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ORIGINAL RESEARCH ARTICLE

Safety of Perflutren Ultrasound Contrast Agents: A Disproportionality Analysis of the US FAERS Database

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Abstract

Introduction Perflutren microbubble/microsphere ultrasound contrast agents have a black-box warning based on case reports of serious cardiopulmonary events. There have been several subsequent observational safety studies. Large spontaneous reporting databases may help detect/refine signals of rare adverse events that elude other data sources/study designs.

Objective The objective of this study was to supplement existing knowledge of the reported safety of perflutren using statistical analysis of spontaneous reports.

Methods We analyzed information from the US Food and Drug Administration Adverse Event Reporting System using a disproportionality analysis. Analysis of overall reporting for perflutren was supplemented by subset (age, indication) analysis. A signal of disproportionate reporting (SDR) was defined as EB05 >2.

Results Overall, 18/380 Preferred Terms and 1/83 Standardized Medical Queries had SDRs. Most were small (EB05 = 2–4). Back pain and flank pain were the largest SDRs followed by events compatible with signs/symptoms of hypersensitivity. The general pattern of SDRs in the subset analysis was consistent with the overall analysis. Almost all events with SDRs were literally or conceptually labeled. Except for chest pain (higher in the age <65 years subgroup) and back pain (higher in the age ≥65 years subgroup), there were no statistically significant differences between age subsets. Except for the Preferred Terms Pruritus and Urticaria and the narrow Standardized Medical Queries Ventricular tachyarrhythmia, Angioedema, Oropharyngeal allergic conditions, and Hypersensitivity (higher in the stress test subgroup), there were no statistically significant reporting differences between indication subsets. There were no SDRs associated with the major cardiovascular events of death, myocardial infarction/ischemia, angina, arrhythmias, or convulsions in any analysis.

Conclusions Our combined signal detection/evaluation analysis did not identify SDRs of novel adverse events or major cardiovascular events associated with perflutren ultrasound contrast agents. The negative results for major cardiovascular events extend previous signal evaluation exercises supporting the relative cardiovascular safety of these agents.

Key Points

Events with signals of disproportionate reporting identified through a disproportionality analysis of spontaneous reports with perflutren for echocardiography were either literally or conceptually labeled, i.e., there were no statistical signals of novel adverse events.

A disproportionality analysis applied to spontaneous reports of perflutren used for echocardiography extends previous signal evaluation exercises that substantially weakened the initial signal of certain major cardiovascular and convulsion events generated by individual spontaneous case reports.

A disproportionality analysis of spontaneous reports can support both signal detection and signal evaluation exercises.

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1 Introduction

Echocardiography for the diagnosis and evaluation of cardiac disorders is ubiquitous owing to its diagnostic accuracy, portability, and lack of ionizing radiation. Although there have been many significant technological advances that have vastly improved image quality in echocardiography, there remain a significant number of echocardiograms that are deemed technically limited. Approximately 10–15 % of routine studies in non-critically ill patients and up to 30 % of studies in critically ill patients are technically limited [1, 2]. There are currently two approved perflutren microbubble/microsphere ultrasound contrast agents (UCAs). OPTISON™ (GE Healthcare Inc., Pollards Wood, Buckinghamshire, UK), perflutren albumin formulation was approved by the Food and Drug Administration (FDA) in 1997. DEFINITY® (Lantheus Medical Imaging Inc., North Billerica, MA, USA), perflutren lipid formulation was approved by the FDA in 2001. Both are approved for the opacification of the left ventricular chamber, and for the delineation of the left ventricular endocardium, for studies that were otherwise limited [3, 4]. Both UCAs are marketed in various countries around the world. Perflutren has been shown to decrease the number of uninterpretable segments, improve reader confidence in wall motion interpretation for both routine and stress studies, and to decrease the overall number of uninterpretable studies [2–6].

Since FDA approval of the lipid microsphere formulation in 2001, there have been several important label changes in response to accumulating safety information, analysis, professional consensus statements, and editorials critical of the initial FDA actions. In 2007, a black-box warning was instituted by the FDA, because of four reported deaths and 190 serious reactions that were temporally related to the use of perflutren [7–9]. These reports often involved serious cardiopulmonary events [9]. In 2008, the contraindication in patients with "serious cardiopulmonary conditions" was changed to a warning, and the recommendation for 30-min monitoring was limited to patients with either serious cardiopulmonary conditions or pulmonary hypertension [9]. In 2011, a subsequent label revision stated that the aforementioned cardiopulmonary events were uncommon, and usually occur within 30 min of administration [3]. The recommendation for monitoring was removed entirely. Additionally, the statement regarding lack of evidence for safety and efficacy in stress testing was removed [3]. After the warning in 2007, the use of perflutren plummeted [9, 10]. Despite the subsequent revisions, the black-box warning on perflutren remains. Although use of this agent has steadily increased since then, as of at least 2011 it had not fully rebounded to the rate of use prior to 2007 [11].

