [Plotkin 2010]. Indeed, strain-specific IgA responses are associated with protection in vaccinees, but the level of response may vary by strain and trial, and IV-induced protection may be correlated with other components of the immune response [Ambrose et al. 2012].

Universal vaccine candidates have likewise showed far greater odds of being scrutinized through CMI assays. Universal IVs comprise a large and heterogeneous variety of experimental vaccine formulations with different platforms, targets and mechanisms of action [Pica and Palese 2013; Gilbert 2013; Zhang et al. 2014]. As we have already mentioned, the currently accepted as a CoP assays of HAI and SRH target the viral HA. By contrast, most next-generation universal IVs target some highly conserved proteins that are common across viral (sub)types [Pica and Palese 2013; Gilbert 2013; Zhang et al. 2014]. This is why the recognized CoPs and other widely used immunological assays are likely to prove unsuitable for the universal vaccine candidates. On the other hand, CMI will undoubtedly be quantified in future trials on the next-generation IVs, since cross-reactive CD4+ and CD8+ T cells have already been proposed as future CoPs in human challenge and cohort studies [Krammer et al. 2020].

Unlike the other tests analyzed, and independently from the IV formulations used, VN assays were employed increasingly over the 20-year period considered. As described earlier, the proliferating interest in quantifying neutralizing antibodies is probably determined by the fact that, unlike the conventional assays, VN tests measure the functional capability of antibodies and not just their total quantity, and are more efficient in quantifying cross-reactivity/cross-immunogenicity.

Industry-(co)sponsored trials quantified neutralizing and ELISA antibodies and determined CMI to a significantly lesser extent than non-industry-sponsored studies. By contrast, about 85% of industry-(co)sponsored studies determined the HAI response, while 100% of studies that used the SRH assay were industry-(co)sponsored. The most probable explanation is two-fold: (i) both HAI and SRH have a well-recognized threshold as a CoP [de

Jong et al. 2003; Coudeville et al. 2010; Black et al. 2011; Delem and Jovanovic 1978] and (ii) clinical guidelines are available for industry to support the licensure of seasonal IVs [FDA 2007; EMA 2016]. Indeed, the US document issued by the CBER [FDA 2007] cites some criteria to support accelerated approval of new IVs, and all these criteria are based on the HAI assay. Indeed, in the case of children and adults aged < 65 years, clinical trials should show that the lower limit of the two-sided 95% CI for the percentage of subjects achieving seroconversion (defined as the proportion of vaccinees with at least a 4-fold increase from before to after IV) and seroprotection (defined as the proportion of vaccinees with an HAI titer ≥ 1:40) reaches or exceeds 40% and 70%, respectively. In the case of elderly subjects these proportions are reduced by 10% (to 30% and 60%, respectively) [FDA 2007]. The previous European criteria issued by the EMA's Committee for Medicinal Products for Human Use (CHMP) [EMA 2016] were similar, but were based on the point estimates rather than the 95% CIs. Moreover, unlike in the US guidelines, the endpoints determined by the SRH assay were recommended only from the European perspective [EMA 2016]; this is probably why we found that most SRH assays were performed in Europe.

In all our analyses, a later phase of clinical development was generally associated with a lower use of VN, ELISA and CMI assays. Contrary to our expectations, we did not encounter any problem of collinearity between the study phase and sample size; an adequately powered sample size is usually directly related to the study phase. The observed absence of collinearity issues was probably driven by the sample size dichotomization rule adopted. Early phase trials on vaccines usually have safety endpoints as primary outcomes; however, some exploratory immunogenicity endpoints may also be assessed (usually as secondary outcomes). By contrast, pivotal phase III trials are designed to provide robust clinical data in support of licensure [WHO 2016; WHO 2017b]. Modern phase III immunogenicity trials enroll thousands of individuals, each of whom is tested for the IV-induced immune response at least twice. In these conditions, the HAI assay was most frequently used, not least because this test is both widely recognized as a CoP and relatively cheap [Trombetta et al. 2018]. Indeed, the reproducibility and unbiased assessment of an assay should be weighed against its cost-effectiveness [Sicca et al. 2020]. On the other hand, the EMA guidelines [EMA 2016] state that "*It is essential that neutralizing antibody titers are determined in all studies*", "*Measurement of … CMI is encouraged*" and "*Applicants may consider evaluating anti-NA antibodies*". We therefore believe that more sophisticated techniques should also be used in at least a subset of participants in pivotal clinical trials.

Despite its strengths, such as its large sample size and meaningful set of predictors, the current study may have some notable limitations. First of all, the information retrieved came mostly from the registered information on clinical trials and is therefore is highly dependent on the quality of this latter. In this regard, Viergever et al. (2014) have shown that the quality of registration at ClinicalTrials.gov is suboptimal, although some slight improvements have been seen over time. We tried to attenuate this bias by consulting the available peer-reviewed publications linked to a given trial; however, this was not always possible. Indeed, in 11.4% of the trials included, we failed to identify the humoral immunity assays used (although we believe that most of these used the HAI assay). This is also why we cannot completely rule out mistakes due to misclassification bias of the study outcome. For instance, records indicating that the immune response was measured according to the CBER criteria were assumed to refer to studies that used the HAI assay, given that the US criteria consider only the HAI assay [FDA 2007].

Second, although ClinicalTrials.gov is the world's largest and first-established registry, we acknowledge that a certain number of studies were registered in other supranational (e.g. the European register available at www.clinicaltrialsregister.eu) or country-specific databases, which were not searched systematically. At present, it is not possible to perform a simultaneous search in more databases nor can double-registered trials be directly linked via a single identifier (in order to avoid duplicates). We believe, however, that the sample of registered trials analyzed is globally representative, given the pioneering nature of ClinicalTrials.gov. We also believe that this shortcoming is particularly relevant with regard to the non-industry-sponsored trials; indeed, in our sample approximately 62% of items were industry-(co)sponsored. Vaccine manufacturers are obliged to prospectively register their trials, and the technical documents commonly submitted to the regulatory agencies have to contain a complete list of the studies that support marketing authorization applications [Jørgensen et al. 2018].

Third, considering a relative paucity of studies that measured CMI, no attempt to further categorize CMI assays was made. It is therefore probable that some meaningful associations were “hidden” by the classification rule adopted.

Finally, we were not able to identify determinants of the SRH and anti-NA assays, owing to the paucity of studies using these tests. Indeed, according to a widely applied “rule of thumb” [Harrell et al. 1996], we needed at least 10 events per independent variable. We tried to address this issue by applying Firth’s penalized logistic regression approach; the output was, however, not consistent (results not shown) [Puhr et al. 2017].

In conclusion, the IV-induced immune response may be measured by means of a variety of immunological assays; these are, however, unevenly distributed across the available registered trials. Continuous diversification of the IV market and research into a universal IV will probably produce a gradual shift from the currently preferred HAI test to other more "functional" assays; assays that measure CMI seem particularly promising. Technological innovation can involve high costs and exerts strong financial pressure on health systems. Today's healthcare systems cannot forgo technological innovation, but must take into account the point of view of the various stakeholders: patients should be guaranteed rapid access to more effective healthcare technologies; research and development efforts should be encouraged when oriented towards the production of high-value products; institutions and regulatory agencies should support innovation by using evidence-based tools for their evaluation, such as health technology assessment (HTA); and health systems should promote technological innovation while ensuring their own sustainability [European Commission 2016]. In this regard, governments around the world are increasingly focusing on the use of public–private partnerships that can combine the strengths of private enterprise, such as innovation, technical knowledge and managerial skills, with the role of public institutions, including social responsibility and public accountability, in order to deliver high-quality health services [Roehrich et al. 2014]. Future IV clinical trials will undoubtedly benefit from functional public-private partnerships, especially from the point of view of searching for new CoPs.

CHAPTER 3. STUDY 2: MAPPING HOST-RELATED CORRELATES OF INFLUENZA VACCINE-INDUCED IMMUNE RESPONSE: AN UMBRELLA REVIEW WITH A SERIES OF META-ANALYSES OF THE AVAILABLE SYSTEMATIC EVIDENCE

Declarations

Background and Rationale

Influenza is the world's leading annually occurring infectious disease, and places an enormous burden on public health [WHO 2012]. For instance, in the general European population, it ranks first in terms of both attack and mortality rates [surpassing, for example, tuberculosis and human immunodeficiency virus (HIV) infection], and results in an average annual loss of 81.8 DALYs per 100,000 inhabitants) [Cassini et al. 2018].

Annual IV is the main public health intervention able to reduce the burden of disease [WHO 2012; de Lusignan et al. 2012]. Most currently available IVs are egg-derived, inactivated (either split or subunit), trivalent or quadrivalent [CDC 2019a; ECDC 2019]. The WHO's most recent position paper [WHO 2012] has recognized some priority target groups for annual IV: pregnant women, children aged 6 months to 5 years, the elderly, subjects with specific chronic conditions, healthcare workers and international travelers. Despite the

recommendations of the WHO (which has also adopted the value-based approach) [WHO 2012], seasonal IV policies are well-established only in high- and a few middle-/low- income countries [Sambala et al. 2019]. In most countries of the WHO European Region, for example, vaccination is recommended for the elderly, people with underlying risk conditions, institutionalized populations, healthcare workers and (in fewer countries) children and pregnant women [WHO 2014], while in the US, it is universally recommended (for all subjects aged 6 months and above) [Grohskopf et al. 2018].

IV-induced protection is ideally measured by conducting RCTs in which the clinical endpoint is laboratory-confirmed influenza. Such studies are, however, expensive. Moreover, their execution is hindered by some particularities of influenza, including its variable annual attack rates, extremely high seasonality pattern in most countries, frequent viral mutations, heterogeneity of the circulating virus population, and varying annual vaccine composition [Dewe et al. 2013]. This is why most IV RCTs use immunogenicity parameters as their primary endpoints. In a simplistic way, it may be claimed that IV-induced protection against laboratory-confirmed influenza is entirely mediated by a single surrogate endpoint (i.e. immune response). In other words, the IV-induced protection must correlate with the IV-induced immune response [WHO 2013]. Plotkin [Plotkin 2010] has defined a correlate of vaccine-induced protection (CoP) as "*an immune response that is responsible for and statistically interrelated with protection*"; in this study, we will adopt this definition.

HAI is the most commonly used assay in IV RCTs. Historically, an HAI threshold titer of 1:40 was associated with a 50% reduction in the absolute risk of contracting laboratory-confirmed influenza [Trombetta and Montomoli 2016]; this figure comes from an adult challenge study dating back to the early '70s [Hobson et al. 1972]. More recent meta-analytical models, however, seem to agree to some extent with that estimate. Specifically, de Jong et al. [de Jong et al. 2003] estimated that the HAI titer of 1:192 was associated with a 90% median reduction in laboratory-confirmed influenza. The Bayesian meta-analytical approach adopted by Coudeville et al. [Coudeville et al. 2010] established that the incremental increase in clinical protection was very marked at HAI titers of up to 1:100, while the benefit became marginal at titers > 1:150. Nonetheless, the above-described HAI "universal" threshold of 1:40 may be not appropriate for some population groups. Indeed, it has been estimated [Black et al. 2011] that, in children, the conventional HAI titer cut-off of 1:40 is associated only with a 22% protection rate, while the cut-offs of 1:110, 1:215, 1:330 and 1:629 predict 50%, 70%, 80% and 90% protection, respectively.

Other, less commonly used, immunological assays are: SRH, VN and ELISA [Trombetta and Montomoli 2016]. Among these, only SRH has an established CoP threshold, which is ≥ 25 mm^2 lysis zone; this roughly corresponds to the HAI cut-off titer of 1:40 [Delem and Jovanovic 1978]. Apart from the humoral immune response CoPs, cellular CoPs are increasingly being recognized and used [Gianchecchi et al. 2018].

Schematically, the immunogenicity, efficacy and effectiveness of IVs may be deemed to be a function of the characteristics of the host, virus and vaccine [Belongia and McLean 2019]. Among the host factors, the age of the vaccinee is probably the factor most known to affect the immunogenicity of IVs. While a poor immune response in the youngest age-class is usually ascribed to immaturity of the immune system [Mugitani et al. 2014], in the elderly it has been linked to immunosenescence [Haq and McElhaney 2014]. The factors related to the influenza virus are well-known: intense selection by the host immune system drives antigenic change in both A and B virus types and results in the continuous replacement of circulating strains with new ones that can re-infect hosts that are immune to previously circulating variants (the phenomenon known as "antigenic drift") [Ferguson et al. 2003]. In the past few years, however, the "vaccine-related" factor has received more attention, owing to the continuously diversifying IV market. For instance, adjuvanted, intradermal and high-dose IVs have consistently been shown to enhance immunogenicity in comparison with standard-dose intramuscularly administered non-adjuvanted vaccines [Ng et al. 2019].

Several intrinsic host characteristics (e.g. age, sex, genetic polymorphisms, and many morbidities) can be viewed as unmodifiable factors that may alter the immune response following vaccination. On the other hand, modifiable host-related factors (e.g. lifestyle habits or dietary patterns) may also potentially interfere with IV-induced immunogenicity. A down-to-earth appraisal of these factors and their impact on the IV-induced immune response is essential both to the design of future immunogenicity RCTs and to the potential development of better and/or more personalized IVs [Zimmermann and Curtis 2019] or, at least, IV-related public health policies. A recently published narrative review [Castrucci 2018] examined some host factors that affect the immune response to IV; the following factors were discussed: preexisting immunity, immunosenescence, genetic polymorphism, sex, obesity and the presence of chronic underlying medical conditions. In the present paper, we adopted a comprehensive approach, in that we conducted an analysis of the evidence from the available systematic analyses of the intrinsic host factors affecting IV-induced immunogenicity. Indeed, the objective was to analyze and graphically synthetize the available systematic evidence on the host factors able to modify IV-induced immunogenicity.

Materials and Methods

General methodology

The present paper was conceived as an umbrella review. The umbrella review [also known also as overview/review of (systematic) reviews, meta-review, and similar] is an emerging field of evidence-based medicine and is becoming increasingly common, given the growing number of systematic reviews on the same/similar topics (see Figure 1.2). The key feature of umbrella reviews is that they are focused on the highest possible level of evidence, i.e. SRs and/or MAs. Furthermore, umbrella reviews are seen as a ready means of enabling relevant stakeholders to gain a clear understanding of a broad topic area [Aromataris et al. 2015]. Indeed, the host factors affecting IV-induced immunogenicity constitute a vast and heterogeneous group of modifiers.

No ethical approval was deemed necessary, given the second-hand nature of this research.

Search Strategy

The search strategy was first calibrated by using a simplistic search string implemented in Google Scholar (www.scholar.google.com): [“influenza” AND “vaccine” AND (“immunogenicity” OR “immune response”) AND (“systematic review” OR “meta analysis”)]. We then examined the first 500 search results and selected potentially eligible papers. We selected only the first 500 results, given the low specificity of Google Scholar: the search produced more than 16,000 results. Google Scholar was chosen since, unlike well-established scientific databases, it can work well with the so-called “gray literature” [Haddaway al. 2015]. A more detailed search strategy was then developed and tested on PubMed (www.ncbi.nlm.nih.gov/pubmed) in order to ensure that all records selected in Google Scholar appeared in the PubMed results output. The following PubMed script was then judged appropriate: ((((((“influenza" OR "flu")) OR influenza, human[mh])) AND (((vaccin* OR immuni*)) OR vaccines[mh]))) AND ((review literature as topic[mh]) OR ("systematic review" OR "meta analysis" OR "meta regression")). The above-described search algorithm was adapted to Embase (www.embase.com) and the search output was retrieved. We then searched the Cochrane Library (www.cochranelibrary.com); however, given that this focuses on SRs/SRMAs, the search was limited to the single keyword

"influenza", in order to increase its sensitivity. The automatic search outputs from the three databases were pooled in a single spreadsheet, and duplicates were removed in a semi-automatic modality. The last automatic search was performed on 2 July 2019 by Alexander Domnich.

The automatic search was subsequently followed by a manual search. This included: (i) standard cross-reference checking of the manuscripts included; (ii) checking articles that cited the SR/SRMAs included (through Google Scholar in order to check for possible sources of the "gray literature"); (iii) seeking advice on additional SR/SRMAs from academic experts/industry. We also tried to search the principal RCT registries (www.clinicaltrials.gov and www.clinicaltrialsregister.eu) and "gray literature" databases (www.opengrey.eu), though this proved fruitless.

We then updated (according to the last search time-period declared by the authors of the SRs/SRMAs included) the list of primary studies by applying the same search strategy and the same inclusion and exclusion criteria used in the SRs/SRMAs included.

Eligibility Criteria and Inclusion Process

All SRs or SRMAs concerning the host factors potentially affecting IV-induced immunogenicity were eligible. The inclusion criteria were formulated according to the PICO (population, intervention, control, outcome) framework. Specifically, no restrictions were placed on the population groups (e.g. age or health conditions) and settings. The intervention was IV of any type. Cases were defined as vaccinees with a given health condition that could modify the IV-induced immune response, while controls were vaccinees without that condition (usually healthy controls). The outcome of interest was the humoral immune response, as measured by HAI. This choice was based on the results of Study 1 and our previous experience; moreover, HAI is: (i) a relatively cheap, well standardized assay with a well-established threshold as a correlate of protection [Trombetta and Montomoli 2016] and (ii) is/was required by regulatory agencies in Europe [EMA 2016] and the US [FDA 2007].

The following statistical parameters associated with the HAI are commonly used and/or required: geometric mean titers (GMTs) following IV, seroconversion rate (SC), usually defined as proportion of vaccinees with at least a 4-fold increase from before to after IV, and seroprotection rate (SP), usually defined as the proportion of vaccinees with an HAI titer ≥ 1:40 [EMA 2016; FDA 2007]. All these parameters were planned *a priori* for inclusion in the meta-synthesis.

SRs/SRMAs of both experimental and observational studies could be included in the analysis. RCTs are a well-known means of comparing two or more experimental arms in a relatively unbiased way, which is why the SRMAs of RCTs were our primary choice. However, several host factors that may potentially alter IV-induced immunogenicity are relatively rare in the general population; observational studies may therefore be more convenient than RCTs. Moreover, some ethical issues may arise from not offering IV to people for whom it is recommended. For this reason, we also decided to include SRs/SRMAs of observational studies (both cohort and case-control).

In the first step, we screened titles and/or abstracts of the combined duplicate-free search output for the following exclusion criteria: (i) animal or *in vitro* studies; (ii) no active immunization with IVs, (iii) no immunogenicity endpoints as correlates of protection (e.g. only efficacy, effectiveness, safety, acceptance and other irrelevant outcomes); (iii) non-systematic nature of the manuscript (e.g. narrative or expert-driven reviews), and (iv) conference abstracts/proceedings with little available information. However, the reference lists of any identified narrative reviews on the topic of interest were screened.

All potentially eligible records and those whose eligibility was unclear from the title/abstract underwent full-text assessment. Full texts meeting all the inclusion criteria were included in the analysis unless they met the following exclusion criteria: (i) no predefined control group (e.g. the assessment of IV-induced immunogenicity in a given "ill" population, as in the case of cross-sectional study design); (ii) no separate information on IV-induced immunogenicity (i.e. an SR/SRMA dealing with vaccines against several diseases); (iii) control groups composed of unvaccinated individuals; (iv) SRs/SRMAs aimed at comparing different IV types; (v) MAs without a formal systematic search (in this case, however, the lists of primary studies included were assessed); (iv) SRs/SRMAs entirely focused on immunological assays other than HAI.

The study selection process was made by two reviewers (Alexander Domnich and Ilaria Manini), each working independently. Any disagreement was solved by discussion.

Data Extraction

Data were extracted and imported into an *ad hoc* spreadsheet by two reviewers (Alexander Domnich and Ilaria Manini), each working independently. Any disagreement was solved by discussion. The following data were extracted: first author and year of publication; review design (SR or SRMA); host factor(s) evaluated; study designs included (RCTs,

observational or both); number of studies included (*k*); study population and setting; total number of cases and controls (see definitions above); statistical parameters of interest used to describe the immunogenicity endpoints; principal results of qualitative and quantitative syntheses; authors' conclusions; funding sources; other potentially relevant information. When possible, the immunogenicity endpoints were extracted separately for each virus (sub)type (e.g. A/H1N1, A/H3N2, B).

Quality Appraisal

The SRs/SRMAs included were assessed by means of the AMSTAR-2 instrument (a measurement tool for assessing systematic reviews, version 2) [Shea et al. 2017]. This is an updated version of AMSTAR that is able to assess SRs of both RCTs and non-randomized studies; it consists of 16 items (instead of the 11 available in the previous version) and has simpler voting rules. The response categories are: “Yes”, “Partial yes”, “No” and “0” [i.e. not applicable (NA), if, for example, no MA was performed]. Of note, this instrument is not designed to produce a single overall SR/SRMA rating [Shea et al. 2017].

