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A study of the association between seasonal influenza vaccines and the increased risk of Guillain–Barré syndrome using Vaccine Adverse Event Reporting System, 2018–2019

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The aim of this study was to investigate the association between the incidence of Guillain-Barré syndrome (GBS) and seasonal influenza vaccines using the United States Vaccine Adverse Event Reporting System. Using multiple logistic regression analysis, we calculated the adjusted reporting odds ratio (ROR) of GBS cases associated with seasonal influenza vaccines administered from August 2018 to July 2019. Additionally, we analyzed the time-to-onset profile. The total number of adverse events reported following vaccination during this period was 43,235. Most of the GBS patients received a cell culture-based quadrivalent inactivated influenza vaccine (42.2%), quadrivalent inactivated influenza vaccine (26.6%), or high-dose trivalent inactivated influenza vaccine (15.6%). The adjusted ROR of seasonal influenza vaccines for GBS was 3.44 (2.40–4.95). The adjusted ROR of sex (male) (as reference female) and 0.5–59 years (as reference ≥ 60 years) were 1.90 (0.73–4.95) and 1.57 (0.88–2.78). Male sex and advanced age were not risk factors for GBS. The median duration of GBS was 9.5 (4.0–21.5) days. GBS following seasonal influenza vaccination developed mainly within 14 days and 42 days at most. In sex-stratified analyses, the median durations of GBS in females and males were 12.0 (8.3–28.5) and 5.0 (3.0–15.5) days (P = 0.050). Therefore, our findings indicate that the incidence of GBS is associated with seasonal influenza vaccines, and careful monitoring of GBS is required for up to 42 days, especially in the first 14 days. Moreover, GBS may occur slightly earlier in males than in females.

1. Introduction

Guillain-Barré syndrome (GBS) is a rare, but serious, acute peripheral neurological disease and an autoimmune disease (Sejvar et al. 2011a; Willison et al. 2016; Haber et al. 2009). It causes muscle weakness and paralysis which progresses rapidly, infrequently leading to death (Willison et al. 2016; Haber et al. 2009). Infections and vaccinations may trigger GBS (Sejvar et al. 2011a; Willison et al. 2016; Haber et al. 2009), with the possible association between influenza vaccines and GBS having been a matter of particular concern since 1976 (Schonberger et al. 1979; Langmuir et al. 1984). Several observational studies have demonstrated, albeit without a definitive explanation, that this possible association varied depending on the season and the target population (Perez-Vilar et al. 2019; Grave et al. 2020; Kawai et al. 2014; Burwen et al. 2010; Polakowski et al. 2013; Kwong et al. 2013; Lasky et al. 1998; Lee et al. 2020). A recent meta-analysis reported that influenza vaccines increased the risk of GBS (relative risk [RR] 1.22 [95% confidence interval {CI} 1.01–1.48]) (Martín Arias et al. 2015). A meta-analysis of the influenza (H1N1) 2009 monovalent inactivated vaccine has shown that the risk of GBS was increased in approximately 1.6 cases per million people vaccinated (Salmon et al. 2013). Therefore, evaluating the safety of seasonal influenza vaccines is crucial.

As vaccine antigens change each season, the Advisory Committee on Immunization Practices (ACIP) under the Centers for Disease Control and Prevention (CDC), which are governed by the United States (US) Department of Health and Human Service, update their recommendations for influenza vaccines annually (Grohskopf et al. 2018). Consequently, the duration of clinical use of each seasonal influenza vaccine are relatively short, and new vaccines produced with a diversity of manufacturing techniques frequently enter the US market (Grohskopf et al. 2018). Previous studies have indicated an increased risk of developing GBS within 42 days at the most following vaccination, with the highest risk observed in the first 8–21 days (Schonberger et al. 1979; Langmuir et al. 1984; Polakowski et al. 2013; Kwong et al. 2013; Lasky et al. 1998; Grohskopf et al. 2018; Sejvar et al. 2011b). However, the detailed development of GBS caused by seasonal influenza vaccines is unknown.

The USA has developed the Vaccine Adverse Event (AE) Reporting System (VAERS) to collect spontaneous reports of AE following immunization and to manage vaccine safety. The main objectives of this system are to detect new, unusual (Shimabukuro et al. 2015) or rare vaccine AEs, assess the safety of newly approved vaccines, identify potential risk factors, monitor the increase in known AEs, and determine and address possible reporting clusters (Shimabukuro et al. 2015; Varricchio et al. 2004). Spontaneous reporting systems (SRSs) are valuable tools in post-marketing surveillance that reflect the realities of clinical practice (Hauben and Zhou 2003). Lee et al. (2000) previously evaluated the risk of GBS associated with seasonal influenza vaccines using the VAERS database. They demonstrated that seasonal influenza vaccines were associated with GBS and that GBS reporting was not affected by sex (Lee et al. 2020). Despite the insights provided by this study, the time-to-onset profiles of GBS following influenza vaccination remain uncertain.