Subsequent observational studies and meta-analysis evaluating the aforementioned signal provide important information [12–27]. The multiple observational studies failed to detect an increased risk of the major cardiovascular events of death, myocardial infarction, and arrhythmia due to perflutren [12, 14–27]. However, studies supported the rare occurrence of anaphylactoid reactions due to perflutren [21, 26]. Despite the maturity of the signal evaluation to date (e.g., multiple observational studies performed in response to a signal generated by individual spontaneous reports), we report a dual-purpose statistical aggregate analysis of spontaneous report with perflutren. Our dual-purpose analysis is intended to: (1) search for statistical reporting signals of novel adverse events reported with perflutren; and (2) supplement previous signal evaluation studies of the aforementioned serious cardiovascular events of death, myocardial infarction, and arrhythmias.

The scientific and public health rationale for performing the analysis is based on the following: (1) large SRS databases were created for sensitive initial signal detection, including rare events that may present a challenge to clinical trials and large observational studies. This power for initial detection of rare events can also be exploited for purposes of signal evaluation or refinement, as we do herein, when rare events are a concern; (2) to the best of our knowledge no one has interrogated these sources of data using statistical methodologies to assess the safety of perflutren; and (3) pursuant to preceding rationale, a convergence of findings across multiple available datasets spanning a range of powers to detect diverse events can enhance the robustness of drug safety findings.

We used a disproportionality analysis (DA). DA applied to spontaneous reporting system (SRS) data is typically used to support initial signal detection, but it may be applied as one element of signal evaluation to help refine an index of suspicion for signals detected by other methods and/or datasets [28, 29].

2 Methods

We analyzed a public release version of the FDA Adverse Events Reporting System (FAERS) database [30] consisting of reports of adverse events reported from January 1st, 1969 through March 31st, 2014. The FDA maintains the FAERS, a database that contains information related to post-marketing medication error reports and safety surveillance as well as individually reported adverse events submitted by healthcare providers and consumers themselves, which may include patients, family members, or lawyers. The data were preprocessed per vendor (Oracle

Health Sciences, Redwood City, CA, USA) to mitigate duplicate reporting and redundant drug nomenclature.

Adverse events (AEs) were recorded in the FAERS using the Medical Dictionary for Regulatory Affairs (MedDRA® Version 17.0) Preferred Terms (PTs). MedDRA® terminology is the international medical terminology developed under the auspices of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. PTs are intended to represent a single medical concept and linked with broader Higher Level Terms, Higher Level Group Terms, System Organ Classes, and narrower groupings (reported terms) in a hierarchical structure. PTs are the typical default unit of observation when performing DA to support routine a priori, initial signal detection. When DA of SRS data is performed for ancillary signal detection (e.g., when searching for signals of differential reporting of labeled events between subsets of reports) or signal refinement (i.e., when signals have already been highlighted and possibly already subjected to evaluation), as in this exercise, PTs may be supplemented with composite event terms. The potential utilities of composite terms in the latter scenarios are to mitigate the impact of sub-setting of data (e.g., comparing reporting between age or indication groups) on sample size and for optimizing case definitions, respectively. Two options for composite terms are Standardized Medical Queries (SMQs) and user-defined custom groupings. SMQs are groupings of terms from one or more MedDRA® System Organ Classes that relate to a defined medical condition or area of interest. They are typically used to aid in case finding. MedDRA® SMQs may be more or less sensitive vs specific ("narrow" vs "broad" SMQs) [31, 32]. If not too broad, SMQs represent an attractive option because they are standardized groupings ready for use. The fundamental "unit of observation" in our analysis was the MedDRA® PT [32], but we supplemented analysis of individual PTs by examining narrow SMQs. We incorporated only narrow SMQs in our analysis because the broad SMQs, which are typically used for sensitive case finding for subsequent case-level clinical review prior to clinical review, were considered too non-specific for quantitative signal evaluation using aggregate report data. However, some narrow SMQs may still be possibly overly broad for aggregate analysis.

Our statistical analysis of safety reporting associations of perflutren was a set of two-dimensional (2-D) DA. 2-D DA calculates observed-to-expected (O/E) reporting measures based on 2 × 2 contingency tables. They have an established history of application in pharmacovigilance [33]. Expected reporting measures are calculated based on what might be expected if the drug and event are independently recorded in the database (which is determined by the marginal reporting frequencies in the 2 × 2 table). Usually, this measure would be an O/E reporting frequency, odds ratio, or proportion. A disproportionality measure exceeding a pre-elected threshold is sometimes termed a signal or statistic of disproportionality (SDR), which may be escalated to a signal of suspected causality warranting full signal evaluation depending on the clinical context [34]. All reports recording perflutren as a suspect drug were included in the analysis.

