Original Article

No signal of interactions between influenza vaccines and drugs used for chronic diseases: a case-by-case analysis of the vaccine adverse event reporting system and vigibase

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ABSTRACT

Background: An increasing number of reports indicates that vaccines against influenza may interact with specific drugs via drug metabolism. To date, actual impact of vaccine-drug interactions observed in the real world clinical practice has not been investigated.

Research design and methods: From VAERS and VigiBase, we collected Adverse Event Following Immunization (AEFI) reports for individuals receiving vaccines against influenza recorded as suspect and selected cases where predictable toxicity was recorded with oral anticoagulants, antiepileptics and statins (i.e., hemorrhages, overdosage and rhabdomyolysis, respectively). We applied AEFI and Drug Interaction Probability Scale (DIPS) Algorithms to assess causality of drug-vaccine interactions.

Results: 116 AEFI reports submitted to VAERS and 83 from Vigibase were included in our analysis; antiepileptics and statins were related to the highest number of *indeterminate*/consistent (93.7%; 65.3%) and *possible/probable* (50%; 57.7%) cases according to the AEFI and DIPS, respectively. The majority of cases occurred within the first week after vaccine administration (5-7 days).

Conclusions: The relative paucity of detected interactions does not impact on the benefit of the vaccination against influenza, which remains strongly recommended; this does not exclude that closer monitoring for selected patients exposed to concomitant chronic pharmacological therapies and affected by predisposing factors may be useful.

Keywords: Adverse Event Following Immunization, antiepileptic toxicity, haemorrages, rhabdomyolysis, spontaneous reporting system database, vaccine-drug interactions, influenza vaccination

1.Introduction

Annual vaccination against influenza virus is the primary means of preventing influenza and its complications in high-risk patients *i.e*. children, pregnant women, elderly and patients with chronic disorders. Safe and effective vaccines are available and have been used for more than 60 years [1,2].

Nonetheless, potential interactions between influenza vaccines and drugs have been described. To date, information reported in the summary of product characteristics (SPCs) regards the possible decreased vaccine efficacy in the presence of immunosuppressive, antimetabolites and cytotoxic drugs [3]. An increasing number of clinical observations, however, indicate that vaccines against influenza may influence drug metabolism, in a small number of susceptible patients, leading to significant changes in serum concentrations of drugs [4-8,12, 16-27]. These studies, however are still limited to specific drugs or on small sample sizes [8,12, 16-27]; in addition, published evidence on potential interactions between influenza vaccines and drugs are conflicting (vitamin K anticoagulants) [7-17], scant/uncertain (statins [18-22] and antiepileptics [23-27]) or not available (direct oral anticoagulants).

Also in view of the increased therapy for chronic illnesses and polytherapy, especially in the elderly, understanding how and whether a given vaccine affects pre-existing therapies is thus of great importance. We have investigated this issue relying on postmarketing surveillance data since they represent a real-world source of data through spontaneous reporting of AEs [28]. We used two major international databases collecting data on vaccine-related AEs: the vaccine adverse event reporting system (VAERS) database, a United States of America (USA) nationwide spontaneous reporting system, and the WHO-Vigibase, recording worldwide reports on drug-related adverse events, including vaccines. The analysis was undertaken for three classes of drugs used by large numbers of patients in a chronic fashion, namely antiepileptic drugs, anticoagulants, and statins.

The reason for this choice is three-fold. None of vaccine-drug interactions involving chronic therapies mentioned above is included in the influenza vaccine SPC, and drug-drug interactions (DDIs) reports in Micromedex® (a daily updated database used to identify DDIs) are limited only to those of vaccine against influenza and antiepileptic drugs and warfarin therapy [29,30]. Antiepileptics and statins are used in a large population and chronically, a condition for which frequent monitoring of interactions with vaccination is not likely to be feasible in the clinical practice. Finally, the impact and the AEs characteristics of vaccine-drug interactions observed in the real world clinical practice have not been investigated.

2.Patients and methods

2.1 Data source

VAERS is a national spontaneous reporting system, established in 1990 to fulfill a requirement of the National Childhood Vaccine Injury Act of 1986 [31]. It is co-sponsored by the Centers for Disease Control and Prevention (CDC) and FDA and collects reports of AEs following immunisation (AEFI) for vaccines licensed in the US [32].

AEFI may be any unfavourable or unintended sign, abnormal laboratory finding, symptom or disease that occurs following or during administration of a vaccine. The AEs are temporally associated events that may be either caused by a vaccine or coincidental to it, i.e. not related to vaccination [33]. VAERS receives annually approximately 28,000 reports of AEFI following administration of a licensed vaccine in the USA. The value of VAERS is that it may be used to detect unexpected or unusual patterns of AEFI, especially rare AEFI, unlikely to arise in pre-licensure clinical trials because of the limited number of patients involved [34].

Anyone can report an adverse event to VAERS, including health-care professionals, vaccine manufacturers, patients, parents and caregivers, and others. Reports are submitted voluntarily either directly from individual reporters, who may be reporting for them-selves or others, or secondarily from vaccine manufacturers, which also receive spontaneous reports and in turn submit them to VAERS.