In this study, we evaluated the relationship between seasonal influenza vaccines, including new vaccines produced with new manufacturing techniques, and GBS using the reporting odds ratio (ROR) adjusted using multiple logistic regression analysis (Lee et al. 2020; Suzuki et al. 2015; van Puijenbroek et al. 2000; Hosoya et

al. 2017; Tanaka et al. 2019). The results obtained will provide the clinical onset profile of seasonal influenza vaccine-related GBS. Analysis of time-to-onset data has been proposed as a method to detect signals for AEs in SRSs (Sauzet et al. 2013). Therefore, we also analyzed the time-to-onset of GBS (Tanaka et al. 2019; Sauzet et al. 2013; Nakamura et al. 2015; Hasegawa et al. 2017).

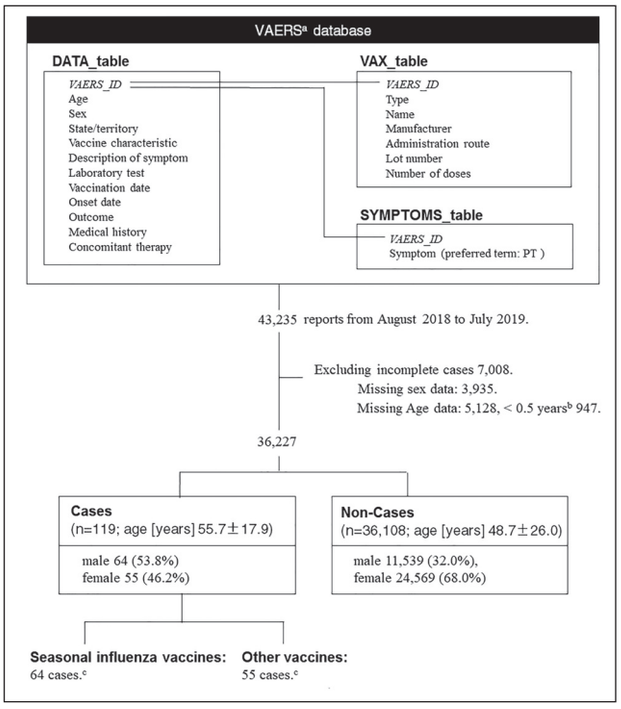

Fig. 1: Flowchart outlining the construction of the dataset for analysis.
[a] VAERS: Vaccine Adverse Event (AE) Reporting System. [b] Age ≥ 0.5 years: Recommended age for seasonal influenza vaccines in the United States. [c] Cases: Patients reporting AEs related to Guillain–Barré syndrome.

55 female patients (46.2%), while the non-case group included 11,539 male patients (32.0%) and 24,569 female patients (68.0%) ($P < 0.001$). The mean (± standard deviation) age was 55.7±17.9 years in the case group and 48.7±26.0 years in the non-case group ($P = 0.003$) (Fig. 1). The univariate analysis showed significant differences in sex and age between the cases and non-cases group. The percentages of patients receiving high-dose trivalent inactivated influenza vaccine (HD-IIV3), quadrivalent inactivated influenza vaccine (IIV4), and quadrivalent cell culture-based inactivated influenza vaccine (ccIIV4) were 15.6%, 26.6%, and 42.2%, respectively (Table 1).

A multiple logistic regression analysis was conducted on the relationship between GBS and risk factors such as the type of seasonal influenza vaccine, age, and sex. The crude ROR (95% CI) of seasonal influenza vaccines for GBS was 3.39 (2.36–4.86), while the adjusted ROR (95% CI) was 3.44 (2.40–4.95). The crude ROR (95% CI) of the trivalent influenza vaccine (TIV), quadrivalent influenza vaccine (QIV), egg culture-based influenza vaccine (egg-based), cell culture-based influenza vaccine (cell-based), and inactivated influenza vaccine (IIV) were 2.33 (1.30–4.20), 3.86 (2.61–5.70), 1.97 (1.27–3.07), 13.70 (8.58–21.70), and 3.28 (2.27–4.75), respectively. The adjusted RORs (95% CI) of TIV, QIV, egg-based vaccines, cell-based vaccines, and IIV were 1.82 (0.99–3.36), 5.25 (3.47–7.94), 1.99 (1.28–3.10), 15.00 (9.27–24.20), and 3.32 (2.29–4.81), respectively (Table 2). In the subgroup analysis, the crude ROR (95% CI) of sex (male) (as reference female) and 0.5–59 years (as reference ≥ 60 years) were 2.48 (1.73–3.56) and 0.78 (0.55–1.12), respectively. The adjusted ROR of sex (male), 0.5–59 years, and age (years) × sex (male) (as reference female) were 1.90 (0.73–4.95), 1.57 (0.88–2.78), and 1.01 (0.99–1.02), respectively (Table 2).