We used a DA method known as the multi-item gamma-Poisson shrinker (MGPS) [33]. MGPS uses an empirical Bayesian measure of disproportionality named the empirical Bayes geometric mean (EBGM), with an associated 90 % credibility interval (EB05–EB95). Very roughly, MGPS calculates a composite O/E that is a precision-weighted average of the overall global mean O/E, which is close to one (Hauben M., unpublished data), and the DEC-specific O/E. This is done to "shrink" large or increase small O/Es based on small numbers of reports towards the global mean O/E because such DEC-specific O/Es may be subject to considerable numerical variability with small numbers of cases [33]. Because large spontaneous reporting databases are sparse (i.e., most DECs are never reported or have one to three reports), many calculated O/E metrics may be numerically unstable, so it is standard to calculate a range of credible values (e.g., a 90 % confidence interval) and use the lower limit of the interval for a disproportionality metric. Intervals may be calculated using asymptotic variance formulas for the various O/E ratios with frequentist DA, or using assumed or fitted prior and posterior distributions in Bayesian implementations [33]. An EB05 >2 [35] was used to define an SDR [34]. We stratified analysis by age, sex, and year of report, to mitigate confounding by these covariates, combining stratum-specific O/Es into a Mantel–Haenszel type summary O/E [33].

DA can be performed using counts of unique reports vs counts of suspected adverse drug reactions (ADRs). Using unique reports, a given suspected ADR report is counted only once in the corresponding 2 × 2 table. Using suspected ADR counts, reports with multiple drugs and events can be counted more than once (i.e., in more than one cell) in the corresponding 2 × 2 contingency table. The sample size is substantially larger when suspected ADR counts are used and the calculated O/Es will be different. Because multiple events recorded in reports are often not independent, some consider this larger sample size to be somewhat inflated, with corresponding "underestimation" of the variance. Both approaches have been used and none is shown to be clearly preferred [36–39]. In this exercise, we use report counts.

For the signal detection component of our analysis, all events with SDRs were assessed for novelty using the latest major version of the United States package inserts (USPIs) for perflutren [3, 4] that was posted on the FDA website as a reference. In performing this adjudication, we considered

whether events terms highlighted by DA matched the USPI either literally and/or conceptually. We also reviewed the overall cumulative data mining results for each reported DEC without a current SDR, looking back in time to identify any persistent and impermanent SDRs during past time intervals. This is particularly indicated because of the substantial decrease in perflutren use subsequent to the published warnings [9, 10], even though results of the DA should be relatively robust to overall changes in drug reporting [40].

Because age ≥65 years and stress indications may be positively associated with the risk of serious cardiopulmonary events [12], we performed supplemental analysis of the corresponding subsets. Our decision rule for comparing results between subsets of reports or between a subset and the overall dataset was based on the calculated credibility intervals. As a conservative heuristic, we considered non-overlapping credibility intervals as an indicator of significant reporting differences between subsets when at least one of the subsets was associated with an SDR [28].

For the signal evaluation component of our analysis, we reviewed all events with SDRs for literal or conceptual identity with the aforementioned major outcome events of death, myocardial infarction, and arrhythmias. To be especially comprehensive, we also examined the results against PTs specific for other acute coronary syndromes (e.g., angina, myocardial ischemia) and convulsions.

3 Results

There were a total of 1832 unique perflutren reports of adverse events through the first quarter of 2014. The vast majority of reports did not specify the shell formulation. Only 15 reports specified the perflutren-albumin formulation. Of the 1832 reports, 783 (42.7 %) recorded female sex, 896 (48.9 %) recorded male sex, and in 153 (8.4 %) cases, sex was unrecorded. The largest percentage of reports (47.3 %) were in patients aged 19–64 years. Among reports in which both age and sex were recorded, the average age and standard deviation for women were 56.6 (±15.7) years and for men were 59.9 (± 13.5) years. Age and sex distribution are detailed in Table 1.