Once sufficiently trained by consulting the available comprehensive user guide [Shea et al. 2017], two reviewers (Alexander Domnich and Giovanna Elisa Calabrò) provided independent votes on each paper included. Any disagreements were solved by involving a third author (Chiara de Waure).

Data Analysis and Synthesis

Once extracted, the data from single manuscripts were first tabulated and summarized qualitatively. Results of SRs without MAs were summarized narratively and separately from SRMAs.

The papers included were then classified according to the host factor studied. If more than one SR/SRMA covered the same/similar host factor, we created citation matrices and quantified the corrected covered areas, as described by [Pieper et al. 2014]. Specifically, the corrected covered area allows the overlap of primary studies included in different SRs to be quantified; it is expressed as $(N – r)/(rc – r)$, where N is the number of papers included in all available SRs, r is the number of original primary studies, and c is the number of SRs. This overlap was categorized as slight (0–5%), moderate (6–10%), high (11–15%), and very high (> 15%) [Pieper et al. 2014].

The results of single MAs were expressed in terms of different ESs, and the models adopted used different estimators. Moreover, some important information was sometimes missing from the meta-analytical output and/or was inadequately reported. In addition, we were able to identify some novel primary research studies. Consequently, we re-applied MAs by extracting the data from single primary studies (also considering the citation matrices described above) in order to be able to visualize the effect of different host factors on the same scale. We decided *a priori* to use both the random-effects and fixed-effects models. If the pooled random- and fixed-effects estimates differed substantially (as usually occurs in the case of high heterogeneity), the random-effects estimate was retained in our conclusions.

The pooled ESs for the binary outcomes of SC and SP were expressed as ORs with corresponding 95% CIs. For the continuous endpoint of post-immunization GMTs, Hedges' *g* with 95% CIs was used. Given the skewed nature of GMTs, pooling was performed by means of loge-transformation, as recommended by the US Advisory Committee on Immunization Practices (2013).

Heterogeneity was quantified by means of I^2 and τ^2. As per Cochrane's Handbook [Cochrane Collaboration 2011], publication bias was assessed only for meta-analyses with $k \geq 10$. Publication bias was assessed by computing Rücker's arcsine and Egger's tests for dichotomous and continuous endpoints, respectively. We decided not to test the excess significance bias formally, as proposed by Ioannidis and Trikalinos (2007), since this test is not currently recommended by the Cochrane Collaboration (2011). Instead, we visually inspected the contour-enhanced funnel plots and noted substantial asymmetries. The 95% prediction intervals were also calculated in order to determine in which range the next data point would probably lie.

Variability measures reported on different scales [e.g. 95% CIs, standard errors (SEs)] were converted to SDs, as recommended by the Cochrane Collaboration (2011).

Missing values of the dispersion measures of GMTs were treated in a *post-hoc* modality. These values were inferred by averaging the available $\log_e$-transformed SDs from similar studies. We excluded studies in which the SDs had been inferred in sensitivity analyses in order to verify the robustness of the base case.

SC, SP rates and GMTs for the three virus (sub)types were pooled separately.

Having envisioned a sufficient number of meta-analyzed studies, we planned *a priori* to conduct a series of sensitivity analyses in order to determine the sources of the heterogeneity observed. For this purpose, we conducted subgroup and/or meta-regression

analyses. The independent variables to include were selected *post hoc* on the basis of *k*, data availability and/or model fitting.

All statistical analyses were carried out by means of R (version 3.6.1) [R Core Team 2020] and the stats packages "meta" [Schwarzer 2019] and "metafor" [Viechtbauer 2019].

Cumulative Evidence Synthesis (CES)

Once all predefined summary ESs had been calculated, the following recommended rules [Fusar-Poli and Radua 2018] were applied in order to categorize the available evidence:

- Convincing (class I): pooled number of cases (N) > 1,000, $P < .000001$, $I^2 < 50\%$, 95% prediction interval excluded the null, no small-study effect/publication bias;
- Highly suggestive (class II): $N > 1{,}000$, $P < .000001$, largest study with a statistically significant effect ($P < .05$) and class I criteria not met;
- Suggestive (class III): $N > 1{,}000$, $P < .001$ and class I–II criteria not met;
- Weak (class IV): $P < .05$ and class I–III criteria not met;
- Non-significant: P for the observed ES > .05.

As highlighted in the previous section, differently from the recommendations made by [Fusar-Poli and Radua 2018], we did not formally test the excess significance bias. However, the absence of excess significance bias is required only for CES class I. In the present paper, only 6/97 (6%) "mappable" estimates fell within CES I, and visual inspection of the contour-enhanced funnel plots did not suggest any excess of the "statistical significance" at $\alpha < .05$.

Evidence mapping was then performed in order to contextualize the available evidence, identify gaps in the systematic research and present the data of this study in a readily intelligible way [Wang et al. 2016]. To do this, we created bubble plots considering the variables (i.e. CES and health condition) of interest.

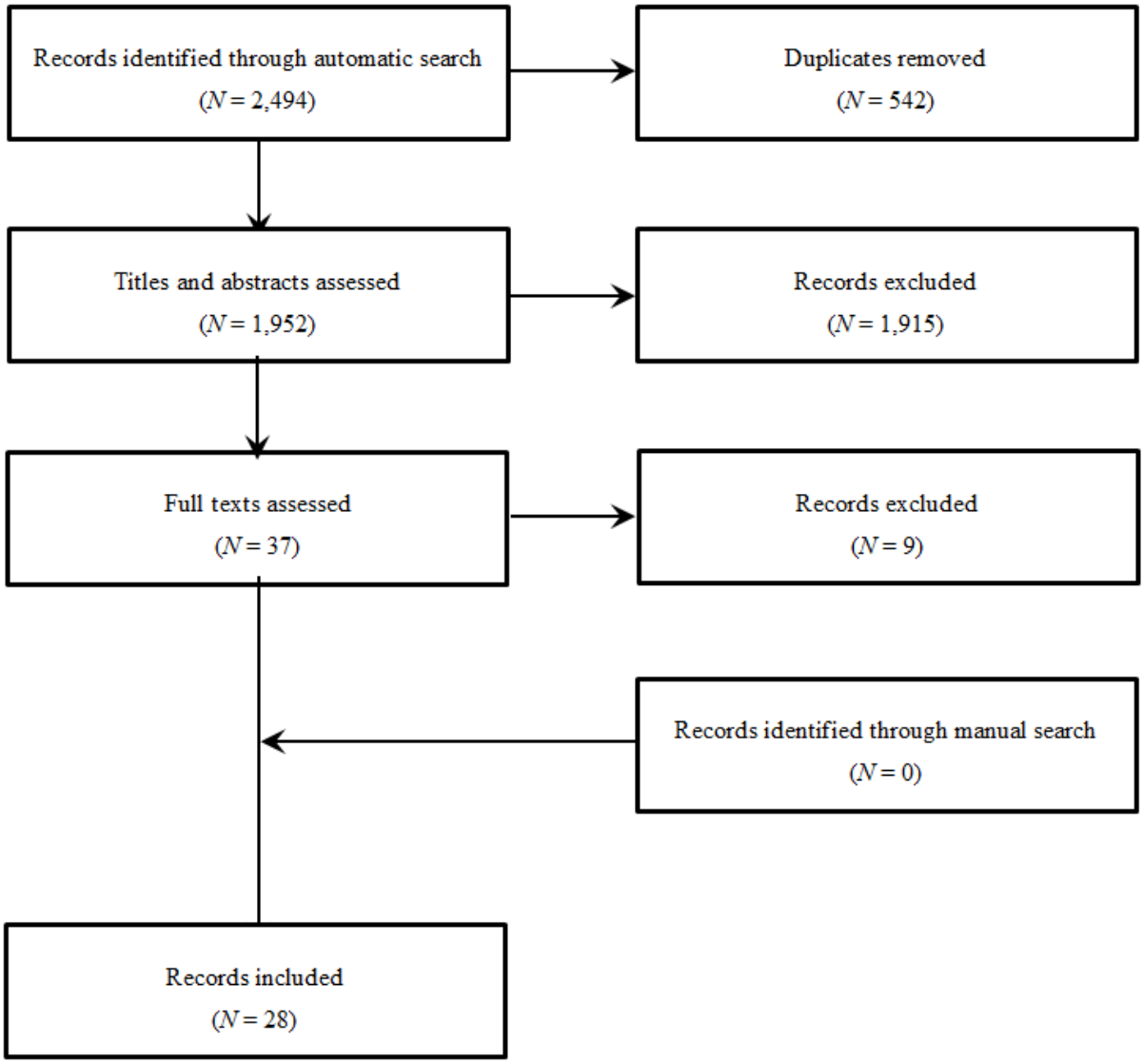
Results

Selection Process and Main Characteristics of the Systematic Reviews and/or Meta-Analyses Included

The whole selection process is depicted in Figure 3.1. Briefly, the automatic search of three databases produced 2,494 records, 542 of which were duplicates. The title/abstract screening procedure allowed us to eliminate a further 1,915 records as clearly ineligible. Of

37 full texts assessed, a total of 28 records [Baral et al. 2007; Pedersen et al. 2009; Beck et al. 2011; Agarwal et al. 2012; Beck et al. 2012; Eckerle et al. 2013; Goossen et al. 2013; Hua et al. 2014; McMahan and Bingham 2014; Pascoe et al. 2014; Posteraro et al. 2014; Shehata and Karim 2014; Karbasi-Afshar et al. 2015; Nguyen et al. 2015; Huang et al. 2016; Liao et al. 2016a; Pugès et al. 2016; Huang et al. 2017; Lei et al. 2017; Sousa et al. 2017; Vollaard et al. 2017; Dos Santos et al. 2018; Lee et al. 2018; Subesinghe et al. 2018; Yeh et al. 2018; Zimmermann and Curtis 2018a; Zimmermann and Curtis 2018b; van den Berg et al. 2019] were retained in the present analysis. No eligible records were identified through manual search and expert/industry consultation. A list of the excluded studies, with reasons for exclusion, is provided in Annex B.

Figure 3.1. Record selection process.



The main characteristics of the SRs/SRMAs included are reported in Table 3.1. Most (61%, 17/28) of the records included were SRMAs, while the remaining 39% were SRs without any quantitative synthesis. The following host factors were covered: intravenous drug use ($N = 1$), psychological stress ($N = 1$), acute exercise (i.e. a short bout of intensive physical activity) and chronic physical exercise (i.e. habitual physical activity, such as fitness training) ($N = 1$), genetic polymorphisms ($N = 1$), use of pre-/pro-/symbiotics ($N = 3$), BCG (Bacillus Calmette–Guérin) vaccination ($N = 1$), diabetes mellitus ($N = 1$), vitamin D supplementation/deficiency ($N = 1$), latent cytomegalovirus (CMV) infection ($N = 1$) and various forms of immunosuppression ($N = 17$). This last category included immunosuppressive conditions and/or drugs associated with rheumatic diseases, cancer, organ transplantation and inflammatory bowel disease (IBD), HIV, etc. (Table 3.1). However, we should point out that the classification adopted is a working one, since, for example, diabetes and intravenous drug use may also be seen as immunosuppressive conditions; we split these conditions into single categories on account of their particular public health burden.

The number of the IV-related studies included in each SR/SRMA was highly skewed (range: 1–209) and presented a median of 15 (interquartile range: 9–18). With regard to the quality of reporting, no SR/SRMA met all 16 AMSTAR-2 criteria (Table 3.1). The most "problematic" AMSTAR-2 items were those regarding the explanation of the study designs for inclusion (item 3), an explicit list of the studies excluded (item 7) and consideration of the funding source of the primary studies included (item 10) (Figure 3.2). No correlation emerged between the number of "Yes" AMSTAR-2 votes and the year of SR publication (Spearman's $\rho = 0.07$, $P = .74$). Detailed information on the AMSTAR-2 votes on the SRs included is provided in Annex C.

Table 3.1. Main characteristics of the systematic reviews and/or meta-analyses included.

First author	Year	Factor(s) assessed	Type of study	Age[†]	*k*[†]	Meta-analysis	AMSTAR-2[‡]
Baral	2007	Intravenous drug use	Obs	Adults	2	No	2/2/8/4
Pedersen	2009	Psychological stress	Obs	All	13	Yes	7/2/7/0
Beck	2011	IS of any etiology	RCT, obs	All	209	Yes	12/0/4/0
Agarwal	2012	IS drugs	RCT, obs	All	11	No	3/1/8/4
Beck	2012	IS by etiology	RCT, obs	All	209	Yes	12/0/4/0

Table 3.1 (*continued*). Main characteristics of the systematic reviews and/or meta-analyses included.

First author	Year	Factor(s) assessed	Type of study	Age†	*k*†	Meta-analysis	AMSTAR-2‡
Eckerle	2013	Solid organ transplants	RCT, obs	All	36	Yes	6/1/9/0
Goossen	2013	Chemotherapy in cancer patients	RCT, CCT	Children	9	No	10/0/2/4
Hua	2014	Antirheumatic drugs in RA patients	Obs	Adults	7	Yes	5/2/9/0
McMahan	2014	Biological and non-biological drugs in rheumatic patients	Unclear	All	18	No	1/0/11/4
Pascoe	2014	Acute and chronic physical exercise	RCT, obs	All	15	No	5/1/6/4
Posteraro	2014	Genetic variations	Obs	Adults	1	Yes	7/1/8/0
Shehata	2014	Cancer patients on systemic treatment	Unclear	All	16	No	2/0/10/4
Karbasi-Afshar	2015	Transplant recipients	Obs	Unclear	15	Yes	1/0/15/0
Nguyen	2015	IS drugs in IBD patients	Obs	All	2	Yes	2/3/11/0
Huang	2016	SLE	Obs	All	15	Yes	11/1/4/0
Liao	2016a	SLE	Obs	All	18	Yes	8/2/6/0
Pugès	2016	SLE	Obs	Adults	17	Yes	10/1/5/0
Huang	2017	RA	Obs	Adults	13	Yes	8/1/7/0
Lei	2017	Probiotics, prebiotics and symbiotics	RCT	Adults	20	Yes	9/1/6/0
Sousa	2017	Rheumatic diseases	RCT, obs	Children	11	No	3/0/9/4
Vollaard	2017	Solid tumor patients on chemotherapy	Obs	Adults	20	No	1/0/15/0
Dos Santos	2018	Diabetes mellitus	RCT, obs	All	15	No	6/0/6/4
Lee	2018	Vitamin D deficiency	RCT	All	9	Yes	6/2/8/0

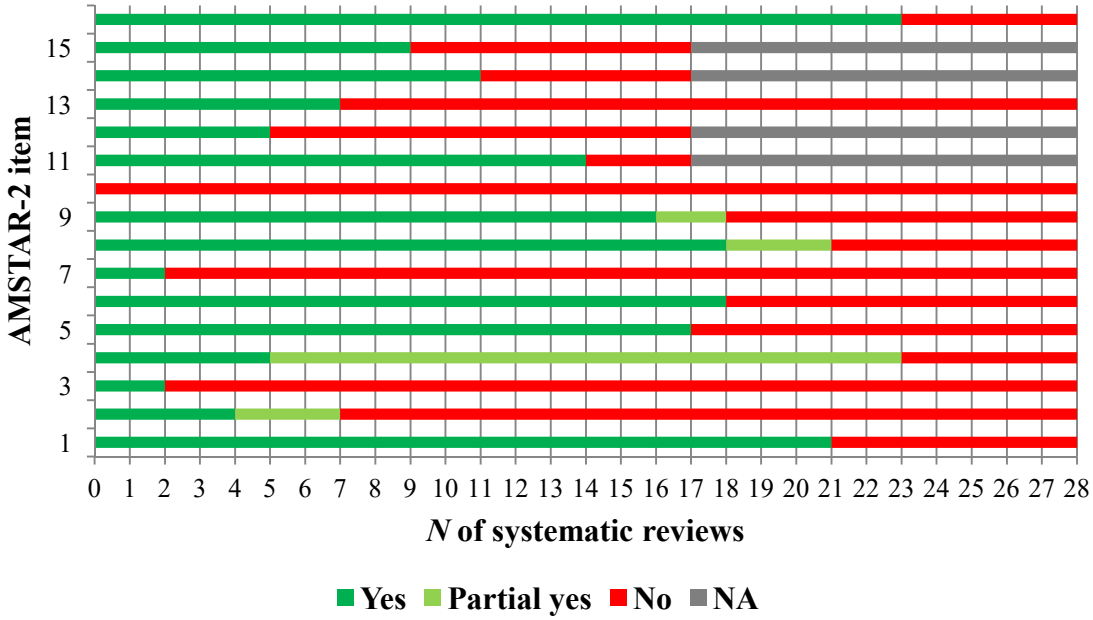
Table 3.1 (*continued*). Main characteristics of the systematic reviews and/or meta-analyses included.

First author	Year	Factor(s) assessed	Type of study	Age†	*k*†	Meta-analysis	AMSTAR-2‡
Subesinghe	2018	Antirheumatic drugs in RA patients	Obs	Adults	7	Yes	9/1/6/0
Yeh	2018	Probiotics, prebiotics and symbiotics	RCT	Adults	20	Yes	8/2/6/0

Zimmermann	2018a	Probiotics	RCT	Adults	12	No	3/1/8/4
Zimmermann	2018b	BCG vaccination	RCT, CCT	Adults	3	No	3/1/8/4
van den Berg	2019	Latent CMV infection	RCT, obs	All	15	Yes	12/0/4/0

†Considering only studies on influenza vaccines (if a systematic review also considered other vaccines); ‡Results are reported as Yes/Partial yes/No/Not applicable; AMSTAR: measurement tool for assessing systematic reviews; BCG: Bacillus Calmette–Guérin; CCT: controlled clinical trial; CMV: cytomegalovirus; IBD: inflammatory bowel disease; IS: immunosuppression/immunosuppressive; RCT: randomized controlled trial; Obs: observational study; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus

Figure 3.2. AMSTAR-2 (measurement tool for assessing systematic reviews, version 2) ratings, by item.



Use of Probiotics, Prebiotics or Symbiotics

Three SRs [Lei et al. 2017; Yeh et al. 2018; Zimmermann and Curtis 2018a] evaluated the effect of using pro-/pre-/symbiotics on IV-induced immunogenicity. All three papers included only RCTs with at least two intervention arms, i.e. (i) intervention group: use of pro-/pre-/symbiotics and (ii) control group: placebo or other dietary supplements not containing pro-/pre-/symbiotics.

The SRMA by Lei et al. (2017) pooled binary outcomes (SCs and SRs), while Yeh et al. (2018) pooled post-vaccination HAI titers that were deemed to be "mean titers" throughout the results. Zimmermann and Curtis (2018a) did not conduct any MA. Some concerns

regarding the pooling technique used by Yeh et al. (2018) emerged. Specifically: (i) no transformation (e.g. $\log_e$) of the skewed summary antibody titers was undertaken, as shown by forest plots; (ii) an enormous variation in SDs; (iii) arithmetic and geometric means were pooled together.

A total of 23 meta-analytical estimates with at least 2 pooled primary RCTs were extracted from the two SRMAs [Lei et al. 2017; Yeh et al. 2018]; the number of pooled studies ranged from 2 to 12, and more than half were statistically significant ($P < .05$) (Table 3.2). Briefly, Lei et al. (2017) reported a statistically significant advantage of taking pro-/prebiotics in terms of immunogenicity with regard to all-age SC against type B and SP against A/H1N1 and A/H3N2. Yeh et al. (2018) reported better results in the treatment arms in terms of the magnitude of the HAI titer against all three (sub)types; however, heterogeneity was extremely high (I^2 = 94–100%), casting doubt on the appropriateness of pooling single estimates. The narrative synthesis by Zimmermann and Curtis (2018a) concluded that the beneficial effect of probiotics on the IV-induced immune response was seen in 5 out of 12 (42%) studies analyzed. As expected (since all three SRs were published in a one-year period), the corrected covered area was very high (57.9%).

Given the above-described inconsistencies in ES estimates, we re-pooled the available primary studies according to our methodology. Briefly, the total number of RCTs included ranged from 8 to 13. Statistically significant ORs were seen with regard to SP against type A viruses: subjects taking any pro-/pre-/symbiotic were significantly more likely to be protected against A/H1N1 and A/H3N2 (by 68% and 93%, respectively). With regard to SCs and post-vaccination HAI titers against all three (sub)types, there were no statistically significant differences between cases and controls. Table 3.3 reports the evidence synthesis of the effect of pro-/pre-/symbiotics on IV-induced immunogenicity. The level of evidence for the statistically significant estimates was, however, categorized as "class IV".

Table 3.2. Pooled estimates extracted from the available meta-analyses on the effect of probiotic, prebiotic or symbiotic use in order to enhance the influenza vaccine-induced immune response (all models are random-effects).