The analysis of time-to-onset profiles is summarized in Table 3. The median duration (interquartile range) of GBS for seasonal influenza vaccines was 9.5 (4.0–21.5) days. The median durations (interquartile range) of GBS caused by TIV and QIV were 12.5 (9.3–27.5) and 7.0 (3.8–19.5) days, respectively (Table 3 and Fig. 2A), but this difference was not statistically significant ($P = 0.153$). The median durations (interquartile range) of GBS

Table 1: Vaccine types used when Guillain-Barré syndrome developed after seasonal influenza vaccination and number of cases

Vaccine type	Abbreviation	The number of influenza virus included	Type of manufacturing process	Type of influenza virus	Recommended age (years)[a]	Route	Case (n [%])
Cell culture-based quadrivalent inactivated influenza vaccine	ccIIV4	Quadrivalent	Cell culture-based	Inactivated	≥ 4	IM[b]	27 (42.2)
High-dose trivalent inactivated influenza vaccine	HD-IIV3	Trivalent	Egg culture-based	Inactivated	≥ 65	IM[b]	10 (15.6)
Standard-dose trivalent inactivated influenza vaccine	SD-IIV3	Trivalent	Egg culture-based	Inactivated	≥ 5	IM[b]	3 (4.7)
Adjuvanted trivalent inactivated influenza vaccine	aIIV3	Trivalent	Egg culture-based	Inactivated	≥ 65	IM[b]	1 (1.6)
Quadrivalent inactivated influenza vaccine	IIV4	Quadrivalent	Egg culture-based	Inactivated	≥ 0.5	IM[b]	17 (26.6)
Quadrivalent live attenuated influenza vaccine	LAIV4	Quadrivalent	Egg culture-based	Live-attenuated	2–49	NAS[c]	–[d]
Quadrivalent recombinant influenza vaccine	RIV4	Quadrivalent	Rcecombinant	–	≥ 18	IM[b]	3 (4.7)
Influenza virus vaccine, name unknown	FLUX	–	–	–	–	–	3 (4.7)

[*] Influenza virus strain A/H3N2/: A/Singapore/INFIMH-16-0019/2016 (H3N2)
[a] By Advisory Committee on Immunization Practices (ACIP) in the 2018–2019 season.
[b] Intramuscular. [c] Intranasal. [d] Not reported.

2. Investigations and results

The VAERS database contains 43,235 reports from August 2018 to July 2019. After excluding reports from patients aged under 0.5 years and reports without sex information, the total number was 36,227. The number of seasonal influenza vaccines was 9,300. GBS cases were reported to be 119 in the dataset for analysis (Fig. 1). The case group included 64 male patients (53.8%) and

following vaccination with egg-based and cell-based vaccines were 12.5 (5.0–29.0) and 5.0 (3.0–12.0) days, respectively (Table 3 and Fig. 2B), and this difference was statistically significant ($P = 0.031$). The median duration (interquartile range) of GBS in IIV was 9.0 (4.0–20.5) days (Table 3). The Weibull shape parameter (WSP) β (95% CI) of GBS for seasonal influenza vaccines was 0.98 (0.81–1.20) (Table 3). Percentages of GBS within 42 and 14