These 1832 reports recorded 380 PTs that span 83 SMQs. Table 2 displays all events associated with SDRs in the overall analysis, including corresponding case counts, EBGM values, and associated 90 % credible intervals (EB05-EBGM-EB95). For the overall analysis, 18 of the 380 (4.7 %) reported PTs and 1 out of 83 (1.2 %) SMQs were associated with SDRs. Of note are the large double-digit SDRs for the clinically similar PTs, Back pain and Flank pain. We see there is also an SDR for the clinically similar term Renal pain PT, though the magnitude of the SDR is much lower. After back-pain type events, the strongest reporting associations were observed for events compatible with signs and symptoms of allergic/hypersensitivity reactions (e.g., PTs: Flushing, Urticaria, Pruritus, Dyspnoea, and narrow SMQ: Angioedema). There were no SDRs involving PTs specific for anaphylaxis/anaphylactoid reactions (as opposed to PTs compatible with individual signs/symptoms of, but not specific for, anaphylaxis/anaphylactoid reactions). The magnitude of the EB05 for most SDRs in overall and subset analysis was 2–4. We looked back at previous time points in the evolution of the data and did not identify impermanent SDRs. There was one transient (i.e., 1 year) SDR for the labeled PT Arthralgia. Table 3 displays the assessment of expectedness of events associated with SDRs in any analysis (overall and/or subset) against events in the USPIs [3, 4]. With the exception of the Vitreous haemorrhage PT (see below), all events with SDRs were judged to be either literally or conceptually labeled (one PT, Muscle spasm is only labeled in the DEFINITY® USPI). From the perspective of signal evaluation notable negative findings for perflutren overall included an absence of SDRs involving the aforementioned major cardiovascular and convulsion PTs (Table 4).

Table 5 displays results from the age subset analysis. In the age ≥65 years subset, 9/197 (4.6 %) of reported PTs were associated with an SDR compared with 15/272 (5.5 %) in the age <65 years subset and 10/146 (6.8 %) in the age-unknown subset. The corresponding figures for SMQs are: 0/65 (0 %), 1/66 (1.5 %), and 2/52 (3.8 %). Renal pain PT was the only event with an SDR that was observed in the age ≥65 years subset, but not in the age <65 years subset. There were several events with SDRs in the age <65 years subset that were not associated with SDRs in the age ≥65 years subset including PTs: Chest discomfort, Dyspnoea, Musculoskeletal pain, pain extremity, Pruritus, Throat tightness, Unresponsive to stimuli, and narrow SMQ: Angioedema. The magnitudes of the SDRs and of the O/E ratios in general,

Table 1 Sex and age distribution

	Age <18 years	Age 19–64 years	Age >65 years	Unknown	Total
Female	4	424	199	155	783 (42.7 %)
Male	4	438	279	175	896 (48.9 %)
Unknown	0	5	3	145	153 (8.4 %)
Total	8 (0.4 %)	867 (47.3 %)	481 (26.3 %)	476 (26.0 %)	1832

Table 2 SDRs from the perflutren overall analysis

PT/SMQ [narrow]	*N*	EB05	EBGM	EB95	*E*
Back pain	950	***37.99***	40.08	42.26	23.67
Chest discomfort	42	*2.57*	3.33	4.26	11.67
Chest pain	112	*2.60*	3.05	3.55	35.83
Contrast media reaction	7	*2.29*	4.45	8.41	0.65
Dyspnoea	151	*2.05*	2.35	2.68	63.29
Feeling hot	34	*3.46*	4.61	6.06	6.39
Flank pain	28	***16.06***	22.23	30.12	1.21
Flushing	119	***8.85***	10.46	12.31	10.93
Muscle spasms	82	*3.33*	4.00	4.78	19.53
Musculoskeletal discomfort	11	*3.02*	5.11	8.47	1.21
Neck pain	34	*3.84*	5.12	6.74	5.66
Oxygen saturation decreased	21	*2.33*	3.37	4.74	5.29
Pain in extremity	79	*2.32*	2.80	3.36	27.29
Pruritus	74	*2.12*	2.57	3.10	27.85
Renal pain	18	**6.29**	11.64	19.51	1.23
Throat tightness	24	*3.46*	4.88	6.75	3.94
Unresponsive to stimuli	13	*2.18*	3.48	5.34	2.78
Urticaria	149	**6.73**	7.73	8.86	18.37
Angioedema (SMQ)	182	*3.15*	3.56	4.01	50.19

$2 < EB05 \leq 4 < \mathbf{EB05} \leq 8 < \boldsymbol{EB05} < \infty$

PT Preferred Term, *SMQ* Standardized Medical Queries, *EB05* empirical Bayes lower bound of the 90 % credible interval, *EBGM* empirical Bayes geometrical mean, *EB95* empirical Bayes upper bound of the 90 % credible interval, *E* expected number, *SDR* signal of disproportionate reporting

were lower in the age ≥65 years subset compared with the age <65 years subset. Only one SDR in the age ≥65 years subset had EB05 >8 (PT: Back pain) compared with three (PTs: Back pain, Flank pain, Flushing) in the age <65 years subset. Most differences were not judged statistically significant according to our credibility interval-based decision rule but Chest discomfort PT was associated with a significantly higher O/E in the age <65 years group compared with the age ≥65 years group, and Back pain PT had a statistically significantly higher O/E in the age ≥65 years group compared with the age under 65 years group. Vitreous hemorrhage, the one literally or conceptually unlabeled event in any analysis, was associated with a substantial SDR in the age-unknown subset and based on a review of the publically available FAERS case summaries corresponds to the cases in which perflutren gas (not microbubble UCA) was used for intra-vitreal injection for the treatment of retinal hemorrhage and thus possibly representing a procedural complications/confounding by a non-cardiological indication. The unknown-age subset displayed significantly larger SDRs for the PT Back pain than the other two age subsets. There were no SDRs for the aforementioned major cardiovascular and convulsion events in any age subset (Table 4).