First author (Year)	Type	Age	Virus	Parameter (ES)	*k*	ES	95% CI	*P*	I^2, %
Lei (2017)	Pro/prebiotics	Any	A/H1N1	SC (OR)	6	1.52	0.75–3.09	.25	51
Lei (2017)				SP (OR)	7	1.83	1.19–2.82	.006	0
Yeh				Δ mean titers	12	7.14	2.73–11.55	.002	96

(2018)				(MD)					
Lei (2017)		Adults		SC (OR)	2	0.62	0.18–2.14	.45	0
Lei (2017)		Elderly		SC (OR)	3	2.93	1.47–5.87	.002	3
Lei (2017)		Any	A/H3N2	SC (OR)	6	2.54	0.93–6.91	.07	83
Lei (2017)				SP (OR)	7	2.85	1.59–5.10	.0004	0
Yeh (2018)				Δ mean titers (MD)	12	17.19	3.39–30.99	.01	100
Lei (2017)		Adults		SC (OR)	2	3.46	1.22–9.83	.02	0
Lei (2017)		Elderly		SC (OR)	3	3.68	1.11–12.25	.03	75
Lei (2017)		Any	B	SC (OR)	6	2.11	1.38–3.21	.0006	0
Lei (2017)				SP (OR)	7	0.99	0.65–1.52	.97	0
Yeh (2018)				Δ mean titers (MD)	12	4.17	0.37–7.96	.03	94
Lei (2017)	Probiotics		A/H1N1	SC (OR)	4	1.91	0.68–5.38	.22	56
Yeh (2018)				Δ mean titers (MD)	7	4.71	0.53–8.89	.03	97
Lei (2017)			A/H3N2	SC (OR)	4	3.52	1.45–8.53	.005	63
Yeh (2018)				Δ mean titers (MD)	7	16.86	0.87–32.85	.04	100
Yeh (2018)			B	Δ mean titers (MD)	7	3.04	-0.77–6.85	.12	96

Table 3.2 (*continued*). Pooled estimates extracted from the available meta-analyses on the effect of probiotic, prebiotic or symbiotic use in order to enhance the influenza vaccine-induced immune response (all models are random-effects).

Lei (2017)	Prebiotics	Any	A/H1N1	SC (OR)	2	0.99	0.54–1.83	.98	0
Yeh (2018)				Δ mean titers (MD)	5	35.15	0.31–70.00	.05	72
Lei (2017)			A/H3N2	SC (OR)	2	1.31	0.22–7.98	.77	75
Yeh (2018)				Δ mean titers (MD)	5	18.66	-13.24–50.56	.25	69
Yeh (2018)			B	Δ mean titers (MD)	5	20.68	-9.07–50.42	.17	79

CI: confidence interval; ES: effect size; MD: mean difference; OR: odds ratio; SC: seroconversion rate; SP:

Table 3.3. Summary evidence of the effect of using probiotics, prebiotics or symbiotics to enhance the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	10	8	9
N	277/274	235/229	266/250
OR RE (95% CI)	1.55 (0.86–2.90)	1.34 (0.72–2.50)	1.14 (0.75–1.74)
***P* RE**	.14	.35	.54
95% PI	0.38–6.50	0.36–5.00	0.75–1.74
OR FE (95% CI)	1.42 (0.96–2.09)	1.12 (0.75–1.66)	1.14 (0.75–1.74)

***P* FE**	.075	.58	.54
I^2, %	49.6	49.0	0
τ^2	0.42	0.35	0
SSE, *P*	.49	NA	NA
LS	No	No	No
CES	ns	ns	ns
Seroprotection rate			
k	13	11	11
N	845/855	805/812	800/810
OR RE (95% CI)	1.68 (1.02–2.75)	1.93 (1.08–3.44)	0.94 (0.73–1.23)
***P* RE**	.040	.026	.66
95% PI	0.47–5.98	0.64–3.13	0.73–1.23
OR FE (95% CI)	1.25 (0.98–1.59)	1.94 (1.20–3.13)	0.94 (0.73–1.23)
***P* FE**	.067	.006	.66
I^2, %	56.2	24.9	0
τ^2	0.36	0.23	0
SSE, *P*	.18	.58	.38

Table 4.3 (*continued*). Summary evidence of the effect of using probiotics, prebiotics or symbiotics to enhance the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroprotection rate (*cont.*)			
LS	No	No	No
CES	IV	IV	ns
Post-vaccination HAI titer			
k	11	10	10
N	399/398	380/374	380/374
***g* RE (95% CI)**	0.05 (-0.09–0.19)	0.05 (-0.10–0.19)	0.00 (-0.15–0.14)
***P* RE**	.49	.53	.96
95% PI	-0.09–0.19	-0.10–0.19	-0.15–0.14
***g* FE (95% CI)**	0.05 (-0.09–0.19)	0.05 (-0.10–0.19)	0.00 (-0.15–0.14)
***P* FE**	.49	.53	.96
I^2, %	0	0	0
τ^2	0	0	0
SSE, *P*	.84	.78	.009
LS	No	No	No
CES	ns	ns	ns

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; HAI: hemagglutination-inhibition; LS: the largest study has a statistically significant effect size; ns: non-significant; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test.

We then conducted a subgroup analysis according to the type of supplement used (i.e. pro-/pre- or symbiotic) (Tables 3.4–3.6). No definite conclusions could be drawn. Specifically, statistically significant ORs were seen in the following comparisons: (i) probiotic use and SC against A/H1N1 [2.89 (95% CI: 1.19–6.99)] (Table 3.4) and (ii)

prebiotic use and SP against A/H1N1 [2.72 (95% CI: 1.14–6.50)] (Table 3.5). In all meta-analyses performed random- and fixed-effects models showed generally comparable results; indeed, the heterogeneity was usually low-to-moderate.

Sub-analysis by age-class revealed two statistically significant comparisons: SC against A/H3N2 in working-age adults [OR 2.32 (95% CI: 1.07–5.03)] and SP against A/H1N1 in the elderly [OR 2.40 (95% CI: 1.25–4.60)] (Table 3.6). The paucity of the available RCTs did not allow us to perform a meta-regression analysis.

Table 3.4. Subgroup analysis (by supplement type and age-class) of the summary evidence on the effect of probiotic, prebiotic or symbiotic use on seroconversion rates.

Parameter		A/H1N1	A/H3N2	B
Probiotics	***k***	3	2	3
	N	71/73	52/49	96/91
	OR RE (95% CI)	*2.89 (1.19–6.99)*	2.37 (0.41–13.78)	1.81 (0.90–3.65)
	OR FE (95% CI)	*2.89 (1.19–6.99)*	1.59 (0.71–3.58)	1.81 (0.90–3.65)
	I^2 **, %**	0	50.2	0
Prebiotics	***k***	4	3	3
	N	132/125	109/104	96/83
	OR RE (95% CI)	2.21 (0.78–6.21)	1.42 (0.42–4.77)	1.16 (0.58–2.36)
	OR FE (95% CI)	1.58 (0.93–2.71)	0.95 (0.52–1.75)	1.16 (0.58–2.36)
	I^2 **, %**	56.8	48.1	0
Symbiotics	***k***	3	3	3
	N	74/76	74/76	74/76
	OR RE (95% CI)	0.67 (0.24–1.85)	1.14 (0.33–3.92)	0.58 (0.24–1.41)
	OR FE (95% CI)	0.73 (0.36–1.50)	1.06 (0.53–2.10)	0.60 (0.27–1.34)
	I^2 **, %**	45.9	68.9	14.3
Adults	***k***	4	3	3
	N	89/94	70/70	70/70
	OR RE (95% CI)	1.28 (0.61–2.72)	*2.32 (1.07–5.03)*	0.84 (0.39–1.79)
	OR FE (95% CI)	1.28 (0.61–2.72)	*2.32 (1.07–5.03)*	0.84 (0.39–1.79)
	I^2 **, %**	0	0	0
Elderly	***k***	6	5	6
	N	188/180	165/159	196/180
	OR RE (95% CI)	1.86 (0.77–4.47)	0.92 (0.48–1.77)	1.31 (0.79–2.18)
	OR FE (95% CI)	1.47 (0.94–2.32)	0.86 (0.55–1.37)	1.31 (0.79–2.18)
	I^2 **, %**	65.1	35.6	0

Statistically significant estimates are evidenced *in italics*; CI: confidence interval; FE: fixed-effects model; OR: odds ratio; RE: random-effects model

Table 3.5. Subgroup analysis (by supplement type and age-class) of the summary evidence on the effect of probiotic, prebiotic or symbiotic use on seroprotection rates.

Parameter		A/H1N1	A/H3N2	B
Probiotics	***k***	5	4	4
	N	630/644	611/622	611/620
	OR RE (95% CI)	1.76 (0.92–3.37)	1.20 (0.49–2.94)	1.02 (0.75–1.40)
	OR FE (95% CI)	1.15 (0.86–1.53)	1.37 (0.71–2.66)	1.02 (0.75–1.40)
	I^2 **, %**	51.8	34.8	0
Prebiotics	***k***	4	3	3
	N	132/125	109/104	104/103
	OR RE (95% CI)	*2.72 (1.14–6.50)*	*3.86 (1.33–11.19)*	0.88 (0.48–1.61)
	OR FE (95% CI)	*2.36 (1.36–4.08)*	*3.86 (1.33–11.19)*	0.88 (0.48–1.61)
	I^2 **, %**	43.1	0	0

Table 3.5 (*continued*). Subgroup analysis (by supplement type and age-class) of the summary evidence on the effect of probiotic, prebiotic or symbiotic use on seroprotection rates.

Parameter		A/H1N1	A/H3N2	B
Symbiotics	***k***	4	4	4
	N	83/86	85/86	85/87
	OR RE (95% CI)	0.69 (0.24–2.00)	2.38 (0.60–9.44)	0.60 (0.21–1.74)
	OR FE (95% CI)	0.64 (0.28–1.43)	2.27 (0.91–5.65)	0.66 (0.31–1.42)
	I^2 **, %**	32.5	54.5	37.6
Adults	***k***	5	4	4
	N	620/636	601/612	601/612
	OR RE (95% CI)	0.92 (0.68–1.25)	1.97 (0.79–4.91)	0.96 (0.68–1.34)
	OR FE (95% CI)	0.92 (0.68–1.25)	1.85 (0.87–3.94)	0.96 (0.68–1.34)
	I^2 **, %**	0	29.0	46.4
Elderly	***k***	8	7	7
	N	225/219	204/200	199/198
	OR RE (95% CI)	*2.40 (1.25–4.60)*	1.87 (0.78–4.49)	0.92 (0.61–1.40)
	OR FE (95% CI)	*2.17 (1.45–3.26)*	*2.00 (1.08–3.71)*	0.92 (0.61–1.40)
	I^2 **, %**	52.7	38.8	0

Statistically significant estimates are evidenced *in italics*; CI: confidence interval; FE: fixed-effects model; OR: odds ratio; RE: random-effects model

On applying a *post-hoc* modality to each study included in the available SR/SRMAs, it emerged that most studies had been funded by dietary supplement producers, though this was not considered in the available SRMAs [Lei et al. 2017; Yeh et al. 2018; Zimmermann and Curtis 2018a]. We therefore conclude that the estimates provided may be prone to the so-called "industry sponsorship" bias.

Table 3.6. Subgroup analysis (by supplement type and age-class) of the summary evidence on the effect of probiotic, prebiotic or symbiotic use on post-vaccination hemagglutination-inhibition titers.

Parameter		A/H1N1	A/H3N2	B
Probiotics	***k***	6	5	5
	N	233/232	214/208	214/208
	***g* RE (95% CI)**	0.08 (-0.10–0.27)	0.06 (-0.13–0.26)	0.00 (-0.19–0.19)
	***g* FE (95% CI)**	0.08 (-0.10–0.27)	0.06 (-0.13–0.26)	0.00 (-0.19–0.19)
	I^2 **, %**	0	0	0
Prebiotics	***k***	2	2	2
	N	92/90	92/90	92/90
	***g* RE (95% CI)**	0.07 (-0.23–0.36)	0.03 (-0.26–0.32)	0.04 (-0.25–0.33)
	***g* FE (95% CI)**	0.07 (-0.23–0.36)	0.03 (-0.26–0.32)	0.04 (-0.25–0.33)
	I^2 **, %**	0	0	0

Table 3.6 (*continued*). Subgroup analysis (by supplement type and age-class) of the summary evidence on the effect of probiotic, prebiotic or symbiotic use on post-vaccination hemagglutination-inhibition titers.

Parameter		A/H1N1	A/H3N2	B
Symbiotics	***k***	3	3	3
	N	74/76	74/76	74/76
	***g* RE (95% CI)**	-0.08 (-0.40–0.24)	0.01 (-0.31–0.33)	-0.06 (-0.38–0.26)
	***g* FE (95% CI)**	-0.08 (-0.40–0.24)	0.01 (-0.31–0.33)	-0.06 (-0.38–0.26)
	I^2 **, %**	0	0	0
Adults	***k***	3	2	2
	N	67/73	48/49	48/49
	***g* RE (95% CI)**	0.15 (-0.19–0.49)	0.08 (-0.31–0.48)	-0.02 (-0.42–0.37)
	***g* FE (95% CI)**	0.15 (-0.19–0.48)	0.08 (-0.31–0.48)	-0.02 (-0.42–0.37)
	I^2 **, %**	3.6	0	0
Elderly	***k***	8	8	8
	N	332/325	332/325	332/325
	***g* RE (95% CI)**	0.03 (-0.12–0.18)	0.04 (-0.11–0.19)	0.00 (-0.15–0.15)
	***g* FE (95% CI)**	0.03 (-0.12–0.18)	0.08 (-0.11–0.19)	0.00 (-0.15–0.15)
	I^2 **, %**	0	0	0

CI: confidence interval; FE: fixed-effects model; RE: random-effects model

BCG (Bacillus Calmette–Guérin) Vaccination

One SR [Zimmermann and Curtis 2018b] investigated the effect of previous or concomitant BCG vaccination on the immunogenicity of various vaccines, including influenza. Three studies were included in the qualitative synthesis of this SR. The authors reported that these studies had found an enhanced effect of inactivated IVs on both the magnitude HAI antibodies and SC rates in recipients of a BCG vaccine. By contrast, no such

effect was seen in subjects who received live attenuated IVs. It was not feasible to pool the results from the three studies, owing to the paucity of the available evidence, different study designs (randomized and non-randomized), different IVs (live, inactivated or both) and different timing of IV administration (concomitantly or separately from BCG).

Genetic Polymorphisms

An SR by Posteraro et al. (2014) was the only one to investigate the potential role of genetic variations in the vaccine-induced immune response, including that induced by IVs. However, only one IV-related study was found and analyzed. Four statistically significant associations were reported. Positivity for HLA-DQB1*03:03 [OR 20.40 (95% CI: 1.10–376.80)] and HLA-DRB1*07 [OR 4.00 (95% CI: 1.30–12.20)] were associated with a negative IV-induced response, while positivity for HLA-DRB3*0X [OR 0.36 (95% CI: 0.10–0.88)] and HLA-DRB1*13 [OR 0.18 (95% CI: 0.05–0.69)] were associated with a positive response. No further studies were identified.

Intravenous Drug Use

Only one SR [Baral et al. 2007] evaluated the immunogenicity of different vaccines in intravenous drug users. Regarding influenza, only two primary studies were discussed. It is, however, difficult to draw any conclusion, as these two studies included former intravenous drug users who were all HIV-positive. HIV positivity as a host factor will be discussed below.

Vitamin D Supplementation/Deficiency

A recent SRMA by Lee et al. (2018) investigated the effect of vitamin D deficiency on the IV-induced immune response. On pooling the binary outcomes of SCs and SPs, the authors obtained inconclusive results. Specifically, vaccinated subjects with normal vitamin D serum levels had a lower probability of being seroprotected against A/H3N2 and B (sub)types; no other significant results emerged (Table 3.7).

Table 3.7. Pooled estimates extracted from the available meta-analyses on the effect of vitamin D deficiency on the influenza vaccine-induced immune response (all models are random-effects).

Parameter		A/H1N1	A/H3N2	B
Seroconversion rate	***k***	6	4	4
	N	383/1178	358/1079	358/1079
	OR (95% CI)	1.10 (0.79–1.54)	0.98 (0.76–1.25)	0.98 (0.75–1.27)
	I^2 , %	25	0	0
Seroprotection rate	***k***	6	4	4
	N	373/1144	358/1079	358/1019
	OR (95% CI)	1.00 (0.52–1.92)	*0.63 (0.43–0.91)*	*0.68 (0.50–0.93)*
	I^2 , %	75	36	1

Statistically significant estimates are evidenced *in italics*; CI: confidence interval; OR: odds ratio

However, we did not retain the results obtained by Lee et al. (2018), since RCTs (vitamin D supplementation vs placebo or no treatment) and cohort studies based on the vitamin D serum concentration (deficient vs normal) were pooled together.

Among the primary studies, four RCTs were identified; their sample sizes were, however, low. We did not find any significant ($P > .27$) association, regardless of the HAI measure used and the virus (sub)type (Table 3.8). Owing to the paucity of studies, no further analyses were conducted.

Table 3.8. Summary evidence of the effect of vitamin D supplementation in order to enhance the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	3	3	3
N	176/276	176/276	176/276
OR RE (95% CI)	0.79 (0.52–1.20)	1.02 (0.69–1.51)	0.93 (0.57–1.52)
***P* RE**	.27	.94	.77
95% PI	0.52–1.20	0.69–1.51	0.57–1.52
OR FE (95% CI)	0.79 (0.52–1.20)	1.02 (0.69–1.51)	0.93 (0.57–1.52)
***P* FE**	.27	.94	.77
I^2**, %**	0	0	0
τ^2	0	0	0
SSE, *P*	NA	NA	NA
LS	No	No	No
CES	ns	ns	ns
Seroprotection rate			
k	3	3	3
N	176/276	176/276	176/276
OR RE (95% CI)	0.85 (0.51–1.41)	0.98 (0.60–1.58)	0.75 (0.44–1.28)
***P* RE**	.53	.92	.29
95% PI	0.51–1.41	0.60–1.58	0.44–1.28
OR FE (95% CI)	0.85 (0.51–1.41)	0.98 (0.60–1.58)	0.75 (0.44–1.28)
***P* FE**	.53	.92	.29
I^2**, %**	0	0	0
τ^2	0	0	0
SSE, *P*	NA	NA	NA
LS	No	No	No
CES	ns	ns	ns
Post-vaccination HAI titer			
k	3	3	3
N	154/153	154/153	154/153
***g* RE (95% CI)**	0.07 (-0.17–0.30)	-0.05 (-0.27–0.17)	0.02 (-0.2– 0.24)
***P* RE**	.58	.66	.89

Table 3.8 (*continued*). Summary evidence of the effect of vitamin D supplementation in order to enhance the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Post-vaccination HAI titer (*cont.*)			
95% PI	-0.18–0.31	-0.27–0.17	-0.21–0.24
***g* FE (95% CI)**	0.06 (-0.16–0.29)	-0.05 (-0.27–0.17)	0.02 (-0.21–0.24)
***P* FE**	.57	.66	.89
I^2, %	4.2	0	0
τ^2	0	0	0
SSE, *P*	NA	NA	NA
LS	No	No	No
CES	ns	ns	ns

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; HAI: hemagglutination-inhibition; LS: the largest study has a statistically significant effect size; NA; non available; ns: non-significant; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Observational studies on IV immunogenicity in vitamin D-deficient patients versus controls with normal concentrations did not usually reveal a significant difference. We decided against pooling since the available studies used different cut-offs to define vitamin D deficiency.

Immunosuppressive Conditions

The category of any immunosuppressive condition was the most populated: as mentioned above, a total of 17 SRs/SRMAs on this topic were included in the qualitative assessment.

A total of 96 pooled estimates (immunosuppressed patients vs healthy controls) based on at least 2 studies were extracted from the nine SRMAs; the number of pooled studies ranged from 2 to 50, and 58% ($N = 56$) of the meta-analytical estimates reported a $P < .05$. Of these latter, all but one ($N = 55$) reported a negative effect of immunosuppression on the SC and/or SP against influenza (Table 3.9).

From the included SRs without MAs, the following qualitative evidence synthesis was drawn. Agarwal et al. (2012) concluded that approximately 50% of the primary studies included had indicated a negative effect of IV in patients using immunosuppressive medications. Three SRs [Goossen et al. 2013; Shehata and Karim 2014; Vollaard et al. 2017] involving cancer patients (either pediatric or adult) advocated the use of IVs in such patients,

although uncertainty regarding the negative effect of the condition was substantial. Finally, the SR by Sousa et al. (2017) on the effect of rheumatic diseases on IV immunogenicity concluded that the biological therapy of rheumatic diseases could hamper the immune response, but that IV could be beneficial in such patients.

Table 3.9. Pooled estimates extracted from the available meta-analyses on the effect of immunosuppressive conditions on the influenza vaccine-induced immune response.