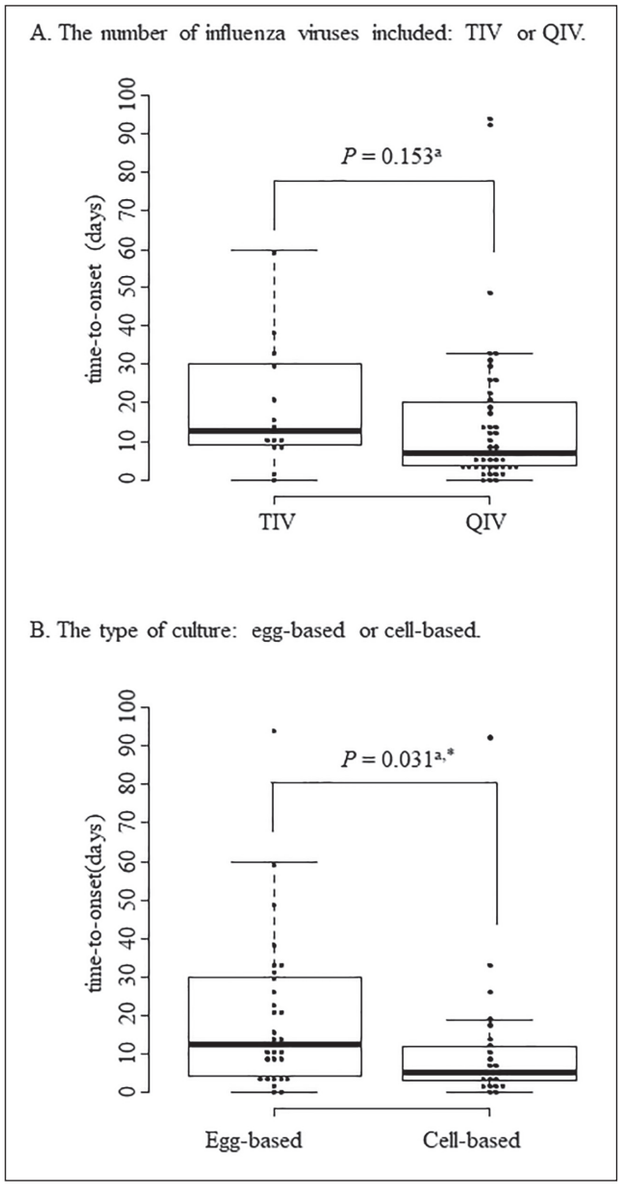

Fig. 2: Box plot of time-to-onset of Guillain-Barré syndrome (GBS) associated with seasonal influenza vaccines in trivalent influenza vaccine (TIV) or quadrivalent influenza vaccine (QIV) (A) and egg culture-based inactivated influenza vaccine (egg-based) or cell culture-based inactivated influenza vaccine (cell-based) (B).
The time-to-onset of GBS induced by cell-based vaccines was shorter than that induced by egg-based vaccines in this study. [a] Medians of time-to-onset were compared using Mann–Whitney U test. * $P < 0.05$.

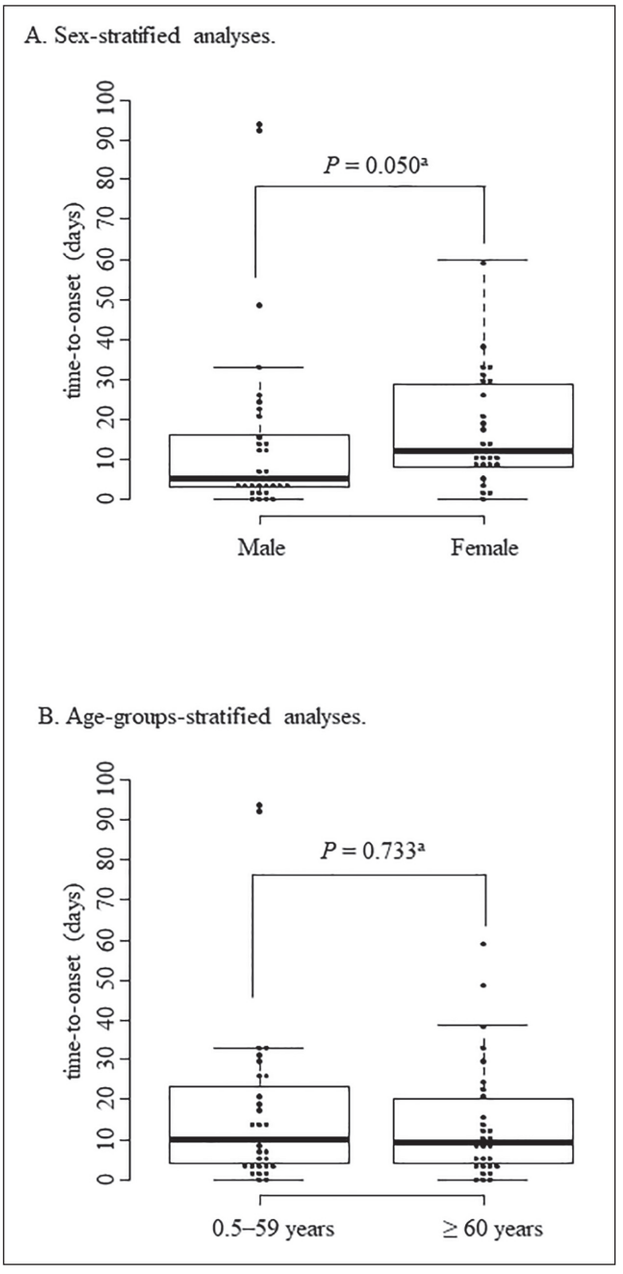

Fig. 3: Box plot of time-to-onset of Guillain-Barré syndrome associated with seasonal influenza vaccines based on sex-stratified analyses (A) and age-stratified analyses (B).
Males had a shorter time-to-onset than females. [a] Medians of time-to-onset were compared using Mann–Whitney U test.

days following vaccination were 93.3% (56/60) and 66.7% (40/60), respectively. Percentages of GBS within 7 days following vaccination with TIV, QIV, egg-based vaccines, cell-based vaccines, and IIV were 14.3% (2/14), 52.3% (23/44), 26.7% (8/30), 59.3% (16/27), and 43.6% (24/55), respectively. Figure 2 shows the box plot of time-to-onset of GBS following vaccination with TIV or QIV (Fig. 2A), and egg-based or cell-based vaccines (Fig. 2B). In approximately 60% of the GBS cases due to cell-based vaccines, GBS developed within 7 days.