A report may be classified as serious based on the Code of Federal Regulations if one or more of the following are reported: death, life-threatening illness, hospitalisation, prolongation of hospitalization, or permanent disability [35]. Signs and symptoms of AEs are coded using the standardised Medical Dictionary for Regulatory Activities (MedDRA) terminology [36]. Each report may be assigned to one or more MedDRA Preferred Terms (PTs). Notably, each report may be also characterised by a free text description of AEs, including patient demographic information, medical history, information on the reporter, detailed description of the AE, health outcomes, date of vaccination, type of vaccine, onset of AE symptoms (and time-to-onset, TTO), recovery status, laboratory data and current illness, if available (known as narratives), which may be used to further assess causality (see below).

VigiBase is the largest and most comprehensive pharmacovigilance database, maintained by the Uppsala Monitoring Centre (UMC) in Sweden [37,38]; it contains the so-called Individual Case Safety Reports (ICSR) from 110 countries over the five continents. Those National Pharmacovigilance Centres (NPCs) which are also members of the WHO Programme for International Drug Monitoring submit their spontaneous reports of adverse drug reactions (ADRs) to VigiBase.

Data originate from a variety of sources, i.e. health care professionals, pharmaceutical companies and patients, and are evaluated by the relevant NPCs prior to submission and registration in VigiBase. Over 10 million ICSRs are currently recorded in VigiBase, and about 1 million ICSRs are added each year. The reports usually contain reporter information, patient characteristics (age, gender), ADR, seriousness, outcome, drug exposures with dates, dosages, and indications. The MedDRA are used to code ADRs [36]. Drugs are coded according to the WHO Drug Dictionary Enhanced and classified using the Anatomical Therapeutic Chemical (ATC) classification [39].

For each vaccine/drug, the characterization of the vaccine/drug role indicated by the primary reporter (i.e. the original source of the information) includes three categories: Suspect, Concomitant and Interacting. All spontaneous reports should have at least one suspect vaccine/drug; i.e. involved

presumably in the occurrence of AEFI. If the reporter indicates a suspected interaction, all interacting vaccines/drugs are considered to be suspect vaccines/drugs. Concomitant therapy indicates drugs not involved in the insurgence of AEFI reported by the reporter.

2.2 Data extraction

The sequences of the steps involved in data extraction were the following:

I) we collected reports for individuals receiving all available types of vaccines against the influenza virus (J07BB) involved as a *suspect* in the AEFI submitted to VAERS (from July 1990 to April, 2016) and VigiBase (from December 2009 to August 2016).

II) We extracted those reports in which patients were concomitantly exposed to *influenza vaccines* and lipid modifying agents (ATC code C10AA) *or* antiepileptics (ATC code N03) *or* antithrombotic agents (ATC code B01A), indicated both as *suspected/interacting* agents (in VigiBase) or concomitant or suspected/interacting drugs (in VAERS).

III) Final dataset included in our analysis comprised reports in which patients concomitantly exposed to vaccines and drugs of interest (mentioned before) manifested at least an outcome of interest (*i.e.* AEFI related to a suspect increased toxicity of interacting drugs).

Specifically, with the aim of increasing sensitivity of the search strategy, for antithrombotic agents and lipid modifying agents, the outcomes of interested were detected through Standardised MedDRA Queries (SMQs) for haemorrhages (excluded laboratory terms; code 20000039) and rhabdomyolysis/myopathy (code 20000002) applying a narrow search for specificity of case retrieval [40].

Since the SMQs for the increased toxicity of antiepileptic drugs is not available to date, outcomes of interest related to the suspect increased toxicity of antiepileptics in patients taking influenza vaccines were searched using pre-specified PTs representing a list of signs and symptoms related to antiepileptic overdose [41-43]: ataxia, altered conscious state, blurred vision, hallucinations, drowsiness, coma, convulsions, nystagmus, seizures, mydriasis, dysarthria, nausea, vomiting,

hypotension, arrhythmias, respiratory depression. AEFI reports describing a detectable increase of drug serum level were also searched and included in the analysis.

Of importance, we identified and excluded from the analysis all duplicate reports in the VAERS and VigiBase; however, AEFI reports in the VAERS and VigiBase databases may have overlapped in some instances.

2.3 Causality assessment and Evaluation of Drug-Drug Interaction

To investigate potential interactions we performed a case-by-case analysis, which allows a better evaluation of reports in terms of a causality assessment (especially considering peculiarities of the administration of vaccines), compared to a disproportionality approach.

Causality assessment and evaluation of drug-vaccine interaction were performed through a multistep process. In the preliminary step we classified the causality of reports using WHO AEFI criteria [44]; next, among cases we assessed the probability of a drug-vaccine interaction using DIPS [45].