First author (Year)	Condition	Age	Virus	Parameter (ES)	*k*	Model	ES	95% CI
Beck (2011)	Any IS		H1N1	SC (OR)	50	RE	0.55	0.43–0.71
			H3N2		47	RE	0.55	0.41–0.73
			B		44	RE	0.48	0.36–0.62
			H1N1	SP (OR)	37	RE	0.36	0.26–0.51
			H3N2		35	RE	0.39	0.26–0.59
			B		37	RE	0.37	0.25–0.53
Beck (2012)	HIV	Any	H1N1	SC (OR)	17	FE	0.51	0.40–0.66
			H3N2		15	RE	0.47	0.27–0.79
			B		15	FE	0.34	0.26–044
			H1N1	SP (OR)	7	RE	0.28	0.12–0.65
			H3N2		7	RE	0.28	0.09–0.80
			B		7	RE	0.24	0.09–0.60
	Cancer		H1N1	SC (OR)	12	FE	0.31	0.22–0.43
			H3N2		12	RE	0.39	0.21–0.71
			B		8	RE	0.37	0.20–0.68
			H1N1	SP (OR)	10	RE	0.30	0.15–0.61
			H3N2		10	RE	0.30	0.14–0.63
			B		9	RE	0.30	0.14–0.67
	Transplantation		H1N1	SC (OR)	10	RE	0.76	0.38–1.51
			H3N2		10	RE	0.38	0.23–0.62
			B		10	RE	0.48	0.27–0.86
			H1N1	SP (OR)	10	FE	0.28	0.16–0.47
			H3N2		7	RE	0.29	0.09–0.92
			B		9	RE	0.36	0.19–0.70
	Autoimmune diseases treated with ISs		H1N1	SC (OR)	8	RE	0.90	0.45–1.80
			H3N2		7	FE	1.54	1.03–2.32
			B		7	RE	0.98	0.43–2.24
			H1N1	SP (OR)	7	FE	0.49	0.29–0.84
			H3N2		9	FE	0.71	0.43–1.17
			B		9	RE	0.48	0.22–1.05
	Respiratory diseases on ISs		H1N1	SC (OR)	2	RE	0.96	0.33–2.79
			H3N2		2	RE	0.93	0.30–2.85
			B		2	FE	0.29	0.14–0.59

Table 3.9 (*continued*). Pooled estimates extracted from the available meta-analyses on the effect of immunosuppressive conditions on the influenza vaccine-induced immune response.

First author (Year)	Condition	Age	Virus	Parameter (ES)	*k*	Model	ES	95% CI
Eckerle (2013)	Solid organ transplantation (any)	Any	H1N1	Response rate (RD)†	19	FE	-0.12	-0.16–-0.08
					19	RE	-0.16	-0.25–-0.06
			H3N2		18	FE	-0.15	-0.19–-0.11
					18	RE	-0.15	-0.23–-0.07
			B		19	FE	-0.12	-0.16–-0.07
					19	RE	-0.10	-0.19–-0.01
	Renal transplantation		H1N1		9	FE	-0.12	-0.19–-0.06
					9	RE	-0.15	-0.32–0.02
			H3N2		9	FE	-0.15	-0.22–-0.09
					9	RE	-0.13	-0.22–-0.04
			B		9	FE	0.03	-0.03–0.09
					9	RE	0.00	-0.09–0.09
	Liver transplantation		H1N1		5	FE	-0.07	-0.14–0.01
					5	RE	-0.07	-0.13–-0.01
			H3N2		5	FE	-0.06	-0.13–0.00
					5	RE	-0.10	-0.25–0.06
			B		5	FE	-0.03	-0.10–0.04
					5	RE	-0.04	-0.12–0.03
	Heart transplantation		H1N1		3	FE	-0.06	-0.14–-0.01
					3	RE	-0.19	-0.75–0.37
			H3N2		2	FE	-0.27	-0.37–-0.17
					2	RE	-0.27	-0.37–-0.17
			B		3	FE	-0.46	-0.57–-0.35
					3	RE	-0.41	-0.59–-0.23
Hua (2014)	RA patients on methotrexate	Adults	H1N1	ARR (OR)	2	FE	1.36	0.69–2.68
			H3N2		2	FE	1.33	0.70–2.53
			B		2	FE	1.28	0.64–2.56
	RA patients on rituximab		H1N1		2	FE	0.44	0.17–1.12
			H3N2		2	FE	0.11	0.04–0.31
			B		2	FE	0.29	0.10–0.81
	RA patients on αTNF		H1N1		4	RE	0.93	0.36–2.37
			H3N2		4	RE	0.79	0.34–1.83
			B		4	RE	0.79	0.37–1.70
Huang (2016)	SLE	Any	H1N1	SC (RR)	13	RE	0.71	0.62–0.81
			H3N2		7	RE	0.73	0.53–1.01
			B		4	RE	0.66	0.52–0.82
			H1N1	SP (RR)	12	RE	0.79	0.73–0.87
			H3N2		6	RE	0.84	0.68–1.03
			B		5	RE	0.75	0.65–0.87

Table 3.9 (*continued*). Pooled estimates extracted from the available meta-analyses on the effect of immunosuppressive conditions on the influenza vaccine-induced immune response.

First author (Year)	Condition	Age	Virus	Parameter (ES)	*k*	Model	ES	95% CI
Liao (2016)	SLE	Any	H1N1	SC (OR)	15	RE	0.39	0.27–0.57
			H3N2		6	RE	0.62	0.21–1.79
			B		6	FE	0.47	0.29–0.76
			H1N1	SP (OR)	18	RE	0.36	0.27–0.50
			H3N2		8	RE	0.48	0.24–0.93
			B		6	RE	0.55	0.24–1.25
Pugès (2016)		Adults	H1N1	SC (OR)	12	RE	0.38	0.27–0.54
			H3N2		11	RE	0.66	0.36–1.23
			B		5	RE	0.51	0.20–1.28
			H1N1	SP (OR)	11	RE	0.36	0.28–0.47
			H3N2		6	FE	0.26	0.14–0.50
			B		5	RE	0.93	0.42–2.08
Huang (2017)	RA		H1N1	SC (RR)	12	RE	0.78	0.68–0.90
			H3N2		8	RE	1.11	0.93–1.32
			B		9	RE	0.84	0.62–1.14
			H1N1	SP (RR)	9	RE	0.72	0.60–0.86
			H3N2		5	RE	0.96	0.82–1.13
			B		5	RE	0.95	0.84–1.08
Subesinghe (2018)	RA patients on methotrexate		H1N1	SP (RR)	5	RE	0.88	0.69–1.11
			H3N2		2	RE	0.94	0.85–1.04
			B		2	RE	1.15	0.63–2.10
	RA patients on αTNF		H1N1	SP (RR)	7	RE	0.86	0.72–1.04
			H3N2		4	RE	0.98	0.74–1.31
			B		4	RE	1.38	0.70–2.72

[†]Seroconversion and seroprotection rates were probably pooled together; ARR: absolute risk reduction; CI: confidence interval; ES: effect size; FE: fixed-effects model; HIV: human immunodeficiency virus; IS: immunosuppression/immunosupressant; OR: odds ratio; RCT: randomized controlled trial; RA: rheumatoid arthritis; RE: random-effects model; RR: risk ratio; SC: seroconversion rate; SLE: systemic lupus erythematosus; SP: seroprotection rate; TNF: tumor necrosis factor

Among the SRs/SRMAs that covered a similar topic, the observed corrected covered areas varied greatly. Indeed, the corrected covered area of three SRMAs on systemic lupus erythematosus [Huang et al. 2016; Liao et al. 2016a; Pugès et al. 2016] and another three SRMAs on rheumatoid arthritis [Hua et al. 2014; Huang et al. 2017; Subesinghe et al. 2018] showed very high corrected covered areas (73.7% and 21.1%, respectively), while three SRs on cancer patients [Goossen et al. 2013; Shehata and Karim 2014; Vollaard et al. 2017] and two SRMAs on transplant patients [Eckerle et al. 2013; Karbasi-Afshar et al. 2015] showed

substantially lower corrected covered areas, which were deemed “moderate” (6.6% and 8.9%, respectively).

Table 3.10 summarizes the evidence synthesis on the effect of any immunosuppressive condition on IV-induced immunogenicity. The total number of studies included varied from 69 to 116, and the corresponding number of cases ranged from 3,720 to 8,673. As expected, the highest number of studies involved A/H1N1, since several studies were conducted during the last H1N1pdm09 pandemic, when the MIV was used. Despite the large patient numbers and ESs, the CES assigned was generally of class II (exception: SP and post-vaccination HAI titer for type B, which were assigned to class III). This was primarily attributed to the relatively high heterogeneity observed and the consequent large 95% prediction intervals, which downgraded the CES (Table 3.10).

Table 3.10. Summary evidence of the effect of immunosuppressive conditions on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	116	94	85
N	8673/4638	4193/3023	3888/2944
OR RE (95% CI)	0.50 (0.42–0.59)	0.51 (0.41–0.63)	0.53 (0.44–0.64)
***P* RE**	$2 \cdot 10^{-15}$	$2 \cdot 10^{-10}$	$4 \cdot 10^{-11}$
95% PI	0.13–1.99	0.11–2.37	0.16–1.75
OR FE (95% CI)	0.53 (0.49–0.59)	0.56 (0.50–0.62)	0.54 (0.48–0.61)
***P* FE**	$< 1 \cdot 10^{-14}$	$< 1 \cdot 10^{-14}$	$< 1 \cdot 10^{-14}$
I^2, %	65.7	65.8	53.9
τ^2	0.49	0.60	0.36
SSE, *P*	.30	.30	.75
LS	Yes	Yes	Yes
CES	II	II	II
Seroprotection rate			
k	102	76	75
N	8452/4272	3780/2605	3759/2505
OR RE (95% CI)	0.42 (0.35–0.51)	0.35 (0.27–0.45)	0.53 (0.41–0.69)
***P* RE**	$< 1 \cdot 10^{-15}$	$1 \cdot 10^{-15}$	$3 \cdot 10^{-6}$
95% PI	0.12–1.54	0.08–1.46	0.10–2.76
OR FE (95% CI)	0.44 (0.39–0.49)	0.38 (0.32–0.45)	0.51 (0.44–0.60)
***P* FE**	$< 1 \cdot 10^{-15}$	$< 1 \cdot 10^{-15}$	$< 1 \cdot 10^{-15}$
I^2, %	54.5	45.7	59.9
τ^2	0.43	0.52	0.69
SSE, *P*	> .99	.78	.12

Table 3.10 (*continued*). Summary evidence of the effect of immunosuppressive conditions on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Seroprotection rate (*cont.*)			
LS	Yes	Yes	Yes
CES	II	II	III
Post-vaccination HAI titer			
k	99	77	69
N	7909/4438	3889/2922	3720/2751
***g* RE (95% CI)**	-0.36 (-0.45–-0.28)	-0.44 (-0.55–-0.34)	-0.34 (-0.43–-0.24)
***P* RE**	$< 1 \cdot 10^{-15}$	$2 \cdot 10^{-15}$	$2 \cdot 10^{-12}$
95% PI	-1.05–0.32	-1.25–0.36	-0.94–0.26
***g* FE (95% CI)**	-0.33 (-0.37–-0.29)	-0.43 (-0.49–-0.38)	-0.32 (-0.38–-0.27)
***P* FE**	$< 1 \cdot 10^{-15}$	$< 1 \cdot 10^{-15}$	$< 1 \cdot 10^{-15}$
I^2**, %**	73.8	75.0	63.6
τ^2	0.12	0.17	0.09
SSE, *P*	.17	.64	.42
LS	Yes	Yes	No
CES	II	II	III

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; HAI: hemagglutination-inhibition; LS: the largest study has a statistically significant effect size; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

With regard to post-vaccination HAI GMT titers, we conducted a sensitivity analysis after excluding studies with imputed SDs. Although *k* dropped significantly, the Hedges' *g*s observed were in line with the main analysis (Table 3.10) and no substantial changes occurred; the CES grouping did not change (Table 3.11).

In order to explain the observed heterogeneity reported in Table 3.10, we conducted a series of meta-regression analyses (Tables 3.12–3.14). The following variables were explored: year of publication (< 2000 vs ≥ 2000), total study sample (< 100 vs ≥ 100), population age (children vs adults), a categorical variable of the virus (sub)type (A/H1N1, A/H3N2 and B, where A/H1N1 is the reference category) and a categorical variable of immunosuppressive condition. This last category was classified *post-hoc* in order to ensure a significant number of observations for each category. The classification was: transplant patients (reference category), cancer, HIV, rheumatic diseases and other/mixed conditions. In the fully adjusted models, only patients with rheumatic diseases generally showed a better response than transplant recipients, although the regression coefficients reached an $\alpha < .05$ only with regard to the post-vaccination HAI titer (Tables 3.12–3.14).

Table 3.11. Sensitivity analysis (by excluding studies with the imputed standard deviations) on the effect of any immunosuppressive condition on post-vaccination hemagglutination-inhibition titers.

Parameter	A/H1N1	A/H3N2	B
k	52	35	35
N	5616/2425	2116/1174	2198/1234
***g* RE (95% CI)**	-0.34 (-0.45–-0.23)	-0.53 (-0.68–-0.39)	-0.36 (-0.50–-0.21)
***P* RE**	$9 \cdot 10^{-10}$	$3 \cdot 10^{-13}$	$1 \cdot 10^{-6}$
95% PI	-0.98–0.30	-1.23–0.16	-1.05–0.34
***g* FE (95% CI)**	-0.29 (-0.34–-0.24)	-0.51 (-0.58–-0.43)	-0.30 (-0.38–-0.23)
***P* FE**	$< 1 \cdot 10^{-15}$	$< 1 \cdot 10^{-15}$	$3 \cdot 10^{-15}$
I^2 **, %**	73.9	68.6	69.7
τ^2	0.10	0.12	0.12
SSE, *P*	.11	.25	.062
LS	Yes	Yes	No
CES	II	II	III

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; PI: prediction interval; RE: random-effects model; SSE: small study effect's test

Table 3.12. Multivariable meta-regression analysis in order to predict the observed pooled estimates on seroconversion rates.

Parameter	Variable	Univariable model		Full model	
		***b* (SE)**	***P***	***b* (SE)**	***P***
Publication in year	<2000	Ref	–	Ref	–
	≥2000	0.06 (0.13)	.64	0.03 (0.13)	.84
Total sample size	<100	Ref	–	Ref	–
	≥100	0.14 (0.11)	.21	0.14 (0.12)	.25
Age	Children	Ref	–	Ref	–
	Adults	-0.02 (0.14)	.86	-0.11 (0.14)	.44
Virus	A/H1N1	Ref	–	Ref	–
	A/H3N2	0.03 (0.13)	.82	0.08 (0.13)	.56
	B	0.07 (0.14)	.62	0.11 (0.14)	.42
Immunosuppression category	Transplantation	Ref	–	Ref	–
	Cancer	-0.27 (0.17)	.12	-0.26 (0.18)	.14
	HIV	-0.05 (0.17)	.79	-0.07 (0.18)	.69
	RDs	0.28 (0.15)	.070	0.27 (0.16)	.091
	Other/Mixed	-0.11 (0.19)	.57	-0.17 (0.20)	.41

HIV: human immunodeficiency virus; RD: rheumatic disease; SE: standard error

Table 3.13. Multivariable meta-regression analysis in order to predict the observed pooled estimates on seroprotection rates.

Parameter	Variable	Univariable model		Full model	
		***b* (SE)**	***P***	***b* (SE)**	***P***
Publication in year	< 2000	Ref	–	Ref	–
	≥ 2000	0.32 (0.18)	.076	0.13 (0.20)	.50
Total sample size	< 100	Ref	–	Ref	–
	≥ 100	-0.02 (0.14)	.88	0.03 (0.15)	.85
Age	Children	Ref	–	Ref	–
	Adults	-0.17 (0.18)	.35	-0.18 (0.18)	.32
Virus	A/H1N1	Ref	–	Ref	–
	A/H3N2	-0.19 (0.17)	.26	-0.14 (0.17)	.39
	B	0.23 (0.16)	.16	0.26 (0.16)	.11
Immunosuppression category	Transplantation	Ref	–	Ref	–
	Cancer	0.27 (0.23)	.23	0.24 (0.24)	.31
	HIV	-0.11 (0.25)	.66	-0.13 (0.25)	.62
	RDs	0.42 (0.19)	.026	0.39 (0.20)	.051
	Other/Mixed	0.35 (0.24)	.14	0.26 (0.26)	.31

HIV: human immunodeficiency virus; RD: rheumatic disease; SE: standard error

Table 3.14. Multivariable meta-regression analysis in order to predict the observed pooled estimates on post-vaccination hemagglutination-inhibition titers.

Parameter	Variable	Univariable model		Full model	
		***b* (SE)**	***P***	***b* (SE)**	***P***
Publication in year	< 2000	Ref	–	Ref	–
	≥ 2000	0.16 (0.07)	.016	0.06 (0.07)	.38
Total sample size	< 100	Ref	–	Ref	–
	≥ 100	0.05 (0.06)	.42	0.04 (0.06)	.51
Age	Children	Ref	–	Ref	–
	Adults	0.01 (0.07)	.85	0.00 (0.07)	.99
Virus	A/H1N1	Ref	–	Ref	–
	A/H3N2	-0.08 (0.07)	.80	-0.05 (0.06)	.39
	B	0.02 (0.07)	.80	0.05 (0.07)	.45
Immunosuppression category	Transplantation	Ref	–	Ref	–
	Cancer	0.14 (0.10)	.13	0.14 (0.10)	.18
	HIV	0.01 (0.10)	.89	0.01 (0.10)	.92
	RDs	0.41 (0.08)	<.0001	0.40 (0.09)	<.0001
	Other/Mixed	0.22 (0.10)	.32	0.20 (0.10)	.060

HIV: human immunodeficiency virus; RD: rheumatic disease; SE: standard error

We then conducted several subgroup analyses according to the type of immunosuppression. As summarized in Table 3.15, most pooled estimates were statistically significant. However, although the ESs were usually large, the overall CES was often of class IV, as it was driven by the total sample size. Indeed, a class-I CES was assigned only to some pooled estimates that regarded the subtype A/H1N1. In any case, these subgroup analyses

allowed us to reduce heterogeneity in several instances. A full description of the subgroup analyses conducted is reported in Tables 3.16–3.22.