We further analyzed GBS stratified by sex and age (0.5–59-year-old and ≥ 60-year-old groups). The median durations (interquartile range) of GBS in females and males were 12.0 (8.3–28.5) and 5.0 (3.0–15.5) days, respectively (Table 3 and Fig. 3A). Although not significant, the median durations in males tended to be shorter than in females ($P = 0.050$). The median durations (interquartile range) of GBS in the 0.5–59 years old and ≥ 60 years old groups were 10.0 (4.0–23.5) and 9.0 (4.0–20.0) days, respectively (Table 3 and Fig. 3B), but this difference was not statistically significant ($P = 0.733$). Similarly, percentages of GBS within 7 days following vaccination in females, males, 0.5–59 years old group, and ≥ 60 years old group were 19.2% (5/26), 61.8% (21/34), 45.2% (14/31), and 41.4% (12/29), respectively. Figure 3 shows the box plot of time-to-onset of GBS following seasonal influenza vaccination in sex-stratified (Fig. 3A) and age-stratified analyses (Fig. 3B). In approximately 60% of the vaccinated males, GBS developed within 7 days.

3. Discussion

Our study suggested that seasonal influenza vaccines were associated with an increased risk of GBS (Kwong et al. 2013; Lasky et al. 1998; Lee et al. 2020; Martín Arias et al. 2015) in the 2018–2019

Table 2: Adjusted reporting odds ratio of Guillain-Barré syndrome using multiple logistic regression analysis

	Vaccine types included	Total	Case	Crude reporting odds ratio (95% CI[a])	Adjusted reporting odds ratio (95% CI[a])
All seasonal influenza vaccines		9,300	64	3.39 (2.36–4.86)*	3.44 (2.40–4.95)*
The number of influenza virus included					
Trivalent influenza vaccine	HD-IIV3, SD-IIV3, aIIV3	2,947	14	2.33 (1.30–4.20)*	1.82 (0.99–3.36)
Quadrivalent influenza vaccine	ccIIV4, IIV4, LAIV4, RIV4	6,002	47	3.86 (2.61–5.70)*	5.25 (3.47–7.94)*
The type of culture					
Egg culture-based influenza vaccine	HD-IIV3, SD-IIV3, aIIV3, IIV4	7,706	31	1.97 (1.27–3.07)*	1.99 (1.28–3.10)*
Cell culture-based influenza vaccine	ccIIV4	993	27	13.70 (8.58–21.70)*	15.00 (9.27–24.20)*
The type of influenza virus					
Inactivated influenza vaccine	ccIIV4, HD-IIV3, SD-IIV3, aIIV3, IIV4	8,699	58	3.28 (2.27–4.75)*	3.32 (2.29–4.81)*
Sex					
Sex (female) (as reference)		6,300	29	1	1
Sex (male)		3,000	35	2.48 (1.73–3.56)*	1.90 (0.73–4.95)
Age (years)					
0.5–59 (years)		5,463	32	0.78 (0.55–1.12)	1.57 (0.88–2.78)
≥ 60 (years)(as reference)		3,837	32	1	1
Interaction term; age (years)×sex					
Age(years)×sex (female) (as reference)					1
Age(years)×sex (male)					1.01 (0.99–1.02)

[a]Confidence interval.
* The reporting odds ratio estimates and lower limits of the 95% confidence interval > 1.

Table 3: Time-to-onset analysis of Guillain-Barré syndrome associated with seasonal influenza vaccines

	Vaccine types included	Cases (number for analysis)	Median duration (interquartile range, days)	Shape parameter, β (95% CI[a])
Total		60 (55)	9.5 (4.0–21.5)	0.98 (0.81–1.20)
The number of influenza virus included				
Trivalent influenza vaccine	HD-IIV3, SD-IIV3, aIIV3	14 (13)	12.5 (9.3–27.5)	1.30 (0.86–1.98)
Quadrivalent influenza vaccine	ccIIV4, IIV4, LAIV4, RIV4	44 (41)	7.0 (3.8–19.5)	0.91 (0.73–1.14)
The type of culture				
Egg culture-based influenza vaccine	HD-IIV3, SD-IIV3, aIIV3, IIV4	30 (28)	12.5 (5.0–29.0)	1.10 (0.83–1.47)
Cell culture-based influenza vaccine	ccIIV4	27 (23)	5.0 (3.0–12.0)	0.87 (0.65–1.16)
The type of influenza virus				
Inactivated influenza vaccine	ccIIV4, HD-IIV3, SD-IIV3, aIIV3, IIV4	55 (51)	9.0 (4.0–20.5)	0.96 (0.78–1.18)
Sex				
Female		26 (25)	12.0 (8.3–28.5)	1.28 (0.94–1.75)
Male		34 (30)	5.0 (3.0–15.5)	0.85 (0.66–1.11)
Age				
0.5–59 (years)		31 (29)	10.0 (4.0–23.5)	0.93 (0.71–1.22)
≥ 60 (years)		29 (26)	9.0 (4.0–20.0)	1.08 (0.80–1.45)

[a]Confidence interval.