The causality assessment of AEFI is critical in terms of clinical and public health implications. We applied established criteria for AEFI causality, as recommended by the WHO [44]. The following items were considered: temporal relationship, alternate explanations, proof of association, prior evidence, population-based evidence and biological plausibility [44].

Cases without adequate information were classified as unclassifiable; cases with adequate information were categorised as i) "consistent with a causal relationship" when the available evidence supported a causal relationship between the vaccine and the AEFI in the individual but it did not rule out the possibility that the AEFI may have been caused by a factor other than the vaccine, ii) "inconsistent with a causal association" *when* the available evidence did not support a causal relationship between vaccine administration and the reported AEFI in the individual, iii)

"indeterminate" *when* the temporal relationship was consistent but the available evidence insufficient to support or rule out a causal relationship in the individual.

To assess the likelihood of interaction, we used a published and validated algorithm to assess drug-drug interactions (DDIs), known as Drug Interaction Probability Scale (DIPS), a 10-item tool specifically developed to assess the likelihood of DDIs [45]. A vaccine was considered as the potential “precipitant drug” (acute administration), whereas any concomitant agent was the “object drug” (chronic use). To avoid over-emphasis on the role of vaccine, the answers to the second question were always “unknown” (knowledge on the mechanism of the interaction is hypothesised and literature data are very scarce/uncertain); in addition, questions n. 5, 6, 10 were not assessable/applicable (dechallenge, rechallenge, and dose adjustments are unfeasible for vaccines considering the peculiarities of their administration). Therefore, the final summary score can reach 10 points. Higher total scores correspond to higher likelihood of drug-vaccine interaction (i.e., >8 = Highly Probable; 5-8 = Probable; 2-4 = Possible; <2 = Doubtful) [42]. Considering that metabolic interference is postulated to be the underlying mechanism of the interaction, a time to onset (TTO) between 3 and 21 days was considered compatible with the hypothesis of vaccine-drug interaction. Because we are aware that this individual inspection of cases may be subjective, a multidisciplinary team of experts cross-checked the data: the adapted DIPS algorithm was applied first by the Bologna Team (comprising clinical pharmacologists) and verified by the Milan Team (comprising toxicologists and experts in pharmacovigilance). Disagreements were solved by a consensus with a third reviewer (Ugo Moretti, Verona Team) with expertise in vaccine safety.

The DIPS was not apply to reports with *inconsistent* causal association according to AEFI Algorithm.

3. Results

3.1 Analysis of AEFI reports extracted from VAERS

525,791 AEFI reports were submitted to VAERS from 1990 to 2016; of these 124,095 (23.6%) were related to vaccinations against influenza.

3.1.1 Antiepileptic drugs

In 289 AEFI reports (0.2%), patients were concomitantly exposed to vaccines against influenza and phenytoin and/or carbamazepine and/or phenobarbital. Of these, 16 case reports (5.6%) described one or more events that could also be related to an increased antiepileptic drug toxicity: phenytoin use was recorded in 6 reports (37.5%), 5 reports (31.2%) were related to carbamazepine and 5 reports (31.2%) to phenobarbital use.

According to AEFI causality assessment, we categorised the reports as follows: 13 cases as *indeterminate* (81.2%), 2 (12.5%) as *consistent* and 1 report was defined *unclassifiable* (6.3%). The application of DIPS algorithm led to the classification of the reports as *probable* (1 report; 6.2%), *possible* (7 reports; 43.8%) and *doubtful* (8 reports; 50%).

In terms of outcome, 6 patients recovered, 5 patients were hospitalised, and in 5 cases the outcome was undefined. The median age of patients was 59.2, with a male predominance (68.7%). We detected an average TTO of 7.4 days; 8 reports of antiepileptic drug toxicity were serious (Figure 1). Table 1 shows the detailed case description of serious reports. No data related to types of influenza virus vaccines (adjuvanted, live virus vaccines, split virus vaccines) were reported.

3.1.2 Warfarin and DOACs

The use of SMQ for haemorrhages retained 9,810 reports, of which 2,398 related to influenza vaccines administration (28.3%).

In 751 AEFI reports, patients were concomitantly exposed to warfarin or/and DOACs (0.6%). Of these, 74 (9.8%) manifested at least one AEFI related to SMQ for haemorrhages, resulting in 3.1% of similar AEFI reports involving influenza vaccines (*i.e.* 2398) (Figure 1).

In all these cases, warfarin was the only concomitant drug involved. No reports related to haemorrhages involving DOACs and influenza vaccines were identified.

The most frequently reported PTs were: contusion (26 reports), injection site haematoma (8 reports), ecchymosis (7 reports), injection site haemorrhage (3 reports). The detailed case description of serious reports (14; 19%) is shown in Table 3.

According to AEFI algorithm, we categorised the reports as follows: 45 (60.8%) as *indeterminate*, 24 (32.4%) as *inconsistent* and 5 (6.7%) as *unclassifiable*. In terms of DIPS, reports were defined as *possible* (41 reports; 55.4%) and *doubtful* (9 reports; 12.1%). In 24 cases (32.4) DIPS was not applicable (i.e. AEFI Algorithm revealed a *inconsistent* causal correlation with immunisation).