Table 3.15. Summary evidence of the effect of single immunosuppressive conditions on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Virus	Parameter		Any RD	RA	SLE	IBD	HIV	Cancer	Transplant
A/H1N1	SC	OR	0.57	0.64	0.35	0.54	0.51	0.49	0.49
		CES	III	IV	II	ns	IV	III	IV
	SP	OR	0.47	0.39	0.38	0.80	0.35	0.28	0.28
		CES	II	II	I	ns	IV	I	I
	HAI titer	*g*	-0.21	-0.37	-0.42	-0.30	-0.51	-0.61	-0.61
		CES	III	I	III	IV	IV	III	III
A/H3N2	SC	OR	0.78	0.97	0.55	NA	0.46	0.44	0.35
		CES	ns	ns	ns	NA	IV	IV	IV
	SP	OR	0.40	0.37	0.39	0.74	0.21	0.37	0.26
		CES	II	IV	IV	ns	IV	IV	IV
	HAI titer	*g*	-0.26	-0.26	-0.23	NA	-0.77	-0.54	-0.89
		CES	III	IV	ns	NA	IV	IV	IV
B	SC	OR	0.75	0.72	0.57	NA	0.37	0.41	0.54
		CES	IV	ns	IV	NA	IV	IV	IV
	SP	OR	0.76	0.84	0.60	1.12	0.33	0.46	0.40
		CES	ns	ns	IV	ns	IV	IV	IV
	HAI titer	*g*	-0.05	-0.11	NA	NA	-0.54	-0.54	-0.49
		CES	ns	ns	NA	NA	IV	IV	IV

CES: cumulative evidence synthesis class; HAI: hemagglutination-inhibition; HIV: human immunodeficiency virus; IBD: inflammatory bowel disease; NA; non available; ns: non-significant; OR: odds ratio; RA: rheumatoid arthritis; RD: rheumatic disease; SC: seroconversion rate; SLE: systemic lupus erythematosus; SP: seroprotection rate

Table 3.16. Summary evidence on the effect of rheumatic diseases on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	38	26	21
N	4676/1825	1245/657	1091/568
OR RE (95% CI)	0.57 (0.43–0.76)	0.78 (0.56–1.10)	0.76 (0.54–1.06)
***P* RE**	.0001	.16	.11

Table 3.16 (*continued*). Summary evidence on the effect of rheumatic diseases on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate (*cont.*)			
95% PI	0.15–2.22	0.22–2.81	0.26–2.18
OR FE (95% CI)	0.57 (0.49–0.65)	0.83 (0.67–1.03)	0.75 (0.59–0.95)
***P* FE**	$< 1 \cdot 10^{-15}$	.090	.016
I^2 **, %**	69.8	54.6	45.5
τ^2	0.46	0.39	0.26
SSE, *P*	.75	.83	.46
LS	Yes	No	Yes
CES	III	ns	IV
Seroprotection rate			
k	42	28	27
N	5108/1971	1618/770	1563/707
OR RE (95% CI)	0.47 (0.36–0.60)	0.41 (0.28–0.61)	0.76 (0.47–1.25)
***P* RE**	$3 \cdot 10^{-9}$	.00001	.28
95% PI	0.19–1.16	0.13–1.31	0.11–5.52
OR FE (95% CI)	0.47 (0.40–0.56)	0.40 (0.30–0.55)	0.84 (0.64–1.11)
***P* FE**	$< 1 \cdot 10^{-15}$	$8 \cdot 10^{-9}$	.22
I^2 **, %**	38.7	30.3	64.1
τ^2	0.20	0.31	0.96
SSE, *P*	.96	.17	.31
LS	Yes	Yes	Yes
CES	II	II	ns
Post-vaccination hemagglutination-inhibition titer			
k	38	27	22
N	4479/1810	1591/833	1415/668
***g* RE (95% CI)**	-0.21 (-0.30–-0.11)	-0.26 (-0.39–-0.12)	-0.05 (-0.21–0.10)
***P* RE**	.00002	.0003	.50
95% PI	-0.63–0.21	-0.80–0.29	-0.61–0.51
***g* FE (95% CI)**	-0.23 (-0.29–-0.17)	-0.28 (-0.37–-0.19)	-0.05 (-0.15–0.05)
***P* FE**	$6 \cdot 10^{-15}$	$6 \cdot 10^{-10}$	.31
I^2 **, %**	55.5	57.0	58.5
τ^2	0.04	0.07	0.08
SSE, *P*	.29	.26	.85
LS	Yes	No	Yes
CES	III	III	ns

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; ns: non-significant OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small study effect's test

Table 3.17. Summary evidence of the effect of rheumatoid arthritis on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	16	12	11

N	1030/719	517/303	502/273
OR RE (95% CI)	0.64 (0.45–0.91)	0.93 (0.62–1.40)	0.75 (0.45–1.26)
***P* RE**	.014	.73	.27
95% PI	0.23–1.80	0.39–2.25	0.22–2.55
OR FE (95% CI)	0.55 (0.45–0.68)	0.97 (0.71–1.32)	0.72 (0.51–1.01)
***P* FE**	$8 \cdot 10^{-8}$	.84	.060
I^2**, %**	53.8	33.7	47.0
τ^2	0.24	0.16	0.32
SSE, *P*	.13	.49	.63
LS	Yes	No	Yes
CES	IV	ns	ns
Seroprotection rate			
k	19	15	15
N	1330/827	816/412	815/403
OR RE (95% CI)	0.37 (0.23–0.53)	0.36 (0.20–0.63)	0.84 (0.40–1.79)
***P* RE**	.00001	.0004	.65
95% PI	0.11–1.26	0.09–1.48	0.08–8.96
OR FE (95% CI)	0.39 (0.20–0.51)	0.37 (0.25–0.55)	0.95 (0.64–1.41)
***P* FE**	$9 \cdot 10^{-13}$	$9.96 \cdot 10^{-7}$	.81
I^2**, %**	47.0	39.8	67.7
τ^2	0.35	0.44	1.31
SSE, *P*	.74	.60	.53
LS	Yes	Yes	Yes
CES	II	IV	ns
Post-vaccination hemagglutination-inhibition titer			
k	15	13	12
N	1283/832	769/417	753/378
***g* RE (95% CI)**	-0.34 (-0.47–-0.21)	-0.27 (-0.42–-0.11)	-0.11 (-0.32–0.10)
***P* RE**	$3 \cdot 10^{-7}$	.0009	.31
95% PI	-0.37–-0.04	-0.60–0.07	-0.69–0.47
***g* FE (95% CI)**	-0.37 (-0.47–-0.27)	-0.26 (-0.38–-0.13)	-0.10 (-0.23–0.03)
***P* FE**	$1 \cdot 10^{-13}$	.00008	.14
I^2**, %**	30.8	29.3	58.1
τ^2	0.02	0.02	0.08
SSE, *P*	.22	.62	.71
LS	Yes	No	No
CES	I	IV	ns

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; ns: non-significant OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Table 3.18. Summary evidence of the effect of systemic lupus erythematosus on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	17	10	5
N	1767/924	344/251	231/129
OR RE (95% CI)	0.35 (0.23–0.53)	0.55 (0.27–1.12)	0.57 (0.35–0.93)

***P* RE**	$9 \cdot 10^{-7}$	.10	.025
95% PI	0.10–1.29	0.08–3.67	0.35–0.93
OR FE (95% CI)	0.38 (0.31–0.46)	0.50 (0.34–0.75)	0.57 (0.35–0.93)
***P* FE**	$< 1 \cdot 10^{-15}$	.0008	.025
I^2**, %**	68.8	65.2	0
τ^2	0.40	0.80	0
SSE, *P*	.79	.14	NA
LS	Yes	Yes	No
CES	II	ns	IV
Seroprotection rate			
k	16	7	6
N	1784/945	303/201	249/147
OR RE (95% CI)	0.38 (0.27–0.54)	0.40 (0.21–0.76)	0.52 (0.25–1.09)
***P* RE**	$2 \cdot 10^{-8}$	.0054	.083
95% PI	0.17–0.85	0.14–1.12	0.12–2.21
OR FE (95% CI)	0.38 (0.30–0.47)	0.39 (0.23–0.68)	0.60 (0.36–0.99)
***P* FE**	$< 1 \cdot 10^{-15}$	.0008	.045
I^2**, %**	36.3	22.8	49.3
τ^2	0.14	0.17	0.40
SSE, *P*	.75	NA	NA
LS	Yes	Yes	Yes
CES	I	IV	IV
Post-vaccination hemagglutination-inhibition titer			
k	13	9	NA
N	1181/758	323/258	NA
***g* RE (95% CI)**	-0.42 (-0.59–0.25)	-0.23 (-0.48–0.03)	NA
***P* RE**	$1 \cdot 10^{-6}$	.082	NA
95% PI	-0.88–0.04	-0.82–0.37	NA
***g* FE (95% CI)**	-0.47 (-0.56–-0.37)	-0.26 (-0.43–-0.09)	NA
***P* FE**	$< 1 \cdot 10^{-15}$	.0026	NA
I^2**, %**	56.7	52.0	NA
τ^2	0.05	0.08	NA
SSE, *P*	.87	NA	NA
LS	Yes	Yes	NA
CES	III	ns	NA

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; NA; non available; ns: non-significant; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Table 3.19. Summary evidence of the effect of inflammatory bowel disease on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	2	NA	NA
N	154/65	NA	NA
OR RE (95% CI)	0.54 (0.15–1.96)	NA	NA
***P* RE**	.35	NA	NA
95% PI	0.07–4.08	NA	NA
OR FE (95% CI)	0.50 (0.26–0.98)	NA	NA
***P* FE**	.043	NA	NA

I^2, %	73.0	NA	NA
τ^2	0.63	NA	NA
SSE, *P*	NA	NA	NA
LS	Yes	NA	NA
CES	ns	NA	NA
Seroprotection rate			
k	5	3	3
N	309/104	108/45	108/45
OR RE (95% CI)	0.80 (0.35–1.86)	0.74 (0.23–2.39)	1.12 (0.07–17.76)
***P* RE**	.61	.61	0.94
95% PI	0.18–3.46	0.23–2.39	0.01–120.4
OR FE (95% CI)	0.80 (0.45–1.41)	0.74 (0.23–2.39)	0.28 (0.10–0.78)
***P* FE**	.44	.61	0.015
I^2, %	43.7	0	61.5
τ^2	0.37	0	3.7
SSE, *P*	NA	NA	NA
LS	No	No	Yes
CES	ns	ns	ns
Post-vaccination hemagglutination-inhibition titer			
k	2	NA	NA
N	201/59	NA	NA
***g* RE (95% CI)**	-0.30 (-0.59–0.00)	NA	NA
***P* RE**	.043	NA	NA
95% PI	-0.59–0.00	NA	NA
***g* FE (95% CI)**	-0.30 (-0.59–0.00)	NA	NA
***P* FE**	.043	NA	NA
I^2, %	0	NA	NA
τ^2	0	NA	NA
SSE, *P*	NA	NA	NA
LS	No	NA	NA
CES	IV	NA	NA

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; NA; non available; ns: non-significant; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Table 3.20. Summary evidence of the effect of human immunodeficiency virus (HIV) infection on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	20	17	15
N	828/1059	594/803	558/742
OR RE (95% CI)	0.51 (0.35–0.74)	0.46 (0.26–0.82)	0.40 (0.29–0.57)
***P* RE**	.0005	.0082	$2 \cdot 10^{-7}$
95% PI	0.14–1.81	0.06–3.41	0.18–0.88
OR FE (95% CI)	0.54 (0.43–0.67)	0.50 (0.38–0.64)	0.37 (0.29–0.49)
***P* FE**	$4 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	$2 \cdot 10^{-13}$

I^2, %	59.4	75.2	30.4
τ^2	0.38	0.95	0.13
SSE, *P*	.47	.93	.22
LS	Yes	Yes	Yes
CES	IV	IV	IV
Seroprotection rate			
k	14	11	9
N	601/936	367/680	331/619
OR RE (95% CI)	0.35 (0.19–0.64)	0.21 (0.13–0.34)	0.33 (0.15–0.72)
P RE	.0006	$2 \cdot 10^{-10}$	.0059
95% PI	0.06–1.91	0.13–0.34	0.05–2.12
OR FE (95% CI)	0.29 (0.21–0.39)	0.21 (0.13–0.34)	0.25 (0.16–0.39)
P FE	$< 1 \cdot 10^{-15}$	$2 \cdot 10^{-10}$	$6 \cdot 10^{-10}$
I^2, %	64.5	0	59.3
τ^2	0.65	0	0.74
SSE, *P*	.70	.60	NA
LS	Yes	Yes	Yes
CES	IV	IV	IV
Post-vaccination hemagglutination-inhibition titer			
k	17	15	12
N	788/1100	554/844	518/783
g RE (95% CI)	-0.51 (-0.75–-0.27)	-0.77 (-1.11–-0.42)	-0.54 (-0.76–-0.32)
P RE	.00003	.00001	$2 \cdot 10^{-6}$
95% PI	-1.38–0.36	-2.03–0.49	-1.13–0.05
g FE (95% CI)	-0.43 (-0.53–-0.32)	-0.67 (-0.79–-0.55)	-0.51 (-0.64–-0.38)
P FE	$< 1 \cdot 10^{-15}$	$< 1 \cdot 10^{-15}$	$2 \cdot 10^{-14}$
I^2, %	78.9	86.5	57.9
τ^2	0.18	0.38	0.08
SSE, *P*	.26	.43	.48
LS	Yes	Yes	Yes
CES	IV	IV	IV

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Table 3.21. Summary evidence of the effect of transplantation on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	31	26	25
N	1326/1008	955/888	910/820
OR RE (95% CI)	0.49 (0.32–0.75)	0.35 (0.23–0.54)	0.54 (0.36–0.82)
P RE	.0010	$1 \cdot 10^{-6}$	.0040
95% PI	0.07–3.58	0.07–1.87	0.11–2.71
OR FE (95% CI)	0.53 (0.43–0.65)	0.41 (0.33–0.51)	0.59 (0.47–0.73)
P FE	$1 \cdot 10^{-9}$	$< 1 \cdot 10^{-15}$	$3 \cdot 10^{-6}$
I^2, %	74.0	67.3	65.5
τ^2	0.99	0.68	0.63
SSE, *P*	.80	.37	.56
LS	Yes	Yes	No

CES	IV	IV	IV
Seroprotection rate			
k	23	16	17
N	1110/803	693/607	709/606
OR RE (95% CI)	0.28 (0.19–0.42)	0.26 (0.14–0.47)	0.46 (0.29–0.74)
***P* RE**	$6 \cdot 10^{-10}$	$8 \cdot 10^{-6}$	.0013
95% PI	0.08–0.95	0.05–1.45	0.12–1.73
OR FE (95% CI)	0.28 (0.21–0.37)	0.30 (0.21–0.43)	0.40 (0.30–0.54)
***P* FE**	$< 1 \cdot 10^{-15}$	$2 \cdot 10^{-10}$	$4 \cdot 10^{-9}$
I^2**, %**	40.6	53.9	47.9
τ^2	0.32	0.69	0.40
SSE, *P*	.11	> 0.99	.12
LS	Yes	Yes	Yes
CES	I	IV	IV
Post-vaccination hemagglutination-inhibition titer			
k	17	12	12
N	1063/780	642/607	616/652
***g* RE (95% CI)**	-0.61 (-0.86–-0.35)	-0.89 (-1.15–-0.64)	-0.52 (-0.69–-0.36)
***P* RE**	$3 \cdot 10^{-6}$	$4 \cdot 10^{-12}$	$4 \cdot 10^{-10}$
95% PI	-1.57–0.35	-1.64–-0.14	-0.88–-0.16
***g* FE (95% CI)**	-0.50 (-0.61–-0.40)	-0.81 (-0.93–-0.69)	-0.49 (-0.61–-0.37)
***P* FE**	$< 1 \cdot 10^{-15}$	$< 1 \cdot 10^{-15}$	$3 \cdot 10^{-15}$
I^2**, %**	82.3	72.8	35.5
τ^2	0.22	0.13	0.03
SSE, *P*	.12	.15	.27
LS	No	Yes	No
CES	III	IV	IV

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Table 3.22. Summary evidence of the effect of cancer on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	31	21	17
N	1326/1008	910/577	663/480
OR RE (95% CI)	0.49 (0.32–0.75)	0.44 (0.29–0.66)	0.41 (0.30–0.57)
***P* RE**	.0010	.00007	$6 \cdot 10^{-8}$
95% PI	0.07–3.58	0.10–1.86	0.26–0.67
OR FE (95% CI)	0.53 (0.43–0.65)	0.50 (0.39–0.64)	0.41 (0.31–0.56)
***P* FE**	$1 \cdot 10^{-9}$	$4 \cdot 10^{-8}$	$1 \cdot 10^{-8}$
I^2**, %**	74.0	59.6	7.5
τ^2	0.99	0.51	0.03
SSE, *P*	.80	.48	.19
LS	Yes	Yes	Yes
CES	III	IV	IV
Seroprotection rate			
k	23	14	

N	1110/803	565/375	565/375
OR RE (95% CI)	0.28 (0.19–0.42)	0.37 (0.19–0.73)	0.46 (0.26–0.79)
***P* RE**	$6 \cdot 10^{-10}$	.0038	.0052
95% PI	0.08–0.95	0.05–2.84	0.10–2.08
OR FE (95% CI)	0.28 (0.21–0.37)	0.52 (0.37–0.75)	0.52 (0.36–0.74)
***P* FE**	$< 1 \cdot 10^{-15}$	.00042	.00024
I^2**, %**	40.6	65.8	52.7
τ^2	0.34	0.95	0.52
SSE, *P*	.11	.42	.83
LS	Yes	Yes	Yes
CES	I	IV	IV
Post-vaccination hemagglutination-inhibition titer			
k	17	16	13
N	1063/780	565/506	480/401
***g* RE (95% CI)**	-0.61 (-0.86–-0.35)	-0.54 (-0.77–-0.31)	-0.54 (-0.75–-0.36)
***P* RE**	$3 \cdot 10^{-6}$	$6 \cdot 10^{-6}$	$3 \cdot 10^{-7}$
95% PI	-1.57–0.35	-1.33–0.26	-1.11–0.03
***g* FE (95% CI)**	-0.50 (-0.61–-0.40)	-0.47 (-0.60–-0.35)	-0.52 (-0.66–-0.38)
***P* FE**	$< 1 \cdot 10^{-15}$	$2 \cdot 10^{-13}$	$3 \cdot 10^{-13}$
I^2**, %**	82.3	69.0	52.5
τ^2	0.22	0.15	0.07
SSE, *P*	.12	.082	.49
LS	No	No	Yes
CES	III	IV	IV

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Latent Cytomegalovirus (CMV) Infection

A recent SRMA by van den Berg et al. (2019) revealed an unclear relationship between CMV seropositivity and IV-induced response in both adults and the elderly. In the MA, the OR of SC against any virus (sub)type did not reach an $\alpha < .05$ [OR = 0.65 (95%: 0.40–1.08); $P = .11$]; heterogeneity was moderate ($I^2 = 33.2\%$). A subgroup analysis by age (adults < 60 years and older adults ≥ 60 years) showed similar results [adults: OR = 0.41 (95% CI: 0.11–1.45); older adults: OR = 0.57 (95% CI: 0.26–1.25)].

In keeping with our aims, we re-performed the meta-analysis by single virus (sub)type. No significant association was found in either the main (Table 3.23) or subgroup analysis by age (Table 3.24), independently from the virus (sub)type and age-class.

Again, these results shod be seen cautiously, considering the between-study methodological differences and their paucity.

Table 3.23. Summary evidence of the effect of latent cytomegalovirus (CMV) infection on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroconversion rate			
k	6	7	9
N	192/83	670/203	99/43
OR RE (95% CI)	0.46 (0.14–1.56)	1.05 (0.74–1.49)	0.64 (0.27–1.56)
***P* RE**	.21	.79	.33
95% PI	0.03–6.65	0.74–1.49	0.27–1.56
OR FE (95% CI)	0.58 (0.29–1.16)	1.05 (0.74–1.49)	0.64 (0.27–1.56)
***P* FE**	.12	.79	.33
I^2, %	65.7	0	0
τ^2	1.47	0	0
SSE, *P*	NA	NA	NA
LS	Yes	No	No
CES	ns	ns	ns
Seroprotection rate			
k	NA	5	NA
N	NA	616/191	NA
OR RE (95% CI)	NA	1.08 (0.76–1.54)	NA
***P* RE**	NA	.42	NA
95% PI	NA	0.76–1.54	NA
OR FE (95% CI)	NA	1.08 (0.76–1.54)	NA
***P* FE**	NA	.42	NA
I^2, %	NA	0	NA
τ^2	NA	0	NA
SSE, *P*	NA	NA	NA

Table 3.23 (*continued*). Summary evidence of the effect of latent cytomegalovirus (CMV) infection on the influenza vaccine-induced immune response, by immunogenicity parameter and viral (sub)type.

Parameter	A/H1N1	A/H3N2	B
Seroprotection rate (*cont.*)			
LS	NA	No	NA
CES	NA	ns	NA
Post-vaccination HAI titer			
k	7	7	NA
N	371/221	716/260	NA
***g* RE (95% CI)**	-0.25 (-0.58–0.08)	-0.06 (-0.22–0.11)	NA
***P* RE**	.14	.50	NA
95% PI	-0.99–0.50	-0.31–0.20	NA
***g* FE (95% CI)**	-0.13 (-0.31–0.04)	-0.06 (-0.20–0.09)	NA
***P* FE**	.13	.45	NA

CES: cumulative evidence synthesis class; CI: confidence interval; FE: fixed-effects model; LS: the largest study has a statistically significant effect size; NA; non available; ns: non-significant; OR: odds ratio; PI: prediction interval; RE: random-effects model; SSE: small-study effect test

Table 3.24. Subgroup analysis (by age-class) of the summary evidence of the effect of latent cytomegalovirus (CMV) infection on the influenza vaccine-induced immune response.

Parameter		A/H1N1	A/H3N2	B
Seroconversion rate				
Adults	***k***	3	NA	NA
	N	85/46	NA	NA
	OR RE (95% CI)	0.33 (0.04–2.70)	NA	NA
	OR FE (95% CI)	*0.35 (0.12–0.99)*	NA	NA
	I^2 **, %**	71.5	NA	NA
Elderly	***k***	3	6	2
	N	107/37	662/187	91/27
	OR RE (95% CI)	0.63 (0.13–3.04)	1.04 (0.73–1.49)	0.56 (0.19–1.70)
	OR FE (95% CI)	0.86 (0.35–2.11)	1.04 (0.73–1.49)	0.58 (0.21–1.55)
	I^2**, %**	63.9	0	18.8
Seroprotection rate				
Adults	***k***	NA	NA	NA
	N	NA	NA	NA
	OR RE (95% CI)	NA	NA	NA
	OR FE (95% CI)	NA	NA	NA
	I^2**, %**	NA	NA	NA
Elderly	***k***	NA	4	NA
	N	NA	571/160	NA
	OR RE (95% CI)	NA	1.08 (0.74–1.56)	NA
	OR FE (95% CI)	NA	1.08 (0.74–1.56)	NA
	I^2**, %**	NA	0	NA

Table 3.24 (*continued*). Subgroup analysis (by age-class) of the summary evidence of the effect of latent cytomegalovirus (CMV) infection on the influenza vaccine-induced immune response.