influenza season. We also show the importance of evaluating the safety profiles of vaccines using post-marketing real-world data. Additionally, male sex (Schonberger et al. 1979; Kwong et al. 2013; Lasky et al. 1998; Lee et al. 2020) and advanced age (Schonberger et al. 1979; Grave et al. 2020; Burwen et al. 2010; Kwong et al. 2013; Lasky et al. 1998; Lee et al. 2020) were not risk factors for GBS following seasonal influenza vaccination. To the best of our knowledge, no time-to-onset analysis of GBS following seasonal influenza vaccination has been performed using SRS databases. GBS develops within 14 days, and at most, 42 days, according to the SRS database (Schonberger et al. 1979; Langmuir et al. 1984; Polakowski et al. 2013; Kwong et al. 2013; Lasky et

	Adverse event of interest	Other adverse event of interest	Total
Vaccine of interest	a	b	a+b
Other vaccine of interest	c	d	c+d
Total	a+c	b+d	a+b+c+d

$$\text{Reporting Odds Ratio (ROR)} = \frac{a/c}{b/d} = \frac{ad}{bc}$$

$$95\%\ \text{confidence interval (CI)} = e^{\ln(ROR) \pm 1.96\sqrt{1/a+1/b+1/c+1/d}}$$

Fig. 4: Two-by-two contingency table used for the calculation of reporting odds ratio.

al. 1998; Grohskopf et al. 2018; Sejvar et al. 2011b). These are reasonable results in the context of available literature. The median duration of GBS in the group that received cell-based vaccines was significantly shorter than that in the group that received egg-based vaccines. Approximately 60% of the GBS induced by seasonal influenza vaccines were developed within 7 days following vaccination with cell-based vaccines and in males.

No signal could be detected only with TIVs. Among the three types of TIVs, a signal was detected only in standard-dose trivalent inactivated influenza vaccine (SD-IIV3) (data not shown). HD-IIV3 and trivalent adjuvanted inactivated influenza vaccine (aIIV3) are the trivalent inactivated influenza vaccine approved in the USA for persons with weakened immune function and aged ≥ 65 years (US Food and Drug Administration [FDA] 2009; US FDA 2015). These results were consistent with previous studies (Perez-Vilar et al. 2019; Dodd et al. 2013). Egg-based vaccines are often affected by egg adaptation (particularly for H3N2) in seed strains, which may lead to decreased effectiveness (Rajaram et al. 2020; Skowronski et al. 2014; Skowronski et al. 2015). Consequently, the effectiveness of cell-based vaccine was higher than that of egg-based vaccine (Boikos et al. 2021). Cell-based preparation of vaccines circumvent the issue of egg adaptation (Rajaram et al. 2020). One of the reason for the "shorter" median time to GBS onset induced by cell-based vaccines might be that they are more recently started to use than egg-based vaccines (approved in 2012 [US FDA 2012]). This might be the reason why the ROR of cell-based vaccines is higher than egg-based vaccines. However, the mechanism of development of GBS is poorly understood. To reveal the mechanism of cell-based vaccines underlying the short time-to-onset of GBS, further study is necessary.

In our study, GBS was reported for all vaccine types produced using a diversity of manufacturing techniques, except for quadrivalent live attenuated influenza vaccine (LAIV4). LAIV is administered intranasally with a single-use sprayer (Grohskopf et al. 2018), and stimulates both mucosal and systemic immune responses compared to the inactivated influenza vaccine (McNeela and Mills 2001). Our results are not consistent with those of a previous study in which typically more cases of GBS following administration of LAIV were reported than following administration of inactivated vaccines (Lee et al. 2020). The reason for this contradiction is unknown.

Our results suggest that male sex (Schonberger et al. 1979; Kwong et al. 2013; Lasky et al. 1998; Lee et al. 2020) and advanced age (Schonberger et al. 1979; Grave et al. 2020; Burwen et al. 2010; Kwong et al. 2013; Lasky et al. 1998; Lee et al. 2020) were not risk factors for GBS following seasonal influenza vaccination as in previous studies. Moreover, to date, no age and sex differences in the time-to-onset of GBS following seasonal influenza vaccination have been reported. In our study, males had a shorter time-to-onset than females.

Because vaccines work by priming the immune system, it is biologically plausible that immunization may be associated with subsequent GBS. GBS is believed to be an immune disorder resulting from the generation of autoimmune antibodies that cross-react with epitopes on peripheral nerves, leading to nerve damage (Haber et al. 2009; Martín Arias et al. 2015); however, the underlying biological mechanisms remain to be demonstrated (Haber et al. 2009; Martín Arias et al. 2015; Sejvar et al. 2011b). Following seasonal influenza vaccination, females typically develop higher antibody responses (Voigt et al. 2019; Edwards et al. 2007; Furman et al. 2014; Engler et al. 2008; Cook et al. 2006) and report more adverse effects of vaccination than males (Furman et al. 2014; Engler et al. 2008; Cook et al. 2006; Beyer et al. 1996). The immune system likely evolves differently with age in males and females, and thus, their response to vaccines may differ (Tadount et al. 2020). Other factors, such as the previous vaccination history, may interfere (Mosterín Höpping et al. 2016; Jackson et al. 2010). GBS occurs more frequently in males than in females (Sejvar et al. 2011a). According to several reports (Schonberger et al. 1979; Kwong et al. 2013; Lasky et al. 1998), including the report by Lee et al. (2000) using VAERS data from 2005 to 2017, the incidence of GBS is not affected by sex. Our results were the same for these studies. However, although not statistically significant, the reports suggested that GBS occurs more frequently in males than in females (Burwen et al. 2010; Tadount et al. 2020). Therefore, further research is needed to assess sex differences in GBS, including earlier onset in males than in females.