In terms of outcome, 23 patients recovered, 9 patients were hospitalised; we identified 3 reports of death; in 39 cases the outcome was undefined. The median age of patients was 73.6. Female and male predominance was 55.4% and 41.9%, respectively; data were not provided in 2.7% of the reports. We detected an average TTO of 3.4 days; 19% (14) of reports were serious: in all cases of the SMQ for haemorrhages. Table 2 shows the detailed case description of serious reports. Types of vaccine mostly reported were: inactivated split virus vaccine (58; 78.3%). Data were not provided in 16 cases (21.6%). No adjuvanted, live virus vaccines were reported.

3.1.3 Statins

Statins were recorded as concomitant medications in patients taking the influenza vaccine in 4,840 AEFI reports submitted to VAERS (3.9%). The use of SMQ for rhabdomyolysis/myopathy (10 PTs) retained 688 reports, of which 209 related to administration of an influenza vaccine (30.3%). Of these, 26 cases (12.4%) occurred in patients reporting treatment with statins (0.5% of total AEFI reports related to the concomitant exposure of influenza vaccines and statins therapy) (Figure 1).

The most frequently reported statin was simvastatin (18 reports), followed by atorvastatin (5 reports), lovastatin, rosuvastatin, cerivastatin (1 report each).

PT mostly reported were rhabdomyolysis (13), blood creatine phosphokinase increased (12), myositis (8), myalgia (7), muscular weakness (7), pain (6), myopathy (6), asthenia (6), pain in extremity (5).

According to AEFI causality assessment, we categorised the reports as follows: 17 cases as *indeterminate* (65.3%), 8 (30.7%) as *inconsistent* and 1 case as *unclassifiable* (3.8%).

Based on the adapted DIPS algorithm, we categorised the reports as follows: *possible* (15 reports; 57.7%) and *doubtful* (3 reports;13 %) and 8 not applicable (30.7%).

In terms of outcome, eight patients recovered, ten were hospitalised, two suffered permanent disability, and one died; in 5 reports outcome was not provided.

There was a male predominance (61.5%) with a median age of 66.2. TTO was 8.4 and 77% of reports were serious (Figure 1). The most frequent types of vaccine were inactivated split virus vaccine (10; 38.4%). Data were not provided in 16 cases (61.5%). No adjuvanted, live virus vaccines were reported.

3.2 Analysis of ICSR extracted from VigiBase

3.2.1 Antiepileptic drugs

Concomitant vaccines (ATC code J07) and antiepileptics (ATC code N03) exposure were retrieved in 239 reports. In 26 cases (11%) patients were exposed to an influenza vaccine and carbamazepine, phenobarbital or phenytoin and in 12 reports (46.1%) patients manifested signs and symptoms related to the increased antiepileptic drug toxicity (Figure 2).

In 5 reports (41.6%) patients were exposed only to an influenza vaccine and carbamazepine/phenobarbital/phenytoin; in 7 reports (58.3%) other concomitant drugs were involved, namely gabapentin (5), acetylsalicylic acid (5) and levetiracetam (3).

Reports were mainly submitted by USA (38.4%), followed by Australia (19.2%), France (11.5%), New Zealand (7.6%), Germany (7.6%), United Kingdom (3.8%), Switzerland (3.8%), the Netherlands (3.8%) and Japan (3.8%). There was a 58.3% of female predominance, with a median

age of 46.4 years; 66.6% (8) of reports were serious. Table 4 lists the detailed case description of reports on serious ADRs related to antiepileptics in patients taking influenza vaccine.

3.2.2 Warfarin and DOACs

In 296 ICSRs patients were described as been exposed to both vaccines and antithrombotic agents (ATC code B01A); in 58 (19.58 %) of these, an influenza vaccine and warfarin/DOACs were involved as suspected or interacting agents. In 42 reports (72.4%), warfarin and an influenza vaccine were involved in the onset of haemorrhages-related symptoms and signs (Figure 2).

Female predominance was 69.5%, with a median age of 69.5 years; 19% (8) of reports were serious. Table 4 lists the detailed case description of serious reports related to DOACs/warfarin in patients taking influenza vaccines.

No reports involving DOACs were detected.

Concomitant drugs mostly reported in reports categorizes as possible according to DIPS, were acetylsalicylic acid (8), clopidogrel (4), lisinopril (4), levothyroxine (4), hydrochlorothiazide (4), digoxin (3), simvastatin (3).

Among the reporter countries, United Kingdom ranked first (42.8%), followed by USA (16.6%), Australia (16.6%), Italy (12%), Sweden (3.4%), France (3.4%) and New Zealand (2.3%).