Parameter		A/H1N1	A/H3N2	B
Post-vaccination hemagglutination-inhibition titer				
Adults	***k***	5	NA	NA
	N	299/168	NA	NA
	OR RE (95% CI)	-0.28 (-0.78–0.20)	NA	NA
	OR FE (95% CI)	-0.12 (-0.32–0.07)	NA	NA
	I^2**, %**	77.2	NA	NA
Elderly	***k***	2	6	NA
	N	72/53	671/229	NA
	OR RE (95% CI)	-0.17 (-0.52–0.19)	-0.08 (-0.26–0.11)	NA
	OR FE (95% CI)	-0.17 (-0.52–0.19)	-0.07 (-0.22–0.08)	NA
	I^2**, %**	0	29.9	NA

Statistically significant estimates are evidenced *in italics*; CI: confidence interval; FE: fixed-effects model; NA; non available; OR: odds ratio; RE: random-effects model

Psychological Stress

An SRMA by Pedersen et al. (2009) investigated the potential role of psychological stress on IV-induced immunogenicity. Stressful conditions were found to be associated with a statistically lower immune response. However, a few observations should be made on the pooled estimates by Pedersen et al. (2009). First, different serological assays (HAI and ELISA) were pooled together. Second, no distinction was made among the three vaccine components. Third, the ES used was the so-called ES correlation coefficient, the interpretation of which is similar to Pearson's *r*. For instance, a statistically significant negative ES correlation coefficient indicates that a given stress condition is associated with lower antibody concentrations. The authors found that chronic stress ($P = .001$), life events ($P = .0001$) and perceived stress ($P = .02$) were all associated with a lower immune response; the ES was, however low, with a correlation coefficient of -0.18 for all three groups of stress condition. Similar results were obtained in the analysis by age-group. The meta-analytical estimates extracted are reported in Table 3.25.

We decided not to re-perform a meta-analysis for several reasons. First, several studies reported the outcome of SC and/or SP for more than one (and sometimes all) of the vaccine strains together (and not separately by strain). Second, only a few studies reported raw data of interest; most used alternative measures and/or ESs (e.g. correlation and regression coefficients, η^2, etc). Third, it was difficult to categorize stressors. In any case, even if the

meta-analysis had been re-performed, the overall CES would have been of class IV at the most, given the small sample sizes.

Table 3.25. Pooled estimates extracted from the available meta-analyses of the effect of psychological stress on the influenza vaccine-induced immune response.

Stressor	Age	*k*	*N*	Model	ESR (95% CI)	*P*
Chronic stress	Any	6	493	FE	-0.18 (-0.28–-0.08)	.001
Life events	Any	6	514	RE	-0.18 (-0.27–-0.09)	.0001
Perceived stress	Any	3	151	RE	-0.18 (-0.33–-0.02)	.02
Any	Younger	6	466	FE	-0.17 (-0.27–-0.07)	.001
Any	Older	6	568	FE	-0.25 (-0.34–-0.15)	.0001

CI: confidence interval; ESR: effect size correlation coefficient; FE: fixed-effects model; RE: random-effects model

Evidence Mapping

A total of 97 meta-analytical estimates obtained were mapped in a single bubble plot (Figure 3.3). While constructing the bubble plot, we realized that the overall CES might not correspond exactly to the magnitude of the observed ES. This non-correspondence was primarily driven by the total sample size. For instance, although the ESs regarding HIV were large, the overall CES was only of class IV, given $N < 1{,}000$. In order to contextualize this, we first transformed the binary outcomes of SC/SP to the standardized mean difference (also known as Cohen's *d*), by using the following formula [Borenstein et al. 2009]: $d = \log_e(\mathrm{OR}) \cdot \sqrt[3]{/\pi}$, where π is a constant approximately equal to 3.14. This transformation was necessary in order to display binary (SCs and SPs) and continuous outcomes (difference in the post-vaccination HAI titers) on the same graph.

As shown in Figure 3.3, most host factors studied exerted a negative effect on IV-induced immunogenicity. Only four pooled estimates were associated with an enhancing effect: this was the case of SPs against A/H1N1 and A/H3N2 among the users of pre/pro/symbiotics and the physically active elderly. These estimates, however, were assigned a CES of only IV.

The distribution of single CESs and average modules of ESs is reported in Table 3.26. As expected (since many studies were conducted during the last 2009 pandemic, when the monovalent A/H1N1pdm09 vaccine was used), most CES I pooled estimates were recorded only for A/H1N1. The omnibus analysis of variance (ANOVA) test did not show any difference between the observed $|d|$ and virus (sub)type ($F_{2,94} = 0.59$, $P = .56$). By contrast,

there was some difference between the ES observed and the CES assigned ($F_{4,92}$ = 5.18, P = .0008).

Figure 3.3. Bubble plot of the cumulative evidence synthesis (CES), by class, direction, (sub)type, serological parameter.

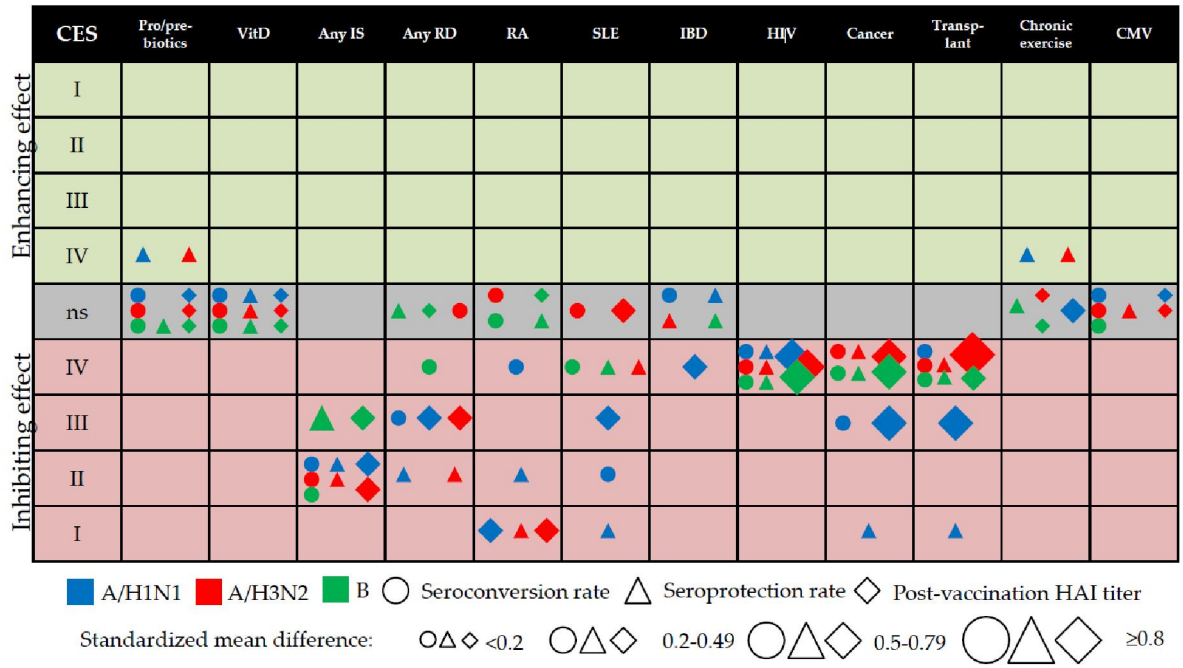


Table 3.26. Distribution of cumulative evidence synthesis classes and mean Cohen's *d*s, by viral (sub)type.

Parameter	A/H1N1 (*k* = 34)	A/H3N2 (*k* = 33)	B (*k* = 30)
CES I, *N* (%)	4 (12)	0 (0)	0 (0)
CES II, *N* (%)	6 (18)	4 (12)	1 (3)
CES III, *N* (%)	6 (18)	1 (3)	2 (7)
CES IV, *N* (%)	8 (24)	14 (42)	12 (40)
CES ns, *N* (%)	10 (29)	14 (42)	15 (50)
Mean |*d*| (SD)	0.17 (0.17)	0.16 (0.21)	0.12 (0.16)

CES: cumulative evidence synthesis class; SD: standard deviation

Discussion

In this study, we examined the available systematic evidence on the relationship between several host-related factors (both modifiable and unmodifiable) and IV-induced

immunogenicity; the analysis was made from both the qualitative and quantitative points of view. The increasing number of SRs/SRMAs on the same/similar topics and performed by different research groups using different systematic approaches may confound the decision-making of the principal stakeholders, including, for example, clinicians, reimbursement agencies and the pharmaceuticals industry. To overcome this problem, the emerging field of umbrella reviews [Aromataris et al. 2015] aims to synthetize the available systematic evidence. In addition to providing a standard representation of pooled estimates, the present research tried to classify this evidence in terms of CESs, and to map the output of interest in a single figure (see Figure 3.3). The diversified categorization strategy used to map the evidence, together with the tabulated data presented, also allowed us to identify some evidence gaps.

Regarding external validity, our pooled estimates were generally in line with those previously published. The main exception concerned the use of pre-/pro-/symbiotics, in that our estimates were more conservative and usually non-significant. The most probable explanation for this is that different extraction modalities and statistical approaches were used. Moreover, as noted above, even the four significant estimates regarding the use of pre-/pro-/symbiotics established in this paper may be prone to the so-called "industry sponsorship" bias; these data should therefore be interpreted with caution.

The present study found that several (but not all) immunosuppressive conditions were associated with a significantly lower immune response to IV. Several parameters analyzed that regarded some immunosuppressive conditions, such as HIV, transplantation and cancer, were assigned only class-IV CESs, despite their moderate-to-high ESs; this was primarily the case of A/H3N2 and B viruses. The most obvious reason for this is that the total number of cases in which these parameters were investigated was often < 1,000; indeed, CES classes I–III require a total sample size of at least 1,000 cases [Fusar-Poli and Radua 2018]. The parameters regarding the subtype A/H1N1 were less affected by this limitation, since many primary studies investigated the immunogenicity of monovalent pandemic H1N1pdm09 vaccines. Indeed, further larger-scale studies on several immunosuppressive diseases are needed in order to upgrade the CESs observed. Moreover, the residual heterogeneity observed (after performing subgroup/meta-regression analyses) could be due to different treatment regimens in the same category of disease. Indeed, we included papers on the same immunosuppressive category published in the range 1970s–2010s; in these 40 years, the standard of care has changed radically [Selmi et al. 2014; Arruebo et al. 2011]. In the future, more detailed analyses (e.g. by medicine type) will be needed.

The levels of available evidence regarding intravenous drug use, genetic polymorphisms, diabetes mellitus, acute physical activity, psychological stress and previous BCG vaccination were not included in the overall evidence synthesis reported in Figure 3.3, owing to the paucity of primary studies available and/or overlapping between the categories included. These factors need to be (re)-examined in the future, given their enormous public health burden.

Our results may have several public health implications. According to the latest report on seasonal IVs, issued by the ECDC (2017), all Member States recommend vaccination for subjects suffering from immunosuppression due to disease or treatment and hematologic/metabolic disorders. On the one hand, these populations are at increased risk of developing severe and life-threatening disease [WHO 2012]; on the other, the immune response to IV may be highly compromised (Figure 3.3). There is some evidence [Iorio et al. 2003; Baldo et al. 2007; McKittrick et al. 2013; GiaQuinta et al. 2015] that alternative (so-called "enhanced") IVs, including adjuvanted and high-dose (quadruple antigen content) formulations, produce a more robust immune response than conventional IVs in immunosuppressed individuals. However, these vaccines are currently indicated for people aged 65 years or more [CDC 2019a]. Alternatively, a two-dose regimen could be considered. However, the effect of a booster dose on IV-induced immunogenicity is controversial. For instance, an SRMA by Liao et al. (2016b) found that a booster did not have any beneficial effect on the immune response in patients on hemodialysis or peritoneal dialysis or in renal transplant recipients. Moreover, the novel egg-independent technologies with several potential advantages are becoming increasingly common [Manini et al. 2017; Trombetta et al. 2019] and should be further investigated also from the immunogenicity point of view.

While the IV formulation and dose regimen in immunocompromised individuals are still to be clarified, the public health strategy aimed at protecting this vulnerable population indirectly is to be pursued. Indeed, vaccinating household contacts and caregivers (including healthcare workers) is highly recommended [ECDC 2020].

Regarding the so-called "natural adjuvants", including the concomitant use of pre-/pro-/symbiotics, vitamin D supplements or moderate physical exercise, we found almost no effect on the IV-induced immune response. The few statistically significant pooled estimates had low ESs (all CESs IV) and were therefore of doubtful practical/public health significance.

Together with its strengths, several limitations of the present study must be acknowledged. Given the umbrella review approach, three major categories of systematic

error could occur, namely: (i) bias regarding our own methodology; (ii) bias regarding the SR/SRMAs included and (iii) bias regarding primary studies.

Considering the first point, we tried to create a more sensitive (and therefore less specific) search script, in order to identify as many as possible of the publicly available SRs. The script created for the automatic search strategy was, in our opinion, sufficient to discover most publicly available SRs/SRMAs; indeed, the manual search did not produce any further results. However, we acknowledge that some SRs that were not dubbed as such by their authors might have been missed by our search strategy.

The second limitation, in our view, is the most challenging, since the reporting quality of the SRs/SRMAs included was generally moderate-to-low, as per the AMSTAR-2 checklist. One explanation may lie in the fact that several SRs/SRMAs were published before the publication of AMSTAR-2 [Shea et al. 2017]. Indeed, if we estimate a two-year lag (as the time needed for the authors to acquaint themselves with the new "quality" scale for their SRs/SRMAs protocols and for peer-review and proof correction) between the publication year of the SRs/SRMAs included and the paper by Shea et al. published in 2017 [Shea et al. 2017], we can see that only 1/28 of the SRs/SRMAs included was published in 2019. Notably, this paper [van den Berg et al. 2019] had a relatively high AMSTAR-2 rating (75% of "Yes" ratings). However, as mentioned in the Methods section, the original AMSTAR scale, which was published in 2007 [Shea et al. 2007], included most of the items contained in the newer version. Therefore, the novelty of AMSTAR-2 is unlikely to have been the main driver of the suboptimal reporting quality of the SRs/SRMAs included.

Again with regard to the second limitation, we must acknowledge that most primary research studies were included on the basis of existing SRs/SRMAs (which is essential in an umbrella review). We strove to mitigate this limitation by performing an updated search by means of the same search strategy and study inclusion criteria as the original SR/SRMAs. We did not, however, test the sensitivity/specificity parameters of the single search strategies, since this was beyond the scope of our study.

Third, we did not investigate any risk of bias among single primary research studies included in the present analysis. Again, this went well beyond our aims. Indeed, as demonstrated by the AMSTAR-2 checklist, only 16 of 28 (57%) SR/SRMAs used a satisfactory technique to assess the risk of bias.

We have some suggestions for future research. First of all, authors of papers on IV-induced immunogenicity should be aware of the currently used terminology, i.e. immunogenicity, efficacy and effectiveness. For instance, we found several studies (even

those conducted in the past few years) dealing with CoPs that were entitled “efficacy” studies, without the surrogate nature of the outcome of protection being mentioned in the abstract/title. This fact may have altered the search output of the SRs/SRMAs included, as well as slowing down the selection/abstracting process. We therefore invite researchers outside IV-related topics to adopt a single terminology, such as, for example, that proposed by the US CDC (2019b).

In conclusion, the present research mapped the available evidence on several modifiers of the IV-induced vaccine response. While the inhibiting effect of several immunosuppressive host factors was evident, the enhancing effect of pro/pre/symbiotics and chronic physical exercise was doubtful and virus type-specific (A but not B); the overall CES was only IV. In other words, the included SR/SRMAs on host factors with potentially enhancing effect on the IV-induced immunogenicity suffered from several limitations (e.g. a low number of included primary studies with a few participants with well-known effects on statistical power in the pooled estimates; possibility of the “industry sponsorship” bias since the funding source of the primary trials was not investigated; etc.). This is why future well designed RCTs or observational studies on the effect of this “natural adjuvants” are needed. On the other hand, we discovered that the pooled ESs observed may not exactly correspond to the CES assigned. This means that further studies are needed in order to upgrade the overall CES grading system.

Furthermore, nowadays the field of vaccinology is still empirical in several aspects [Poland et al. 2018]. The current limited knowledge into qualitative and quantitative paradigms of the immune response generated by vaccines is a serious barrier to understanding poor vaccine immunogenicity in a plethora of physiological and pathological conditions. Today, emerging scientific lines propose a personalized approach to the practice of vaccinology, as it happens for other healthcare fields [Poland et al. 2018; Poland et al. 2011]. Studying the host-related correlates of IV-induced immune response could contribute to the production of new personalized vaccines and to the development of new patient-oriented vaccination strategies in a value-based public health perspective.

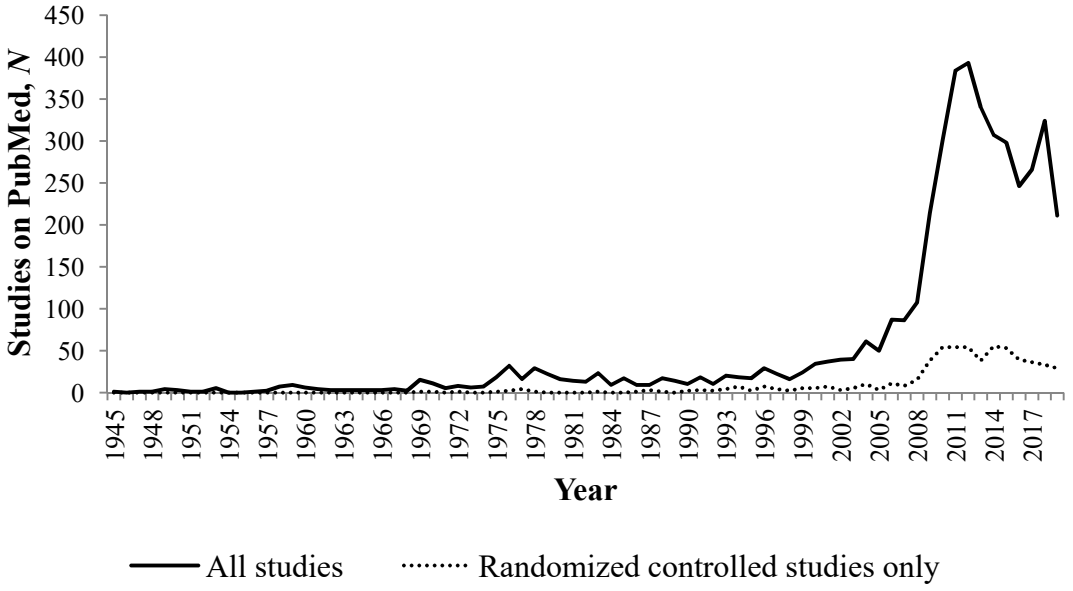
CHAPTER 4. OVERALL SYNTHESIS AND CONCLUDING REMARKS

IV research is a continuously evolving field; as we showed in Chapter 2, several IV candidates are in various stages of clinical development and some of them will likely be commercialized in the next few years (e.g. a dossier on a plant-based quadrivalent virus-like particle recombinant IV has recently been submitted to the Canadian regulatory agency for scientific review [Medicago 2019]). Given the particular epidemiological features of both influenza virus and influenza-related health outcomes, the so-called "hard clinical endpoints" are often unfeasible for various reasons and cannot always reflect the real value of IVs. According to the recent WHO (2017a) framework, the real protection induced by IVs may be quantified by measuring either efficacy or effectiveness parameters. Vaccine efficacy is defined as a reduction of disease among vaccinees resulting from immunization in ideal conditions, as estimated from RCTs, while vaccine effectiveness is the same reduction but derived from real-life conditions, as estimated from observational studies (case-control, cohort and their modifications). In both efficacy and effectiveness studies, several influenza-related clinical outcomes may be considered; these differ in terms of specificity and sensitivity. The "gold standard" is laboratory-confirmed influenza diagnosed by reverse transcription-polymerase chain reaction (RT-PCR), though other laboratory methods may also be used (e.g. culture or rapid tests). However, the use of laboratory confirmation may pose various challenges, and it is often more convenient to use less specific influenza-related outcomes (so-called "proxy" measures). These may include: influenza-like illness (ILI), severe acute respiratory infection (SARI), all-cause pneumonia requiring hospitalization, and all-cause mortality (this last, however, is not recommended) [WHO 2017a]. Theoretically, IV-induced immunogenicity should correlate with both efficacy and effectiveness, but this often does not happen (see below).

As mentioned earlier in the thesis (see Chapter 2), most IV-related RCTs have immunogenicity parameters as primary endpoints, and the use of these parameters seems to be increasing. We verified the above statement by conducting a simple bibliometric analysis in PubMed by using the following script: ("immunogenicity" OR (immun* AND "response")) AND (influenza[MeSH Terms]) (Figure 4.1). The increasing trend is evident in both time series, peaking between 2011 and 2013. Indeed, the Mann-Kendall test rejected the null

hypothesis that there was no monotonic upward trend in either "all studies" ($z = 9.73$, $P < .001$) or "RCTs only" ($z = 8.77$, $P < .001$) time series.