Table 3 Comparison of FAERS reported adverse events with SDRs and the adverse events listed in the perflutren USPIs [3, 4]

Reported adverse event (PT/SMQ[narrow]) with SDR	Labeled adverse reaction on the USPI	Conceptually labeled reaction on the USPI	Unlabeled
Angioedema (SMQ)	X		
Back pain	X		
Chest discomfort		X	
Chest pain	X		
Contrast media reaction		X	
Dyspnoea	X		
Feeling hot	X		
Flank pain		X	
Flushing	X		
Hypersensitivity (SMQ)	X		
Muscle spasms	X[a]		
Musculoskeletal pain	X		
Neck pain		X	
Oropharyngeal allergic conditions (SMQ)	X		
Oxygen saturation decreased	X		
Pain in extremity		X	
Pruritis	X		
Renal pain	X[b]		
Throat tightness	X		
Unresponsive to stimuli	X		
Urticaria	X		
Ventricular tachyarrhythmias (SMQ)	X		
Ventricular tachycardia	X		
Vitreous haemorrhage			X

PT Preferred Term, *SMQ* Standardized Medical Queries, *USPI* United States packaging insert, *SDR* signal of disproportionate reporting, *FAERS* Food and Drug Administration Adverse Events Reporting System

[a] Unlabeled on the OPTISON™ USPI [4]

[b] Conceptually labeled on the OPTISON™ USPI [4]

Table 6 lists reported indications for perflutren. There were 1918 indication codes in 1832 reports, indicating more than one reported indication in some reports. For example, both the procedure and the disease for which the procedure is indicated, may be recorded as indications in the same report. Of note, "stress echo" is listed as the indication for 308 of these reports. Importantly, a large proportion of reports (34.0 %) had an unknown/unrecorded indication. When an indication was recorded and it was not specific for a stress study, it was often non-specific and potentially compatible with either a stress or non-stress study. Subset analysis of reports with specific stress-study indication codes is presented in Table 7,

Table 4 Results for major cardiovascular and convulsion events reported by perflutren