3.2.3 Statins

The ICSRs in which the patients had been exposed both to a vaccine (ATC code J07) and a lipid modifying agents (ATC code C10) were 268. In 108 (40.2%) of these, statins (ATC code C10AA) were recorded as suspected or interacting agents. In 29 ICSRs (26.8%) statins and influenza vaccines (ATC code J07BB) were involved in the onset of myopathy/rhabdomyolysis (Figure 2). Figure 2 shows the overview of AEFI reports submitted to Vigibase related to statins, in patients taking influenza vaccines; the median age, the sex predominance, the completeness score and the reporter qualification are also reported.

Simvastatin was more frequently reported (17 reports; 58.6%), followed by atorvastatin (8 reports; 27.5%), rosuvastatin (1 report; 3.4%), cerivastatin (1 report; 3.4%), lovastatin (1 report; 3.4%) and pravastatin (1 report; 3.4%).

In six cases (20.6%), statins and an influenza vaccine were the only agents reported in the onset of myopathy/rhabdomyolysis; in 23 reports (79%) other concomitant drugs were reported *i.e.* furosemide (9; 31%), acetylsalicylic acid (7; 24.1%) and warfarin (6; 20.6%). There was a 65.5% of female predominance, with a median age of 68.2 years; 72.4% (21) of the reports were serious (Table 4).

The United Kingdom and the USA ranked first among the reporter countries (24.1%), followed by Germany (20.6%), Australia (10.3%), France (10.3%), Israel (3.4%), Italy (3.4%) and Sweden (3.4%).

Table 5 presents the number of patients in whom potential AEs following influenza vaccination have been reported for the three drug categories in the I) published literature, II) VAERS, and III) VigiBase.

4. Discussion

As previously reported vaccines against influenza virus elicits an immune response that mimics the response to the natural disease in order to provide protection against subsequent challenges, including the production of cytokines such as interferon-(INF) [46]. INF, along with pro-inflammatory cytokines such as IL-10, has been reported to reduce the activity of several CYPs relevant to drug metabolism both in vivo and in vitro [47-52]. This support the notion that vaccines against influenza may influence drug metabolism, in a small number of susceptible patients, leading to significant changes in serum concentrations of drugs [4-8,12,16-27].

This is the first study investigating the potential interactions between influenza vaccines and drugs used for chronic diseases in clinical practice, by assessing spontaneous reports submitted to VAERS and Vigibase, the largest archives on post-marketing vaccines safety. Notwithstanding the inherent limitations of the analysis, which are discussed below, four major findings emerged: 1) the total

number of reports included in our analysis is extremely low when compared to the number of doses of influenza virus vaccines administered worldwide each year, suggesting that this occurrence is in line with spontaneous events that are found in normal population [53]. However vaccines against influenza may influence drug metabolism only in a small number of patients susceptible to the effect of the vaccine on CYP 450. Moreover, the effect observed as a result of interaction is typical of the drugs toxicity involved in the analysis (eg myopathy for statins or haemorrhage for anticoagulants); it is therefore more difficult to correlate these effects with influenza vaccination and not just with drugs. All these factors are likely to contribute to the underreporting; 2) the application of standardised AEFI algorithm revealed that, in 63.8% of total reports, causality assessment was *indeterminate* (in the remaining cases was *inconsistent* or *unclassifiable;* only two cases revealed a *consistent* correlation) suggesting that the vaccine is not directly involved in the occurrence of AEs; 3) the application of the DIPS algorithm revealed that in just over half of total reports the interaction was *possibile*.

In these cases, the AEs occurred within the first week after vaccine administration, *i.e*. the estimated time interval within which the vaccine may induce dysregulation of inflammatory cytokines (including IFNc, IL-6, IL-10) on CYPP450 regulation [4-7,26]. In cases with no causal correlation there was no plausible temporal relationship and/or patients had taken other drugs or had comorbidities (e.g., diabetes, renal impairment) potentially involved in the events of interest; 4) although current seasonal influenza virus vaccines possess very low reactogenicity and systemic effects, the inactivated, split-virus vaccine was the type of influenza virus vaccines mostly reported in our analysis (68 reports; 58.6%; data not provided in (41.3%), in line with literature data [5,8,18,19,23-25]. We discuss these aspects for each of the three classes of drugs investigated.

4.1 Antiepileptic drugs

The possibility of an interaction between influenza vaccine and antiepileptic drugs was previously suspected in a case of carbamazepine toxicity 13 days after immunisation [23]. The patient developed nausea, vomiting, ataxia and lethargy with an increased serum carbamazepine level.

Other studies carried out to clarify the risk of interaction between influenza vaccines and antiepileptic drugs have shown conflicting results in significant changes in serum concentrations of antiepileptic drugs [23-27]. Significant increases in mean serum phenytoin concentrations on days 7 and 14 following the vaccination were not observed by Levine et al. [26]. In a subsequent study after seven days from vaccination, mean serum concentrations of phenytoin and phenobarbital were instead significantly higher than baseline [25].

In our analysis, suspected adverse events associated with antiepileptics and influenza vaccination [43-45] were observed within a few days in 5.6% of AEFI reports submitted to VAERS (in which patients were concomitantly exsposed to antiepileptics and influenza vaccine).