Figure 4.1. Number of records on influenza-related immunogenicity parameters available in PubMed from 1945 to 2019.



Current immunization practices mostly assume that the same vaccine is universally suitable for everybody in the target population (unless a contraindication exists). These practices are based on several assumptions. First of all, this approach assumes that every single individual is at approximately the same risk of contracting a vaccine-preventable disease such as influenza. Second, the vaccine formulation and the number of doses needed in order to achieve protection are the same across the target population. Third, it is believed that essentially all vaccinees will respond to the vaccine stimulus (and from the point of view of both humoral and CMI immune responses) [Poland et al. 2008].

Regarding seasonal IV, the above-mentioned "dogmatic" statements can be contradicted, at least partially. Natural (i.e. among unvaccinated individuals) influenza attack rates are usually higher in children than in adults. For instance, an SRMA by Somes et al. (2018) of 32 RCTs showed pooled estimates of natural attack rates of 12.7% (95% CI: 8.5–18.6%), 4.4% (95% CI: 3.0–6.3%) and 7.2% (95% CI: 4.3–12.0%) in children < 18 years, adults of working age and the elderly, respectively. Moreover, there may be some interaction between age and virus type. Indeed, an SRMA by Jayasundara et al. (2014) established that

the natural attack rate in children was 12.27% (95% CI: 8.56–15.97%) and 5.50% (95% CI: 3.49–7.51%) for virus types A and B, respectively. In the adult population, the corresponding rates were 2.32% (95% CI: 1.47–3.17%) and 0.59% (95% CI: 0.28–0.91%), respectively [Jayasundara et al. 2014].

Secondly, the IV formulation and/or the number of doses required may depend highly on several characteristics of the vaccinee; therefore, the so-called "one-size-fits-all" approach may be judged unsuitable. For instance, traditional egg-based standard-dose unadjuvanted IVs may be poorly immunogenic in both young children and older adults, owing to the immature immune system [Moriarty and Omer 2014] of the former and the immunosenescence of the latter [Sambhara and McElhaney 2009]. In this regard, alternative IV formulations have been developed and have proved to be more immunogenic and protective [Chen et al. 2020]. Moreover, these target-specific features of novel IV formulations have recently been incorporated into public health practices. For example, aTIV and hdTIV have been preferentially recommended in the United Kingdom (UK) [Nation Healthcare Service of England 2020] and Australia [Australian Technical Advisory Group on Immunisation 2020]. LAIV is preferentially recommended for children in the UK [Nation Healthcare Service of England 2020]. In Italy, aTIV was preferentially recommended in subjects aged 75 years and above in the 2018/19 [Italian Ministry of Health 2018] and 2019/20 seasons [Italian Ministry of Health 2019].

Thirdly, another particular feature of IV is that secondary exposure (i.e. previous exposure to either the circulating virus or IV antigen) may have a differential effect on humoral and cellular immunity. Indeed, Rosendahl Huber et al. (2019) demonstrated that a single dose of the 2009 pandemic MIV induced both sufficient humoral and T cell responses, while a second dose of the same vaccine further increased only antibody responses, but not cellular responses. On the other hand, both humoral and cellular immune responses were boosted by the next seasonal TIV [Rosendahl Huber et al. 2019].

The preferential recommendations of one IV type over another may be seen as crucial, and is among the first steps towards the personalization of preventive healthcare in general and IV in particular. Indeed, recent advances in personalized medicine are now being applied to vaccinology. In this regard, a mathematical model conceptualized in order to predict some non-random events that lead to a pre-defined vaccine-induced immune response has been proposed [Poland et al. 2013]; this can be expressed by equation (1):

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \varepsilon$$

where

y is the vaccine-induced immunogenicity parameter;
β_0 is the intercept;
β_i is the regression coefficient for the i^{th} independent variable;
X_i is an independent variable indicating the amount of change in y for a one-unit change in X_i;
ε is the random error.

On providing a significant correlation between IV efficacy/effectiveness and the IV-induced immune response, the above equation may be customized to the theoretical framework by Belongia and McLean (2019), in that IV-induced protection is a function of the features of the vaccinee, influenza virus and IV. The modified equation (2) would therefore be:

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i \mathrm{H}_i + \sum_{j=1}^{p} \beta_j \mathrm{V}_j + \sum_{k=1}^{q} \beta_k \mathrm{IV}_k + \varepsilon$$

where

β_i is the regression coefficient for the i^{th} host-related (H) independent variable;
β_j is the regression coefficient for the j^{th} virus-related (V) independent variable;
β_k is the regression coefficient for the k^{th} IV-related independent variable.

As we demonstrated in Study 1, there is wide variation in the adoption of single immunological assays, HAI being the absolute "leader" (see Table 2.1 and Figure 2.1). However, apart from its technical and economic convenience, HAI may present some important limitations, which must be taken into account. Table 4.1 summarizes the most frequently used immunological assays able to detect/quantify the humoral immune response induced by IVs and compares them from the point of view of advantages and disadvantages. In this regard, and from the results of our Study 1, assays measuring neutralizing and anti-NA

antibodies seem to be undervalued, despite their clear advantages over the more "traditional" HAI. In a body of previous research, the VN assay has shown a high correlation ($r < .50$) with the corresponding HAI titers in various populations, including: (i) the general population of all ages [Truelove et al. 2016]; (ii) children [Kim et al. 2012]; (iii) adults [Grund et al. 2011; Trombetta et al. 2018; Sicca et al. 2020]; (iv) the elderly [Ansaldi et al. 2006] and (v) HIV-positive pregnant women [Nunes et al. 2018]. However, as underlined by Sicca et al. (2020), although the correlation coefficient between HAI and VN titers is nominally high, the agreement is poor; these authors therefore urged the adoption of the internationally recognized and standardized protocols for the VN assay [Sicca et al. 2020]. Indeed, some important and clinically significant observations may emerge from using the VN assay. Moreover, in our Study 1 , we established that industry (co)-sponsored trials had, on average, 40% lower odds of quantifying neutralizing antibodies. Considering that most [61.9% (95% CI: 59.0–64.7%)] research reported in Study 1 was industry (co)-sponsored, we believe that IV producers should consider the VN assay more frequently.

Table 4.1. Comparison of the principal immunological assays used to measure influenza vaccine-induced humoral immune response [adapted from Haaheim and Katz 2011 and Trombetta and Montomoli 2016].

Assay	Advantages	Disadvantages
Hemagglutination inhibition (HAI)	• Wide use • Technically simple • Inexpensive • Detects both IgM and IgG • Known titer that correlates with protection • Good correlation with other assays like SRH, ELISA or VN	• Lack of sensitivity in detecting antibodies against some influenza viruses • Inter-laboratory variability
Single radial hemolysis (SRH)	• Technically simple • Inexpensive • Reliable and unbiased • Known titer that correlates with protection • Good correlation with other assays like HAI, ELISA or VN	• Use limited to some European laboratories • Detects mainly IgG

Table 4.1 (*continued*). Comparison of the principal immunological assays used to measure influenza vaccine-induced humoral immune response [adapted from Haaheim and Katz 2011 and Trombetta and Montomoli 2016].

Assay	Advantages	Disadvantages
Virus neutralization (VN)	• Ability to detect a broader range of functional antibodies • Enhanced sensitivity in detecting antibodies against some viruses • Good correlation with other assays like HAI, SRH or ELISA	• Requires live virus • Expensive • Less specific than HAI • No known titer that correlates with protection • High inter- and intra-laboratory variability
Enzyme-linked immunosorbent assay (ELISA)	• Able to detect both serum and nasal wash IgA, IgM and IgG • Unbiased automated process for high-throughput testing • No pretreatment of sera or erythrocytes • Good correlation with other assays like HAI, SRH or VN	• Lack of specificity for virus types A and B • No known titer that correlates with protection
Enzyme-linked lectin assay (ELLA)	• Able to detect specific anti-neuraminidase antibodies • High sensitivity	• No known titer that correlates with protection • Relatively low use

Analogously, despite the advantage of quantifying anti-NA antibodies, these antibodies are still greatly underused in any research, whether sponsored or publicly-funded. This fact is particularly disappointing, given that IVs containing optimal amounts of NA may be particularly useful in addressing the phenomena of antigenic drift and shift, as NA is an antigen that may offer broader protection [Eichelberger and Monto 2019].

In sum, we conclude that the outcome variable *y* in Equation 2 must be different for each immunological assay.

We will now proceed to discuss the three domains of independent variables described in the right-hand part of Equation 2. The total number of host characteristics (*H*s) is probably infinite. In our current work (Study 2) we were able to identify up to ten systematically derived health conditions able to modify (usually inhibit) the IV-induced immune response. It now seems useful to provide a policy-oriented synthesis from the Italian perspective. For this purpose, the evidence-based bubble plot provided in Chapter 3 (see Figure 3.3) was conceptualized and tabulated against the most recent recommendations provided by the Italian Ministry of Health (2020). However, we extracted only those host-related categories that may impact IV-induced immunogenicity. Other risk categories (e.g. higher risk of professional exposure or transmission) were excluded, as they were outside the scope of this study. As shown in Table 4.2, several evidence gaps still have to be addressed from the systematic point

of view. Of note, although we were not able to formally evaluate the effect of older age on IV-induced immunogenicity, we did not consider this category as an evidence gap for the following reasons. First, the phenomenon of immunosenescence and its detrimental effect on the immune response is well recognized [Sambhara and McElhaney 2009]. Second, seasonal IV is recommended for older adults in most high-income countries [ECDC 2018; Grohskopf et al. 2018]. Third, the available pooled quantitative analyses [Seidman et al. 2012] (these were excluded from Study 2 owing to the indirect comparisons made) have suggested a significantly poorer immune response in the elderly than in working-age adults.

Table 4.2. Italian Ministry of Health (2020) recommendations for influenza vaccination in the 2020/21 season, as compared with the results of Study 2.

Population group	Analyzed	CES†			Systematic evidence gap
		A/H1N1	A/H3N2	B	
Subjects aged ≥ 65 years	–	NA	NA	NA	–
Chronic respiratory diseases	–	NA	NA	NA	+
Cardiovascular diseases	–	NA	NA	NA	+
Diabetes mellitus and other metabolic diseases, including obesity	+	NA	NA	NA	+
Chronic renal failure	–	NA	NA	NA	+
Adrenal insufficiency	–	NA	NA	NA	+
Hemoglobinopathies and other hematopoietic disorders	–	NA	NA	NA	+
Tumors	+	III/I/III	III/III/III	III/III/III	–
Congenital or acquired conditions associated with insufficient antibody production	+/–	II/II/II	II/II/II	II/III/III	+/–
Iatrogenic immunosuppression	+/–	II/II/II	II/II/II	II/III/III	–
HIV/AIDS	+	IV/IV/IV	IV/IV/IV	IV/IV/IV	–
Chronic inflammatory diseases	+/–	III/II/III	ns/II/III	IV/ns/ns	+/–
Malabsorption syndromes	+/–	ns/ns/IV	NA/NA/ns	NA/NA/ns	+/–
Pathologies for which major surgery is planned	+/–	I/III/III	IV/IV/IV	IV/IV/IV	–
Pathologies associated with high risk of pulmonary aspiration	–	NA	NA	NA	+
Chronic hepatopathies	+/–	NA	NA	NA	+

Table 4.2 (*continued*). Italian Ministry of Health (2020) recommendations for influenza vaccination in the 2020/21 season, as compared with the results of Study 2.

Population group	Analyzed	CES†	Systematic

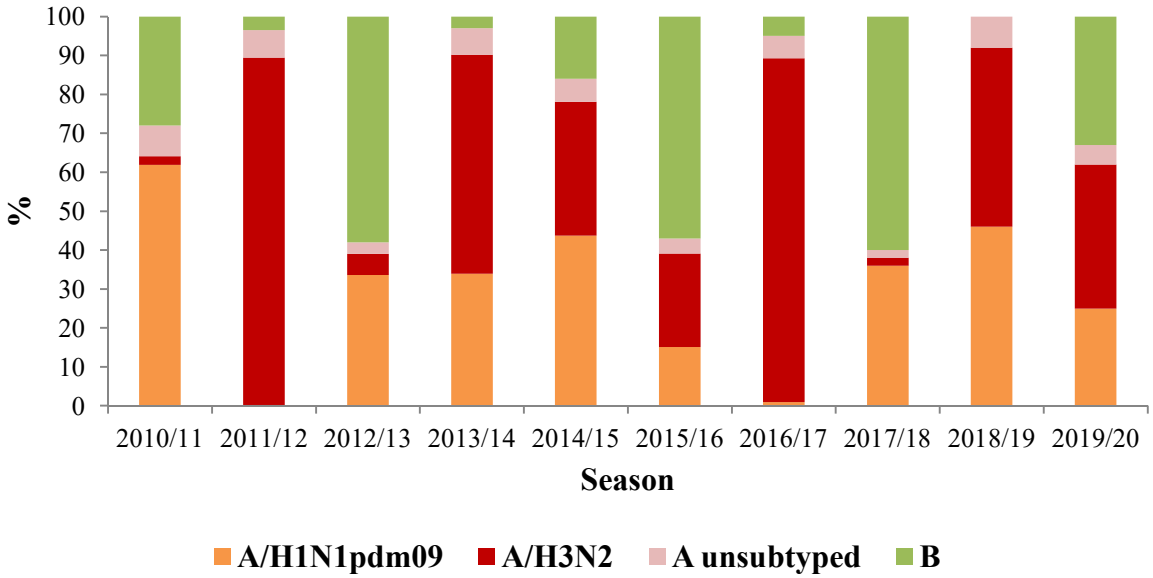
		A/H1N1	A/H3N2	B	evidence gap
Chronic hepatopathies	+/–	NA	NA	NA	+
Children on chronic therapy with acetylsalicylic acid	–	NA	NA	NA	+
Children at risk of developing Reye's syndrome following influenza	–	NA	NA	NA	+

[†]Results are reported as seroconversion rate/seroprotection rate/post-vaccination hemagglutination-inhibition titer; NA: non-available; ns: non-significant.

Another point of interest here is that the reported CESs may be upgraded in the future when new studies become available. Indeed, despite a large ES, some clearly immunosuppressive conditions, such as HIV/AIDS and organ transplantation, were penalized from the point of view of CES, owing to the relatively small pooled number of subjects. Moreover, it should be borne in mind that the categories analyzed in the present Study 2 may still be composed of highly differentiated populations. For example, IBD has three major forms, namely Crohn's disease, ulcerative colitis and indeterminate colitis [Satsangi et al. 2006]. Moreover, patients with any of these three nosological forms may be on different treatment regimens, including either immunosuppressive or non-immunosuppressive therapies. Although most IBD patients take immunosuppressive medicines, a significant proportion of them are on monotherapy with 5-aminosalicylic acid (mesalazine/mesalamine), which is considered a non-immunosuppressive therapy and is clinically well accepted in treating mild-to-moderate ulcerative colitis [Andrews et al. 2009]. This may explain the reason for the non-significance of the majority of CESs observed. The same rule can be applied to other categories investigated, such as the different forms of rheumatic diseases.

The characteristics of influenza viruses (*V*s), and the circulating virus population in particular, constitute, in our opinion, the most "tricky" set of variables, since they are difficult to predict. First of all, the relative distribution of the virus (sub)types varies by year and it is almost unpredictable. Figure 4.2 reports the relative distribution of the virus (sub)types observed in the post-pandemic period (from the 2010/11 to 2019/20 seasons) in Italy [Italian Superior Institute of Health 2020]. In the last ten seasons, virus type B clearly predominated only in three seasons (2012/13, 2015/16 and 2017/18), while the subtype H3N2 was usually more prevalent than H1N1pdm09 (Figure 4.2).

Figure 4.2. Relative distribution of influenza virus (sub)types observed in Italy from 2010/11 to 2019/20 [adapted from Italian Superior Institute of Health (2020)].



Second, different influenza virus (sub)types may infect different age-groups in a different manner. Indeed, in their meta-regression analysis, Panatto et al. (2018) showed that the detection rate of influenza virus B was generally lower in the elderly than in younger populations, especially children and adolescents. A subsequent paper by Caini et al. (2018) documented almost the same trend, but reported a more detailed primary analysis of 358,796 influenza cases observed in a representative set of 29 countries between 1999 and 2014. The final outcome was expressed as a summary relative illness ratio (sRIR), defined as "*the percentage of cases in an age-group in relation to the percentage of the country's population in the same age-group*". In other words, the sRIR is the highest estimate inside a given age-class, and indicates the highest relative prevalence of a given influenza virus (sub)type. As shown in Table 4.3, the highest sRIRs for pre-2009 seasonal A/H1N1, A/H1N1pdm09, A/H3N2 and B were observed among young children, older children, working-age adults and the elderly, respectively [Caini et al. 2018].

Third, the continuous antigenic drift of the influenza virus population, which is largely unforeseeable, gives rise to annual epidemics, and the mutation rate of virus types A and B is different, with type A displaying a higher mutation rate [Nobusawa and Sato 2006; Shao et al. 2017].

Table 4.3. Summary relative illness ratios of influenza virus vaccine (sub)types, by age-group [adapted from Caini et al. 2018].

Age-class, years	A/H1N1 pre-2009		A/H1N1pdm09		A/H3N2		B	
	sRIR	95% CI	sRIR	95% CI	sRIR	95% CI	sRIR	95% CI
0–4	*3.57*	*3.00–4.14*	2.28	2.10–2.46	3.30	2.95–3.64	2.93	2.68–3.19
5–17	1.36	1.19–1.54	1.23	1.02–1.45	1.04	0.93–1.14	*1.69*	*1.53–1.85*
18–39	0.84	0.72–0.96	*0.94*	*0.87–1.01*	0.73	0.68–0.78	0.65	0.59–0.71
40–64	0.49	0.41–0.57	*0.62*	*0.55–0.69*	0.59	0.55–0.63	0.41	0.37–0.45
≥ 65	0.16	0.12–0.20	0.27	0.24–0.31	*0.74*	*0.66–0.83*	0.38	0.33–0.43

The highest summary relative illness ratio (sRIR) in each age-class is shown in *italics*.

Finally, the IV-induced immune response against some virus (sub)types, as measured by means of the HAI assay, may be inefficient. Specifically, HAI lacks the sensitivity to detect antibodies against A/H5, A/H7 and even some B strains [Haaheim and Katz 2011; Trombetta and Montomoli 2016].

The characteristics of IVs may, however, be expressed by a set of dummy-coded variables that represent a variety of characteristics, including for example: valence, inactivation-related issues (inactivated vs live attenuated), antigen characteristics, mode of administration, production platform, presence of adjuvants, antigen quantity, etc [Grohskopf et al. 2018; ECDC 2019]. Regarding IV valence, the currently available seasonal IVs may be either TIVs or QIVs. The former formulation is composed of two A subtypes (A/H1N1 pdm09 and A/H3N2) and one strain belonging to either B lineage (B/Victoria or B/Yamagata), while the latter contains both B lineages. The aim of including the second B strain in the QIV formulation is to reduce the negative effects of B lineage mismatch, which is relatively frequent [Tisa et al. 2016]. Indeed, over 13 consecutive influenza seasons in Italy, 50.6% of B detections were of the lineage not included in the TIV formulation [Puzelli et al. 2019]. On the other hand, the impact of B lineage mismatch on both IV-induced immunogenicity and vaccine effectiveness may depend on the age of the vaccinee. In their large-scale meta-regression analysis, Beyer et al. (2017) found that the impact of B lineage mismatch on IV effectiveness depended on the level of pre-seasonal antibodies, which is highly correlated with age. Specifically, infants and young children, who presumably have low pre-existing antibody titers, are subject to a significant negative impact, while in older adults, whose pre-existing immunity is high, this impact is negligible [Beyer et al. 2017].

The currently available IVs may be either killed/inactivated or LAIVs. The latter are, however, commercialized in a small number of countries. The currently marketed LAIVs are administered intranasally and are based on temperature-sensitive strains that are still able to replicate in the upper respiratory tract (while replication in the lower respiratory tract is limited). LAIVs seems to be particularly suitable for pediatric populations, since (i) children

are important spreaders of the wild-type viruses and (ii) intranasal administration may increase parental compliance. By contrast, there are some concerns about the use of LAIVs in the elderly, as frequent previous contacts with viruses may exert pressure on the immune system and facilitate reversion of the vaccine strain to the wild strain [Gasparini et al. 2011]. Indeed, the most widely used quadrivalent LAIV is indicated for subjects aged from 2 to 49 years [FDA 2019].

On the basis of their antigen characteristics, the inactivated IVs can be further divided into whole-virion, split and subunit formulations, though most available IVs are split or subunit. The whole-virion IVs contain the entire virus, while in the split IVs the virus is fragmented by means of detergents. Subunit IVs are further purified and contain only the main viral glycoproteins of HA and/or NA [Grohskopf et al. 2018; ECDC 2019]. The aim of this further purification is to reduce reactogenicity. However, it seems plausible that that these highly purified formulations may also be less immunogenic, owing to the removal of viral antigens other than HA and NA that are still immunogenic. On the other hand, an MA by Beyer et al. (1998) did not find a clinically significant difference in the sero-response induced by the three formulations, while (as expected) the subunit IVs elicited lower rates of local and systemic adverse events [Beyer et al. 1998].