SRSs, such as the VAERS database, have several limitations, including under-reporting, over-reporting, missing data, bias, confounding factors, lack of a control population as a reference group, lack of adequate data quality, and the possible existence of reports based on indirect information (e.g., heard on TV/read in

newspapers). This type of study does not allow assessing whether a vaccine caused an AE or not. In particular, ROR computing does not allow the quantification of the risk of an AE, but only suggests a statistical association between a vaccine and an AE (Montastruc et al. 2011). Though we adjusted ROR for age, sex and interaction term between age and sex in this study, we cannot exclude the possibility that our results could be due to unadjusted confounders, such as exposure to prior infections (Mosterín Höpping et al. 2016; Jackson et al. 2010). Nevertheless, despite the limitations inherent to spontaneous reporting, our study indicates the importance of characterizing the safety profile of seasonal influenza vaccines, including newer vaccines produced through various manufacturing techniques. Furthermore, the results regarding GBS development was corroborated by previously reported studies, and males had a shorter time-to-onset than females. Our study provides information to improve our understanding of this issue. In this study, we focused solely on the risk of GBS following seasonal influenza vaccination and did not address the benefits of seasonal influenza vaccination.

Despite the limitations inherent to SRS, we demonstrated the potential risk of GBS associated with seasonal influenza vaccines, based on RORs. Male sex and advanced age were not risk factors for GBS following seasonal influenza vaccination as in previous studies. Based on the time-to-onset analysis, it was shorter in males than in females. Further, patients vaccinated with seasonal influenza should be closely monitored for the initial symptom of GBS, namely, muscle weakness and paralysis, for 42 days at the most, particularly in the first 14 days. We believe that our results represent a valuable contribution to clinical knowledge and will help in the management of GBS.

4. Experimental

4.1. Data sources

Data were retrieved from the VAERS database, an American vaccine safety surveillance database of AEs created in 1990 (Shimabukuro et al. 2015; Varricchio et al. 2004) and co-administered by the CDC and the Food and Drug Administration (Varricchio et al. 2004). This SRS compiles reports of suspected AEs either voluntarily reported by patients, clinicians, pharmacists, and other healthcare professionals or mandatorily reported by various pharmaceutical manufacturers (Shimabukuro et al. 2015; Varricchio et al. 2004). It approves all reports without discerning the clinical causality (Shimabukuro et al. 2015). Data from the primary reports, with sensitive patient information removed, are publicly available on the VAERS website (www.vaers.hhs.gov/data/index) and through the CDC's Wide-ranging Online Data for Epidemiologic Research (WONDER) tool (http://wonder.cdc.gov/vaers.html) (Shimabukuro et al. 2015). To protect patient privacy, additional information obtained during follow-up on individual VAERS reports is not included in the publicly available data (Shimabukuro et al. 2015). For the 2018–2019 influenza season, the ACIP recommended that "age-appropriate vaccine should be used" as seasonal influenza vaccines (Grohskopf et al. 2018). In this study, we obtained AE reports from the VAERS website which were limited to those reported by the US population from August 2018 to July 2019.

The Medical Dictionary for Regulatory Activities (MedDRA) is a collection of international medical terminology prepared by the International Council for Harmonization for standardizing and unifying AE reports. MedDRA contains a five-level hierarchical structure: lowest level terms, preferred terms (PTs), high level terms, high level group terms, and system organ classes (MedDRA 2021). In the VAERS database, AEs are coded by assigning one or more PTs of MedDRA.

The VAERS database consists of three tables which contain patient demographic information, such as age, sex, state/territory, vaccine characteristics, description of symptoms, laboratory test, vaccination date, onset date, outcome, medical history, and concomitant therapies (DATA); vaccine information, such as type, name, manufacturer, administration route, lot number, and the number of doses (VAX); and AEs, namely one or more symptoms coded using the assigned PTs of MedDRA (SYMPTOMS) (VAERS 2017). We constructed a relational database that integrated the three data tables using Microsoft Access® Office 365 (Microsoft Corporation, Redmond, WA, USA) with VAERS_ID as a key code.