Only a minority of AEFI reports suggests however a plausible causal correlation (12.5%). The number of unclassified reports (6.3%), although small, suggests that making a definitive diagnosis was challenging and the quality of the data might have been suboptimal. Both indicate a need to improve the quality of reporting. A possible understimation may occur considering that a specific SMQ for search strategy is not available, to date.

According to DIPS algorithm, more than half of our validated AEFI reports were categorised as *probable* (1 report, 6.2%) and *possible* (7 reports, 43.8%), suggesting the hypothesis of a possible causal relationship between the occurrence of specific AEFI related to signs and symptoms of antiepileptics toxicity and vaccine-drugs interaction. The TTO (7.4 days) is in line with aforementioned studies [23-27].

The case-by-case analysis detected a broad spectrum of signs and symptoms related to antiepileptic drugs toxicity [41-43], namely nausea, vomiting, dizziness, disorientation, breathing difficulty, diplopia, photophobia, sweating and headache. A reduction in the absorption of vitamin D, a recognised dose-dependent adverse effect related to antiepileptic therapy (above all for carbamazepine, phenobarbital, phenytoin) was also detected in a serious report with probable causation for a potential drug-vaccine interaction (Table 1) [54].

Past vaccination against influenza in these patients had already been associated with these reactions, in the absence of alternative causes for these events (question 7 of the DIPS). In the analysed reports, there was no mention of recent therapy variation (or there was an explicit statement about the absence of variations); thus the immunisation represents the only likely precipitating event to the toxic effect of antiepileptic drugs.

The possible interference of influenza vaccines with antiepileptic drugs metabolism was also supposed in a minority of reports submitted to the VigiBase in which patients manifested antiepileptics toxicity-related symptoms and signs (12 reports; 46.1% of total reports in which patients were concomitantly exsposed to antiepileptics and influenza vaccines). In all these cases, the reporter (clinicians in 41.6% of reports) had indicated as suspected and/or interacting both the influenza vaccine and the antiepileptic drugs.

In this context it is important to point out some epidemiological aspects. Epilepsy is the commonest serious brain disorder in the world [55]; its prevalence is estimated in about 50 million people worldwide [56] and the antiepileptic drugs still represent the mainstay therapy [57]. Furthermore, a high number of vaccine recipients are exposed yearly to influenza vaccination. Therefore, considering the large cohort of patients potentially involved in our analysis, the number of reports we detected is very low (assuming a very low annual vaccination rate of only 1% in this cohort of individuals with epilepsy, the reporting rate would be of 0.00289%).

Moreover, seasonal influenza virus infection has been associated with various neurological complications [58]. Infections may also interfere with drug pharmacokinetic, leading to changes in drug concentrations; these may be misinterpreted as a direct consequence of vaccination in patients in which an unreported infection would occur closely to vaccination. Based on this observation, in our study we considered only those cases in which there was no mention, or in which there was an explicit statement, about the absence of recent infections or concomitant drug changes.

According to sporadic cross over studies and case reports [23-27] and to our analysis, it appears indeed reasonable to assume that the increased antiepileptic drugs toxicity as result of anti-influenza

vaccination represent an unlikely possibility, which does not impact on clinical practice. Further analyses are however needed, especially in view of the marketing of new anticonvulsivant drugs [59].

4.2 Anticoagulant drugs

The large majority of available data on vaccines-anticoagulants interaction regards warfarin, although most of the published studies are not adequately powered from a statistical viewpoint [12-14,24]. The most recent prospective case-control studies carried out to clarify the risk of interaction between vaccines and warfarin anticoagulation have reported conflicting results: significant differences in INRs and bleeding events (Table 5) [7-17]. As far as DOACs are concerned, no data are yet available.

Our analysis of the VAERS database identified 74 cases of haemorrhages-related symptoms and signs mainly occurring in patients older than 65 years, all under warfarin treatment (corresponding to 3% of total hemorrhage related to influenza vaccines). The majority of ADRs were not clinically relevant events such as contusion, injection site haematoma and ecchymosis (only 19% of total reports were categorised as serious). In less than half of cases, DIPS application yielded a *possible* vaccine-drug interaction; however, in most cases it was very difficult to evaluate the crucial and exclusive role for the vaccine, because of patients were exposed to other concomitant medications including acetylsalicylic acid, lisinopril, levothyroxine, hydrochlorothiazide, clopidogrel. These findings make it difficult to corroborate the hypothesis of a possible interference of the influenza vaccines with warfarin metabolism [8,11].

As detected in VAERS database, the haemorrhages-related symptoms and signs reported in Vigibase in patients exposed to warfarin and influenza vaccine (72.4%), were not clinically relevant; only 8 reports were categorised as serious. All events we detected are commonly reported by patients taking warfarin exsposed to immunisation [60] and it is most likely not a vaccine interaction that causes bleeding, but the vaccination itself.

In 45.2% of these cases, the physician indicated as suspected or interacting both the influenza vaccine and warfarin; however no noteworthy event suggesting a likely vaccine-warfarin was detected in our analysis, *i.e*. significant changes in INR and or relevant bleeding events.