There are three main routes of IV administration, namely intramuscular/subcutaneous, intradermal and intranasal (this last is mostly used for LAIVs; see previous paragraph) [Grohskopf et al. 2018; ECDC 2019]; most IVs are administered intramuscularly. The intradermal route may present some advantages, since it exploits the unique immune "microclimate" of the skin, which is rich in specialized dendritic cells (e.g. Langerhans cells and macrophages) that are highly efficient in the process of antigen presentation [Icardi et al. 2012]. Another advantage of the intradermal formulations is related to compliance, in that they may be more suitable for people who are afraid of needles [Durando et al. 2012]. Although an SRMA by Marra et al. (2013) did not establish that intradermal TIVs induced a significantly greater humoral immune response than intramuscularly administered TIVs in the overall population, it did provide evidence of greater immunogenicity in older adults when the antigen content was higher (e.g. 15 μg vs 7.5 μg) [Marra et al. 2013]. However, the commercially available intradermal IVs have recently been withdrawn from the market [EMA 2018].

To date, most (> 90%) IVs available worldwide are still produced in eggs. While this traditional egg-based platform is well standardized and has proved efficient over decades, it has some important intrinsic limitations. First of all, as it relies on the supply of fertilized hen

eggs, the potential risk of a shortage of eggs is inherently present (e.g. in the event of outbreaks of avian influenza in poultry). Second, the egg-based platform may still be susceptible to microbial contamination, given that it is a relatively "open" process. This is why antibiotics are needed during egg-based (but not cell culture-based) IV manufacturing. Third, some recent studies have suggested that not all wild virus strains can be recovered from eggs; indeed, more than 90% of A/H3N2 human isolates cannot grow in eggs. For this reason, reassortment with a donor A type virus (which is able to replicate in eggs) is needed during the egg-based manufacturing process. Finally (and this shortcoming is strictly linked to the previous one) and probably most importantly from the public health point of view, propagation of the candidate vaccine virus seeds in eggs may generate so-called "egg-adaptive" mutations that may interfere with the IV match and hence vaccine effectiveness [Manini et al. 2015; Barr et al. 2018]. In particular, A/H3N2 strains grown in eggs usually presents an egg-adaptive T160K retro-mutation that introduces a new putative *N*-glycosylation site in the dominant epitope B; indeed, isolates presenting the K160T mutation grow poorly in eggs [Zost et al. 2017]. Similarly, the L194P mutation that emerges during propagation in eggs is able to modify the 3-dimensional conformation of HA, and therefore its antigenic properties [Wu et al. 2017]. A recent Italian study [Galli et al. 2020] seems to confirm the above observations; moreover, the authors concluded that, according to the *p*-epitope model, these substitutions may have lowered the effectiveness of egg-derived IVs (in comparison with a cell-derived IV) by 50% in the 2016/17 influenza season. Similarly, a more extensive worldwide 16-year retrospective study showed that A/H3N2 and B/Victoria strains were more prone to egg-adaptive mutations, and that the cell-propagated A/H3N2 and B/Victoria proposed by the WHO as reference viruses were more antigenically similar to the circulating virus population than their egg-derived counterparts [Rajaram et al. 2020]. Interestingly, Barrett et al. (2011) claimed that an HAI titer of ≥ 1:15 provided a reliable CoP for a cell-based IV, while no additional advantage in predicting protection was seen at HAI titers of ≥ 1:30.

Several egg-independent technologies for IV production are already in place or in various stages of clinical development [Manini et al. 2017; Barr et al. 2018]. One of the first egg-alternative IVs was produced in the Madin-Darby canine kidney (MDCK) cell line [Manini et al. 2017] and its QIV version has recently become available in both the US and Europe [Lamb 2019]. Some preliminary real-world data have suggested that the cell-based QIV may be more effective than the standard egg-based QIV against several influenza-related

proxies, such as ILI [Boikos et al. 2020] or influenza-related hospitalizations [Izurieta et al. 2019].

Another egg-independent option is to use recombinant technology; one recombinant QIV is currently authorized for human use in the US [Grohskopf et al. 2018]. This vaccine is composed of 45 μg of HA, the sequences of which exactly correspond to the reference strains recommended by the WHO. HAs are produced in a proprietary continuous cell line (*expres*SF+® insect cells); the recombinant HAs are expressed in this cell line by means of a baculovirus vector [Cox et al. 2008]. In an RCT [Dunkle et al. 2017] conducted among subjects aged 50 years or more, the recombinant QIV proved to be 30% (95% CI: 10–47%) more efficacious than the egg-derived QIV against any laboratory-confirmed influenza.

Adjuvanted IVs may represent another important milestone in the development of improved vaccine formulations. Some particularly attractive features of the use of adjuvants in IVs include: (i) enhancement of impaired immune response; (ii) boosting of immunogenicity; (iii) more rapid immune response; (iv) longer-lasting immune response; (v) dose sparing and (vi) immunomodulation. Several IV-related adjuvants are currently licensed (e.g. the squalene-based adjuvants MF59®, AS03®, AF03®; virosomes; etc.) [Tregoning et al. 2018]. Among these, the adjuvant MF59® was the first IV adjuvant to be licensed worldwide (in 1997 in Italy) [O'Hagan et al. 2013] and is still in use. A QIV formulation with MF59® has just been authorized for use in subjects aged 65 years or more in Australia [Therapeutics Good Administration 2019], the US [FDA 2020] and Europe [EMA 2020]. Compared with unadjuvanted TIVs, aTIV has consistently been shown to induce a more robust immune response versus both homologous [Banzhoff et al. 2003; Nicolay et al. 2019] and heterologous strains [Ansaldi et al. 2010; Nicolay et al. 2019]. It has also been shown [Frey et al. 2014] that, in subjects vaccinated with aTIV, antibodies are more persistent. Both the absolute and relative effectiveness of aTIV has also been systematically demonstrated [Domnich et al. 2017].

Finally, the quantity of antigen in a single IV dose may have an important role. Most currently commercialized IVs contain 15 μg of HA of three or four strains. However, IV formulations with higher antigen contents are also on the market. For instance, a QIV containing 60 μg of antigen has recently been licensed in Italy for the active immunization of subjects aged ≥ 65 years [Italian Ministry of Health 2020]. A trivalent formulation of this vaccine induces a higher immune response in the elderly [Samson et al. 2019], and its relative effectiveness may be higher than that of the standard-dose TIVs [Lee et al. 2018].

Apart from the type of IV, the subject's vaccination history must be taken into account. Today, there is an ongoing debate on the effects of vaccination in previous seasons on both the immunogenicity and efficacy/effectiveness observed in the current season. This is of particular importance to public health, given that annual administration of IV is currently recommended. Ng et al. (2013) suggested that, in children, previous IV administration was associated with a lower antibody response against type-A strains, while the response against type-B strains was higher among individuals primed in preceding years with strains belonging to the same lineage [Ng et al. 2013]. In this regard, the immunological hypothesis of "original antigenic sin" postulates that exposure to a novel influenza strain induces antibodies primarily to older viral strains at the expense of an immune response to novel antigens [Kim et al. 2009]. Observations from the 2009 A/H1N1 pandemic seem to be in line with this concept. Indeed, the 2009 virus affected older adults to a lesser extent. Owing to the fact that the A/H1N1 subtype of swine origin (which caused the so-called Spanish flu) circulated continuously between 1918 and 1957, most people born before 1957 were exposed to this virus, and could therefore be partially protected against the novel variant of A/H1N1pdm09 [Adalja and Henderson 2010]. Other explanations of the effect of repeated IV administration have also been described [Lewnard and Cobey 2018]. The so-called "ceiling effect" assumes that individuals with initially high antibody titers have smaller boosts. On the other hand, many non-responders start with low-to-moderate titers; one of the reasons for this may be that these individuals do respond but the responses are still unmeasured by an appropriate assay (e.g. in case most antibodies target NA but the HAI assay was used [Lewnard and Cobey 2018]. Indeed, as we demonstrated in Study 1, assays other than HAI are relatively underused. This fact is also of interest, since in the above-described non-responders, in whom HAI is traditionally used, the HAI-based CoP may not work in several individuals, and the effect of IV-induced immunogenicity may be a poor predictor of vaccine effectiveness. An SRMA by Belongia et al. (2017), which covered the post-2009 pandemic period (from the 2010/11 to 2014/15 seasons), concluded that there was marked heterogeneity in the effects of repeated IV administration, both within and between seasons and subtypes. When negative effects were observed, these were mostly ascribable to the A/H3N2 subtype, especially during the season 2014/15, when the vaccine component remained unchanged from the previous season but was antigenically distinct from the predominantly circulating A/H3N2 strain [Belongia et al. 2017]. A more recent SRMA [Ramsay et al. 2019] estimated that, in comparison with IV administration in the previous season only, administration in both seasons elicited greater protection against A/H1N1pdm09 [25% (95% CI: 14%–35%)] and B [18% (95% CI: 3–

33%)], but not A/H3N2 [7% (95% CI: -7–21%)]. By contrast, no difference in vaccine effectiveness against A/H1N1pdm emerged between IV administration in both seasons and administration in the current season only [3% (95% CI: -8–13%)], but significantly lower protection against H3N2 [- 20% (95% CI: -36–-4%)] and B [-11% (95% CI: -20–-2%) was achieved by vaccine administration in both seasons [Ramsay et al. 2019].

In the form reported, equation 2 summarizes the so-called "main" effects of the infinity of independent variables on the immunogenicity parameter of interest. However, the observed effect on y may change through more sophisticated mechanisms, such as effect interaction or mediation [Corraini et al. 2017]. Interaction occurs when there is a joint effect of two or more predictors on the outcome of interest; the effect may be either synergistic (i.e. when the joint effect is higher than that obtained from the sum of the individual effects) or antagonistic otherwise [Corraini et al. 2017]. Interaction effects are always worth checking, as they may reveal clinically important features [Cleophas et al. 2006]. In the case of IVs, many potential interaction terms may be meaningful. For example, there may be a significant interaction between age and pre-vaccination HAI titers: vaccinees with no previous antibodies may show a higher response as age increases, while a lower HAI IV-induced response may be observed with increasing age among vaccinees with a determinable antibody titer [Hirota et al. 1996]. We believe that several other modifiers of the IV-induced immune response may display some significant interaction patterns and should therefore be analyzed in future research.

Statistical mediation, by contrast, focuses on random pathways: here, a mediator is a step on the pathway between exposure and the outcome of interest. In the case of a significant mediation paradigm, the intermediate variable may partially or entirely determine the final outcome [Corraini et al. 2017]. Mediation analysis in IV research is uncommon. Indeed, the only study that we identified was conducted by Voigt et al. (2019); they analyzed a cohort of 159 adults aged 50–74 years (62% females) and found a sex-dependent expression in T and natural killer (NK) cell genes that could be partially attributed to higher CD4+ and lower NK cell fractions in women.

We understand that the personalized IV-induced immunogenicity model conceptualized in equation 2 is hard to populate and would require large and sophisticated datasets. Moreover, IV is a public health intervention that is performed annually, in a relatively short period of time, and involves millions of people. This is why a "precision vaccinology" approach could be more convenient. Personalized medicine is not completely synonymous with precise medicine, although these two terms are often used interchangeably

[Ho et al. 2020]. Indeed, precision medicine "*refers to tailoring medical treatment to individual characteristics of each patient. It does not literally mean the creation of drugs ... unique to a patient, but rather the ability to classify individuals into subpopulations that differ in their ... response to a specific treatment.*" Precision vaccines therefore: (i) consider the different target populations; (ii) are produced in order to activate the immune system in a targeted way by selecting anatomic sites, cells and/or molecular pathways that induce an appropriate and protective immune response; (iii) contain adjuvants able to act optimally in the given target population [Levi 2018]. Therefore, we believe that in the future it may be useful to perform a cluster analysis in order to identify similar traits in terms of IV-induced immunogenicity. Such a procedure may reduce technical complexity and be more convenient from the point of view of public health. As we demonstrated in Study 1, most IV immunogenicity studies are industry (co)-sponsored; however, industry is more prone to utilizing those immunological assays that are required for regulatory purposes. Private-public partnership would therefore be beneficial.

To conclude, a universal IV is the holy grail of current IV research, and much progress has been made in the last few years. Several vaccine candidates that use novel platforms are in various stages of clinical development. For these novel IVs, traditional CoPs may be unsuitable. Indeed, rather than focusing on the induction of high HAI titers, new more exhaustive approaches are needed, since cross-protective, durable IV-induced protection may be achieved by activating several compartments of the immune system [Ward et al. 2018]. A down-to-earth appraisal of the characteristics of host, virus and vaccine will undoubtedly improve our ability to measure IV-induced immunogenicity in a more functional way and establish more efficient CoPs.

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ANNEXES

Annex A. List of clinical studies included in Study 1 (*N* = 1,164).

NCT03603509	NCT00231920	NCT01511419	NCT01465035	NCT02680002	NCT02105519	NCT00849277
NCT04143451	NCT01688297	NCT03275389	NCT01047202	NCT03028974	NCT03658629	NCT00307710
NCT03888989	NCT02141581	NCT00706732	NCT00956202	NCT02586792	NCT00532792	NCT01436370
NCT03282240	NCT00336453	NCT03816878	NCT01444482	NCT03699839	NCT02398097	NCT01707628
NCT03233217	NCT00545025	NCT01640314	NCT01004653	NCT04074928	NCT03743688	NCT01000246
NCT03448705	NCT00170508	NCT01934127	NCT01224613	NCT01761123	NCT02387294	NCT00383539
NCT02819115	NCT00349141	NCT01194297	NCT02555618	NCT00554333	NCT00814385	NCT01368029
NCT03734237	NCT00377611	NCT02218697	NCT01196026	NCT03932682	NCT01319955	NCT00296829
NCT01933048	NCT00522236	NCT00968890	NCT00205270	NCT01086657	NCT03314662	NCT01666535
NCT02480101	NCT01258595	NCT01035749	NCT02988739	NCT01053143	NCT03497845	NCT00828828
NCT03884296	NCT03020628	NCT02655874	NCT00559975	NCT02691130	NCT01811823	NCT00551837
NCT03391193	NCT02249221	NCT00603811	NCT01884519	NCT02251288	NCT00764998	NCT02212990
NCT02285998	NCT03859141	NCT00936468	NCT00645411	NCT01063608	NCT00719043	NCT01008813
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NCT02710409	NCT04144179	NCT00665509	NCT00492063	NCT01938742	NCT00959465	NCT04077424
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NCT00328107 NCT02385123 NCT01561768 NCT00537524 NCT04120194 NCT03399357 NCT02675608
NCT00363870 NCT02438423 NCT00644059 NCT01407978 NCT01040078 NCT01065285 NCT02294487
NCT00995527 NCT01924169

Annex B. List of excluded studies (Study 2), with reasons.

First author, year*	Reason for exclusion
Seidman, 2012	No predefined control group
Beck, 2013	Commentary on two published systematic reviews (both were included)
Hua, 2013	Conference abstract without sufficient details
Ramos, 2015	Non-systematic nature of the review
La Torre, 2016	No predefined control group
Liao, 2016	No predefined control group
Bekkat-Berkani, 2017	No predefined control group
Chong, 2018	Comparison of different influenza vaccine types
Zhang, 2018	Comparison of different influenza vaccine types; no predefined control group

***Excluded studies:**

Seidman JC, Richard SA, Viboud C, Miller MA (2012). Quantitative review of antibody response to inactivated seasonal influenza vaccines. Influenza Other Respir Viruses 6:52–62.

Beck CR, McKenzie BC, Hashim AB, Harris RC, Zanuzdana A, Agboado G, Orton E, Béchard-Evans L, Morgan G, Stevenson C, et al (2013). Influenza vaccination for immunocompromised patients: summary of a systematic review and meta-analysis. Influenza Other Respir Viruses 7 Suppl 2:72–75.

Hua C, Barnetche T, Combe B, Morel J (2013). Influence of methotrexate, anti-TNF and rituximab on the immune response to influenza and pneumococcal vaccines in patients with rheumatoid arthritis: a systematic literature review and meta-analysis. Ann Rheum Dis 72:A408.

Ramos I, Fernandez-Sesma A (2015). Modulating the innate immune response to influenza A virus: Potential therapeutic use of anti-inflammatory drugs. Front Immunol 6:361.

La Torre G, Mannocci A, Colamesta V, D'Egidio V, Sestili C, Spadea A (2016). Influenza and pneumococcal vaccination in hematological malignancies: a systematic review of efficacy, effectiveness, and safety. Mediterr J Hematol Infect Dis 8:e2016044.

Liao Z, Xu X, Liang Y, Xiong Y, Chen R, Ni J (2016). Effect of a booster dose of influenza vaccine in patients with hemodialysis, peritoneal dialysis and renal transplant recipients: A systematic literature review and meta-analysis. Hum Vaccin Immunother 12:2909–2915.

Bekkat-Berkani R, Wilkinson T, Buchy P, Dos Santos G, Stefanidis D, Devaster JM, Meyer N (2017). Seasonal influenza vaccination in patients with COPD: a systematic literature review. BMC Pulm Med 17:79.

Chong PP, Handler L, Weber DJ (2018). A systematic review of safety and immunogenicity of influenza vaccination strategies in solid organ transplant recipients. Clin Infect Dis 66:1802–1811.

Zhang W, Sun H, Atiquzzaman M, Sou J, Anis AH, Cooper C (2018). Influenza vaccination for HIV-positive people: Systematic review and network meta-analysis. Vaccine 36:4077–4086.

Annex C. AMSTAR-2 (measurement tool for assessing systematic reviews, version 2) ratings, by paper and item (Study 2).

First author, year	Item															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Baral, 2007	–	–	–	+/-	–	+	–	+/-	–	–	0	0	–	0	0	+
Pedersen, 2009	+	–	–	+/-	–	+	+	+	+/-	–	+	–	–	+	+	–
Beck, 2011	+	–	+	+	+	+	–	+	+	–	+	–	–	+	+	+
Agarwal, 2012	+	–	–	+/-	+	–	–	–	–	–	0	0	–	0	0	+
Beck, 2012	+	–	+	+	+	+	–	+	+	–	+	–	–	+	+	+
Eckerle, 2013	+	–	–	+/-	+	–	–	+	–	–	+	–	–	+	–	+
Goossen, 2013	+	+	–	+	+	+	+	+	+	–	0	0	+	0	0	+
Hua, 2014	+	+/-	–	+/-	–	+	–	–	+	–	+	–	–	+	–	–
McMahan, 2014	–	–	–	–	–	–	–	–	+	–	0	0	–	0	0	+
Pascoe, 2014	+	–	–	+/-	+	+	–	+	–	–	0	0	–	0	0	+
Posteraro, 2014	+	–	–	+/-	+	+	–	+	+	–	+	–	–	–	–	+
Shehata, 2014	–	–	–	–	–	+	–	–	–	–	0	0	–	0	0	+
Karbasi-Afshar, 2015	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+
Nguyen, 2015	–	–	–	+/-	–	–	–	+/-	+/-	–	–	–	–	+	–	+
Huang, 2016	+	–	–	+/-	+	+	–	+	+	–	+	+	+	+	+	+
Liao, 2016a	+	–	–	+/-	–	+	–	+/-	+	–	+	+	+	–	+	+
Pugès, 2016	+	–	–	+/-	+	+	–	+	+	–	+	+	+	+	–	+
Huang, 2017	+	–	–	+/-	+	+	–	+	+	–	+	–	–	–	+	+
Lei, 2017	+	–	–	+/-	+	+	–	+	+	–	+	–	–	+	+	+
Sousa, 2017	+	–	–	–	+	–	–	+	+	–	0	–	–	0	0	–
Vollaard, 2017	–	–	–	–	–	–	–	–	+	–	0	0	–	0	0	+
Dos Santos, 2018	+	–	–	+	+	–	–	+	+	–	0	0	+	0	0	–
Lee, 2018	–	+/-	–	+/-	+	+	–	+	+	–	+	–	–	–	–	–
Subesinghe, 2018	+	–	–	+/-	+	+	–	+	+	–	+	+	+	–	–	–
Yeh, 2018	+	+/-	–	+/-	+	+	–	+	+	–	–	–	–	+	+	+
Zimmermann, 2018a	+	–	–	+/-	–	–	–	+	–	–	0	0	–	0	0	+
Zimmermann, 2018b	+	–	–	+/-	–	–	–	+	–	–	0	0	–	0	0	+
van den Berg, 2019	+	–	–	+	+	+	–	+	+	–	+	+	+	+	+	+

The ratings are reported as: Yes (+); Partial yes (+/-); No (–) and Not applicable (0)

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