PT/SMQ [narrow]	Overall		Indications subsets						Age subsets (years)					
			Stress		Non-stress		Unknown		≥65		<65		Unknown	
	N	EB05	*N*	EB05	*N*	EB05	*N*	EB05	*N*	EB05	*N*	EB05	*N*	EB05
Acute myocardial infarction	4	0.22	1	0.13	3	0.3			3	0.38	1	0.06		
Angina pectoris	2	0.09	1	0.12	1	0.07			2	0.25				
Arrhythmia	1	0.03	1	0.11					1	0.10				
Atrial fibrillation	12	0.53	2	0.21	8	0.65	2	0.14	7	0.49	4	0.38	1	0.08
Atrial flutter	1	0.12			1	0.18					1	0.2		
Atrioventricular block complete	1	0.12			1	0.18			1	0.20				
Bradycardia	12	0.67	5	0.81	4	0.34	3	0.28	5	0.42	7	0.73		
Bundle branch block right	1	0.13			1	0.19			1	0.22				
Cardiac arrest	22	1.06	4	0.48	12	1.08	6	0.55	8	0.84	13	0.99	1	0.09
Cardio-respiratory arrest	10	0.66			6	0.69	4	0.47	2	0.23	3	0.24	5	1.23
Complex partial seizures	1	0.22									1	0.22		
Convulsion	25	0.64	5	0.36	12	0.61	7	0.36	6	0.61	13	0.42	6	0.41
Coronary artery occlusion	1	0.06	1	0.16					1	0.16				
Death	12	0.18	1	0.04	8	0.25	3	0.08	5	0.18	3	0.1	4	0.12
Extrasystoles	2	0.29	1	0.23			1	0.2	1	0.23	1	0.17		
Grand mal convulsion	2	0.12			1	0.09	1	0.11			2	0.13		
Myocardial infarction	5	0.05	3	0.11	2	0.03			2	0.06	2	0.03	1	0.02
Myocardial ischaemia	1	0.07	1	0.18									1	0.19
Nodal rhythm	3	0.84	1	0.27	1	0.24			1	0.27	2	0.62		
Pulseless electrical activity	4	1.02			4	1.36			2	0.58	1	0.22	1	0.23
Sinus bradycardia	3	0.42			2	0.39					3	0.62		
Sinus tachycardia	5	0.65	2	0.45	3	0.58			1	0.23	4	0.62		
Subendocardial ischaemia	1	0.29	1	0.3									1	0.28
Supraventricular tachycardia	4	0.64	2	0.52	1	0.17	1	0.18	2	0.47	2	0.36		
Tachycardia	19	0.89	8	1.11	3	0.19	8	0.80	3	0.34	14	0.90	2	0.22
Torsade de pointes	1	0.11					1	0.19					1	0.21
Ventricular extrasystoles	8	1.08	4	1.1	2	0.32	2	0.34	1	0.17	7	1.47		
Ventricular fibrillation	7	0.95	3	0.78	3	0.56	1	0.15	3	0.65	4	0.68		
Ventricular tachycardia	14	1.73	8	*2.42*	3	0.45	3	0.50	6	1.20	3	0.40	5	1.94
Bradyarrhythmias (incl conduction defects and disorders of sinus node function) (SMQ)	7	0.23	1	0.07	4	0.24			2	0.12	5	0.29		
Cardiac arrhythmia terms (incl bradyarrhythmias and tachyarrhythmias) (SMQ)	56	0.82	21	1.21	23	0.67	10	0.30	22	0.71	27	0.79	7	0.41
Cardiac arrhythmia terms, nonspecific (SMQ)	5	0.17	1	0.08	4	0.27			3	0.24	1	0.04	1	0.06
Cardiac arrhythmias (SMQ)	56	0.81	21	1.20	23	0.66	10	0.30	22	0.70	27	0.78	7	0.40
Convulsions (SMQ)	32	0.60	5	0.26	17	0.66	9	0.36	6	0.45	18	0.42	8	0.47
Myocardial infarction (SMQ)	9	0.08	4	0.13	5	0.08			5	0.14	3	0.04	1	0.01
Shock-associated circulatory or cardiac conditions (excl torsade de pointes) (SMQ)	43	0.91	7	0.46	27	1.18	9	0.39	12	0.58	22	0.79	9	0.91
Supraventricular tachyarrhythmias (SMQ)	20	0.67	6	0.63	11	0.69	3	0.16	10	0.61	9	0.61	1	0.06
Tachyarrhythmia terms, nonspecific (SMQ)	2	0.19	1	0.19			1	0.15	1	0.18	1	0.12		
Tachyarrhythmias (incl supraventricular and ventricular tachyarrhythmias) (SMQ)	48	1.16	20	1.86	18	0.83	10	0.50	20	0.99	21	1.00	7	0.75
Torsade de pointes, shock-associated conditions (SMQ)	15	0.73	8	1.23	3	0.20	4	0.34	6	0.62	3	0.16	6	1.23
Torsade de pointes/QT prolongation (SMQ)	15	1.00	8	1.57	3	0.27	4	0.45	6	0.80	3	0.22	6	1.52

Table 4 continued

PT/SMQ [narrow]	Overall		Indications subsets						Age subsets (years)					
			Stress		Non-stress		Unknown		≥65		<65		Unknown	
	N	EB05	*N*	EB05	*N*	EB05	*N*	EB05	*N*	EB05	*N*	EB05	*N*	EB05
Ventricular tachyarrhythmias (SMQ)	28	1.67	14	*2.71*	8	0.78	6	0.62	10	1.15	12	1.11	6	1.49