The vaccines selected from the VAERS database for this study were all seasonal influenza vaccines approved in the USA and available during the 2018–2019 influenza season: HD-IIV3, SD-IIV3, IIV4, aIIV3, ccIIV4, LAIV4, quadrivalent recombinant influenza vaccine (RIV4), and influenza virus vaccine, name unknown (FLUX) (Table 1). To compare conventional vaccines (such as inactivated, trivalent, and egg-based vaccines) and other vaccines (such as live-attenuated, quadrivalent, and cell culture-based vaccines), seasonal influenza vaccines were classified based on the following criteria: 1) the number of influenza viruses included: trivalent or quadrivalent, 2) the type of culture: egg-based or non-egg-based (cell-cultured), and 3) the type of influenza virus: live-attenuated or inactivated (Table 1) (Lee et al. 2020).

AEs were retrieved using the following PTs: "Guillain-Barré syndrome" (PT code: 10018767) (MedDRA 2021; Ali 2014).

"Cases" were defined as patients reporting AEs related to GBS, while "non-cases" were defined as patients reporting all other AEs. A *t*-test and Fisher's exact test were conducted to analyze age and sex, respectively. A *P*-value of < 0.05 was considered statistically significant.

4.2. Multiple logistic regression analysis

We evaluated the association between seasonal influenza vaccines and GBS using pharmacovigilance signal detection methods, the ROR. The ROR is the ratio of the odds of reporting an AE versus all other events associated with seasonal influenza vaccines compared with the reporting odds for AEs associated with all other vaccines present in the database. We calculated the ROR using a two-by-two contingency table in the form of $(a \times d)/(b \times c)$ (Figure 4) (van Puijenbroek et al. 2002). RORs were expressed as point estimates with 95% CIs. The detection of a signal was dependent on the signal indices exceeding a predefined threshold. Safety signals are considered significant when the ROR estimates and the lower limits of the corresponding 95% CI exceed 1 (van Puijenbroek et al. 2002).

The ROR was applied to multiple logistic regression analysis and partially adjusted for confounding factors (Suzuki et al. 2015; van Puijenbroek et al. 2000). GBS was considered the objective variable, while the patient characteristics (sex and age) and classification of seasonal influenza vaccines were considered explanatory variables (Lee et al. 2020; Suzuki et al. 2015; van Puijenbroek et al. 2000; Hosoya et al. 2017; Tanaka et al. 2019). Considering the effect of age on the relationship between GBS development and sex, we added the interaction term between age (years) and sex (Jia et al. 2019). To calculate the adjusted ROR, we extracted data with age ≥ 0.5 years (recommended age for seasonal influenza vaccines in the USA [Grohskopf et al. 2018]), excluding reports where information on the sex was missing. The multiple logistic regression models used to calculate the adjusted ROR were as follows:

$$\log(\text{odds}) = \beta_0 + \beta_1 V + \beta_2 A + \beta_3 S + \beta_4 A \times S$$

where V is the type of seasonal influenza vaccine, A is age, S is sex, and β_0, β_1, β_2, β_3 and β_4 are model parameters. Subgroup analysis was performed by stratifying groups according to sex or age: 0.5–59 years and ≥ 60 years. According to the World Health Organization, individuals aged ≥ 60 years are defined as being elderly (World Health Organization 2007).

4.3. Time-to-onset analysis

Time-to-onset from the VAERS database was calculated from the vaccination date to the date when AEs occurred. The median durations (interquartile range) and WSPs were used to evaluate the time-to-onset data (Tanaka et al. 2019; Sauzet et al. 2013; Nakamura et al. 2015; Hasegawa et al. 2017). The WSP test is used for statistical analysis of time-to-onset data and can describe the non-constant rate of incidence of AEs (i.e., the risk of increase or decrease over time) in SRS (Tanaka et al. 2019; Sauzet et al. 2013; Nakamura et al. 2015; Hasegawa et al. 2017). The scale parameter α of the Weibull distribution determines the scale of the distribution function. A larger scale value stretches the distribution, while a smaller scale value shrinks the distribution. The shape parameter β of the Weibull distribution determines the shape of the distribution function. A larger shape value gives a left-skewed curve, while a smaller shape value gives a right-skewed curve. The shape parameter β of the Weibull distribution indicates the hazard without a reference population. When β is equal to 1, the hazard is estimated to be constant over time. If β is greater than 1 and the 95% CI of β excludes 1, the hazard is considered to increase over time. If β is smaller than 1 and the 95% CI of β excludes 1, the hazard is considered to decrease over time (Tanaka et al. 2019; Sauzet et al. 2013; Nakamura et al. 2015; Hasegawa et al. 2017). Subgroup analysis was further performed by stratifying groups according to sex or age: 0.5–59 years and ≥ 60 years. We calculated the *P*-value using the Mann–Whitney U test to analyze the medians of time-to-onset. A *P*-value of < 0.05 was considered significant.

These analyses were performed using EZR (version 1.41; Saitama Medical Center, Jichi Medical University, Saitama, Japan) (Kanda 2013). Time-to-onset analysis using the WSPs only was performed with R (version 3.6.3; The R Foundation for Statistical Computing, Vienna, Austria).

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