Our results extend previous evidence of an unlikely interaction between the vaccination against influenza and warfarin (Table 5). The impact of immunisation on warfarin metabolism is difficult to assess because confounding factors that may lead to warfarin haemorrhages independently of vaccination are not detailed in the reports of VAERS and VigiBase. These range from diet, nutritional status to genetic factors (i.e. polymorphisms in the warfarin metabolising enzyme CYP2C9 and its target Vitamin K epoxide reductase) [61].

The strength and the amount of evidence gathered, although of great mechanistic relevance, should not be used to alert clinicians on a potential increase in haemorrhage-related adverse events in patients following influenza vaccination.

As far as direct anticoagulant drugs are concerned, we did not identify reports of haemorrhages-related ADRs submitted to VAERs and VigiBase.

4.3 Statins

Minor events as myalgias or arthralgia are also commonly related to influenza vaccines use; however, rhabdomyolysis has not been attributed to anti-influenza vaccination in patients who had not been on drugs therapy. Evidence of severe muscle damage following treatment with statins and possibly triggered by influenza vaccination has been previously reported in sporadic case reports (Table 5). Of importance, the involved mechanism has not been clearly established [18-22].

In our analysis of 209 cases of rhabdomyolysis/myopathy submitted to VAERS and related to anti-influenza immunisation, 26 (12.4%) occurred in patients exposed to statin therapy with TTO of 8.4 days; of these 5, cases were reported in the literature [18-22]. TTO is in line with previous studies reporting the occurrence of rhabdomyolysis in patients under treatment with statins within a median onset of 6.5 days after vaccination [18-22]. In these cases, although the authors found no clinical or laboratory evidence that influenza vaccine caused myopathy, they concluded that vaccine acted as a

trigger for rhabdomyolysis on a background of statin therapy [18-22]. Interestingly, in 65.3% of cases extracted by our search, DIPS detected a *possible* a vaccine-statins interaction, suggesting that immunisation may indeed affect statins metabolism.

Although the recorded patient history may not be fully representative of all potential sources of CYP interference (because of the inherent limits of spontaneous reporting system), the case-by-case analysis of these validated reports reported no changes having occurred in the therapy of the patients. These likely exclude alternative contributing causes, as patients suffered from chronic disorders and were exposed to stabilised therapy; this leaves the anti-influenza vaccination as the only known precipitating event to rhabdomyolysis.

It must be acknowledged however that in 12 reports (46.1%), the concomitant therapy may have impacted on the occurrence of serious cases of rhabdomyolysis via pharmacokinetics/pharmacodynamics interactions; likewise predisposing conditions, such as renal failure and diabetes mellitus, may have increased patients' susceptibility to vaccine-drug interaction (see Table 3). Moreover, several risk factors that may predispose patients to develop statin-induced rhabdomyolysis, including older age, hypothyroidism, hypertension and polypharmacy, were detected [62].

The association between anti influenza vaccination and rhabdomyolysis/myopathy in statin-treated patients is reported in twenty-nine reports of rhabdomyolysis/myopathy (i.e. 26.8% of total reports related to statins and influenza vaccination exposure) indicated statins and anti-influenza vaccination as the suspected or interacting agent (in 51.7%, reporters were physicians).

Of all statins, simvastatin was the most frequently reported in cases of rhabdomyolysis/myopathy in both databases, in line with previous studies [18,21,22]. The mechanistic basis may be multifaceted. First, simvastatin *per se* is generally associated with a higher incidence of muscular toxicity than other statins. Simvastatin is more lipophilic than other statins and thus more likely to penetrate muscle and induce myotoxicity [63-66].

In addition, simvastatin is metabolised via CYP3A4, which may be influenced by the influenza vaccine, resulting in increased statins levels, thus potentiating its muscular toxicity.

Moreover, both IFN-γ and TNF-alpha production are strongly associated with the influenza vaccines. These cytokines impair the expression of the influx and efflux drug transporters (OATP) 2B1, OATP1B1, and OATP1B3, which are important determinants of the liver detoxification pathway of statins [67-70].

In addition, inter-individual pharmacokinetic variability has been associated with OATP-1B1 genetic polymorphisms in patients taking statins; this would explain why the toxicity discussed above is no systematically detected in all patients concomitantly exposed to statins and influenza immunisation [71].

Also in this context, a population perspective is needed.

Statins are among the most popular and best-selling drugs in the USA [72]. Rhabdomyolysis, the most severe form of myotoxicity, can occur with all statins, either in monotherapy or in combination therapy, although the exact mechanism of this link is still unknown. The incidence of rhabdomyolysis from all causes is 1.6 per 100,000 person-years; based on FAERS data, the reporting rates of statin-induced rhabdomyolysis is 0.3–13.5 cases per 1,000,000 statin prescriptions [73]. In light of all these aspects, including sporadic case reports from the literature, the reported number of our validated cases is not surprising and very low considering all the individuals taking statins and exposed to influenza vaccines. Although data may have been underestimated and further studies are needed, our finding does not support the likelihood of a relevant increased toxicity of statin drugs in patients exposed to anti-influenza vaccination.