$2 < EB05 \leq 4 < \mathbf{EB05} \leq 8 < \boldsymbol{EB05} < \infty$

PT Preferred Term, *SMQ* Standardized Medical Queries

Table 5 SDRs from the age subset analysis

PT/SMQ [narrow]	Age under 65 years					Age 65 years or greater					Age unknown				
	N	EB05	EBGM	EB95	*E*	*N*	EB05	EBGM	EB95	*E*	*N*	EB05	EBGM	EB95	*E*
Back pain[b,c]	416	***31.52***	34.19	37.03	12.13	294	***38.47***	42.39	46.61	6.89	240	***56.58***	62.99	69.97	3.78
Chest discomfort[a]	27	*2.56*	3.54	4.79	6.75	4	0.51	1.15	2.30	2.76	11	*2.59*	4.36	6.97	1.78
Chest pain	60	*2.24*	2.78	3.42	20.72	35	*2.69*	3.57	4.67	8.67	17	1.89	2.86	4.18	5.21
Dyspnoea	85	*2.19*	2.62	3.12	31.57	38	1.34	1.76	2.28	20.64	28	1.89	2.60	3.50	10.05
Feeling hot	19	*2.84*	4.19	6.01	3.64	11	*2.56*	4.33	7.25	1.46	4	0.89	2.11	4.42	1.17
Flank pain	15	***10.34***	19.14	30.07	0.70	6	*2.32*	6.86	24.42	0.33	7	***16.17***	37.38	71.25	0.15
Flushing	72	***9.38***	11.78	14.58	5.80	24	**5.04**	7.91	12.38	2.43	23	**4.96**	7.13	10.11	2.52
Muscle spasms	43	*3.06*	3.95	5.04	10.00	20	*2.20*	3.20	4.54	5.14	19	*2.83*	4.18	6.00	3.80
Musculoskeletal pain	15	*2.52*	3.91	5.84	2.95	4	0.71	1.59	3.19	1.63					
Neck pain	17	*2.74*	4.14	6.05	3.21	10	*2.40*	4.15	7.05	1.33	7	*2.17*	4.18	7.50	0.94
Pain in extremity	43	*2.29*	2.96	3.77	13.69	19	1.43	2.09	2.99	8.09	17	*2.03*	3.06	4.48	4.81
Pruritus	47	*2.33*	2.97	3.75	14.94	15	1.17	1.80	2.67	7.42	12	1.26	2.07	3.24	5.08
Renal pain	6	1.95	3.93	7.37	0.66	6	*2.74*	10.71	32.80	0.27	6	*3.29*	9.48	32.23	0.25
Throat tightness	18	*3.60*	5.39	7.86	2.45	3	0.76	1.92	4.19	0.61	3	0.72	1.97	4.53	0.81
Unresponsive to stimuli	10	*2.35*	4.03	6.56	1.60	1	0.20	0.77	2.28	0.89	2	0.51	1.73	4.65	0.45
Urticaria	83	**5.44**	6.54	7.82	11.79	28	**4.60**	6.60	9.68	3.36	38	**6.57**	8.76	11.65	3.69
Vitreous hemorrhage											5	***19.73***	54.49	117.96	0.06
Angioedema (SMQ)	103	*2.88*	3.39	3.98	29.49	33	1.92	2.57	3.39	11.78	46	*3.55*	4.55	5.77	9.35
Hypersensitivity (SMQ)	162	1.50	1.70	1.94	94.30	56	0.99	1.24	1.54	44.29	86	*2.01*	2.41	2.86	35.01

$2 < EB05 \leq 4 < \mathbf{EB05} \leq 8 < \boldsymbol{EB05} < \infty$

SDR signal of disproportionate reporting, *PT* Preferred Term, *SMQ* Standardized Medical Queries, *EB05* empirical Bayes lower bound of the 90 % credible interval, *EBGM* empirical Bayes geometrical mean, *EB95* empirical Bayes upper bound of the 90 % credible interval, *E* expected number

[a] Age under 65 years subset ≫65 years or greater subset

[b] Age 65 years or greater subset ≫under 65 years subset

[c] Unknown subset ≫both under 65 years and 65 years or greater subsets

which contains individual PTs and narrow SMQs with SDRs in at least one subset. Of 167 reported PTs in the stress-specific indications, 8 (4.8 %) were associated with an SDR vs 14/273 (5.1 %) for non-stress-specific indications and 9/180 (5.0 %) for nonspecific indications. The corresponding figures for SMQs are: 4/51 (7.8 %), 1/71 (1.4 %), and 1/48 (2.1 %). There were several events with SDR in the stress indication subset, but not in the non-stress subset (PTs: Dyspnoea, Pruritus, and narrow SMQs: Hypersensitivity, Oropharyngeal allergic conditions, Ventricular tachyarrhythmias). There were several events with SDRs in the non-stress subset that were not associated with SDRs in the stress subset (PTs: Chest discomfort, Feeling hot, Flank pain, Muscle spasms, Oxygen saturation decreased, Renal pain, Throat tightness, Unresponsive to stimuli, and Vitreous haemorrhage). The magnitudes of the SDRs and of the O/E ratios in general were lower in the stress test-indication subset compared with the non-stress-indication dataset. Two of these had EB05 >8 (PTs:

Table 6 Indications reported for perflutren

Indication PT	*N* (%)
{Null}	653 (34.0)
Atrial fibrillation	4 (0.2)
Cardiac failure congestive	8 (0.4)
Cardiac imaging procedure	9 (0.5)
Cardiac pharmacologic stress test	7 (0.4)
Cardiac stress test[a]	76 (4.0)
Cardiovascular evaluation	10 (0.5)
Chest pain	44 (2.3)
Coronary artery disease	9 (0.5)
Diagnostic procedure	25 (1.3)
Dyspnoea	9 (0.5)
Echocardiogram	587 (30.6)
Electrocardiogram	4 (0.2)
Exercise test[a]	5 (0.3)
Preoperative care	4 (0.2)
Retinal hemorrhage	14 (0.7)
Scan with contrast	11 (0.6)
Stress echocardiogram[a]	308 (16.1)
Ultrasound doppler	6 (0.3)
Ultrasound scan	34 (1.8)
Others[b]	91 (4.7)
Total	1918