4.4 Limits and Strengths

We acknowledge inherent limitations of both the type and source of data [74]. VAERS and Vigibase may be subject to reporting bias, including both underreporting of AEs, especially those which are common or mild and stimulated reporting, which is the elevated reporting that might

occur in response to intense media attention and increased public awareness [75]. Therefore, both underestimation and overestimation may have occurred in our analysis. These were minimised in our analysis through a comprehensive approach by applying, where feasible, specific SMQs for haemorrhages and rhabdomyolysis, and in the largest databases available (VAERS and VigiBase). Moreover, we do not expect major distortion in reporting frequencies due to specific events.

A strength is that from Vigibase we extracted all reports in which both influenza vaccines and drugs are indicated as suspected or interacting agents; thus we analysed all cases in which a likely vaccine-drug interaction was suspected by the reporter. A critical review of these reports, including the application of algorithms, however, was not carried out due to the lack of information related to medical history, laboratory data and current illness, which are used to assess causality.

Quality and completeness of VAERS reports are variable and many reports lack valid medical diagnoses. Although the lack of complete clinical data did not allow us to assess causality with certainty, in the majority of reports we were unable to identify clear confounders. Moreover, because VAERS data do not include an unvaccinated comparison group, it is not possible to calculate and compare rates of adverse events in vaccinated versus unvaccinated individuals and determine if a vaccination is associated with an increased risk of an adverse event. Additionally since VAERS collects information about AEFI that occur after the administration of vaccines, it is not designed to evaluate adverse *drug* reactions triggered by immunisations. Additionally, the role (i.e. suspect, concomitant or interacting) of drugs reported in the Medications section is not provided.

However, VAERS reports often include detailed information on vaccines given, characteristics of the individual vaccinated, and the adverse event itself. Because of the large and diverse population available to report, VAERS allows for the rapid detection of possible safety problems of newly licensed vaccines leading to identification of potential risk factors for particular types of AEs and issuing of new recommendations for existing vaccines and rare AEs [76].

We recognise that DIPS was not specifically developed to assess drug-vaccine interaction, thus complicating the final causality assessment.

5. Conclusion

The relative paucity of detected interactions does not impact on the benefit of the vaccination against influenza, which is thus to be recommended; this does not exclude that closer monitoring for selected patients exposed to concomitant chronic pharmacological therapies and affected by predisposing factors may be useful.

6. Key issues

- An increasing number of clinical observations indicates that vaccines against influenza may influence drug metabolism, leading to significant changes in serum concentrations of drugs. Further analyses are required to fully elucidate the clinical impact of this phenomenon.
- The published evidence on potential interactions between influenza vaccines and drugs are conflicting (vitamin K anticoagulants), scant/uncertain (statins and antiepileptics) or not available (direct oral anticoagulants).
- The application of standardised AEFI algorithm revealed that, in 64.6% of reports, the causality assessment was *indeterminate* suggesting that the vaccine is not directly involved in the occurrence of AEs. The application of the DIPS algorithm revealed that in just over half of total reports the interaction was *possibile*.
- The relative paucity of detected interactions does not impact on the benefit of the vaccination against influenza, which remains strongly recommended; this does not exclude that closer monitoring for selected patients exposed to concomitant chronic pharmacological therapies and affected by predisposing factors may be useful.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Author contributions

Carla Carnovale conceptualized and designed the study, carried out the analyses, drafted the manuscript, revised and approved the final manuscript as submitted. Emanuel Raschi conceptualized and designed the study, interpreted the data, revised the manuscript and approved the final manuscript as submitted. Luca Leonardi participated in the conceptualization and design of the study, participated in the analysis and interpretation of the data, revised the article, and approved the final article as submitted. Ugo Moretti participated in the conceptualization and design of the study, participated in the analysis and interpretation of the data, revised the article, and approved the final article as submitted. Fabrizio De Ponti participated in the conceptualization and design of the study, participated in the analysis and interpretation of the data, revised the article, and approved the final article as submitted. Marta Gentili participated in the analysis and interpretation of the data, revised the article, and approved the final article as submitted. Marco Pozzi participated in the analysis and interpretation of the data, revised the article, and approved the final article as submitted.

Emilio Clementi participated in the conceptualization and design of the study, participated in the analysis and interpretation of the data, revised the article, and approved the final article as submitted. Elisabetta Poluzzi conceptualization and design of the study, participated in the analysis and interpretation of the data, coordinated and supervised data collection, critically reviewed the manuscript and approved the final manuscript as submitted. Sonia Radice conceptualized and designed the study, participated in the analysis and interpretation of the data, coordinated and supervised data collection, critically reviewed the manuscript and approved the final manuscript as submitted.

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Reference annotations

*** Of interest**

**** Of considerable interest**

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