PT Preferred Term

[a] Included in the perflutren stress test-indication subset

[b] Indications with three or fewer reports are summarized under others

Back pain, Urticaria) compared with 3 (PTs: Back pain, Flank pain, Flushing) in the non-stress test-indication dataset. Some allergy-related events were disproportionately more frequent in the stress-indication subset (PTs: Pruritus, Urticaria and narrow SMQs: Angioedema, Hypersensitivity, Oropharyngeal allergic conditions). However, the PTs themselves are not necessarily serious in nature and the corresponding allergic SMQs with SDRs contain a broad array of PTs covering a range of seriousness and severities. Of the aforementioned major cardiovascular and convulsion events, only two, Ventricular tachycardia PT and Ventricular tachyarrhythmia SMQ [narrow] had SDRs in the stress subset compared with the non-stress subset. However, only the SMQ was statistically significantly higher by our decision rule.

4 Discussion

An initial signal of serious cardiopulmonary events with perflutren UCA arose from routine surveillance of spontaneous reports [9]. Subsequent observational studies refined (i.e., substantially weakened) the signal for the major events of death, myocardial infarction, and arrhythmias but supported a low risk of anaphylactoid reactions [12, 14–27]. The product label evolved dynamically in response to this accumulating information.

Our analysis extends the previous signal detection and evaluation exercises by virtue of a paucity of statistical findings from a DA applied to a dataset originally implemented for registering rare events that might elude other datasets. Given the relevant patient populations, and the high potential for confounding by indication in SRS data, the paucity of findings seems noteworthy. There were no SDRs of novel AEs either in overall or subset analysis for perflutren UCAs. With the exception of Ventricular tachycardia PT and Ventricular tachyarrhythmia SMQ [narrow], there were no SDRs for the key major cardiovascular events reported in the multiple observational studies. The only positive findings of note were a significantly higher reporting frequency of PTs: Pruritus, Urticaria and narrow SMQs: Angioedema, Hypersensitivity, Oropharyngeal allergic conditions, and Ventricular tachyarrhythmias in the stress-indication-specific subset. The generally low magnitude of SDRs points to an increased possibility for confounding as an alternative explanation. Consistent with this, given the multiple studies, including ours, reporting the relative safety of perflutren, it has been postulated that some adverse events attributed to perflutren, were "pseudocomplications" or complications that resulted from the underlying condition that necessitated perflutren use, and not the use of the agent itself, i.e., cofounding by treatment indication. Those patients intubated on a ventilator, for example, have notoriously poor echocardiography windows, and more frequently require perflutren [41]. By virtue of being intubated, these patients are likely to be sicker and have a high mortality, which is unrelated to the use of echo contrast.

Multiple studies [12, 14–27] have demonstrated the safety of perflutren, focusing on death, cardiac arrhythmias, and myocardial infarction, including three studies that were specifically requested by the FDA [12–14]. The first was the CaRES (Contrast Echocardiography Registry for Safety Surveillance) study [12]. This was a multi-center, prospective, nonrandomized study designed to evaluate the association of perflutren with adverse events and enrolled a total of 1053 patients. There were no deaths or life-threatening events during or after perflutren administration. The overall adverse event rate was 10.8 %, of which 96.5 % were categorized as mild to moderate in severity. The rates of adverse events were highest in those patients undergoing pharmacologic stress, followed by patients undergoing exercise stress, and the lowest adverse event rate in patients with rest-only studies. This brought the conclusion that many adverse events may have been due to the stress protocol rather than perflutren, and even when all events were included, perflutren was well tolerated.

Table 7 SDRs from the specific stress test indications subset analysis

PT/SMQ [narrow]	Perflutren (stress test indications)					Perflutren (without stress test indications)					Perflutren (unknown indication)				
	N	EB05	EBGM	EB95	*E*	*N*	EB05	EBGM	EB95	*E*	*N*	EB05	EBGM	EB95	*E*
Back pain	183	***30.80***	34.84	39.28	5.21	427	***37.65***	40.80	44.15	10.43	340	***38.68***	42.32	46.23	8.00
Chest discomfort	10	1.63	2.78	4.50	2.68	18	*2.00*	2.98	4.31	5.10	14	1.85	2.91	4.40	3.88
Chest pain	29	*2.44*	3.33	4.47	7.76	45	*2.16*	2.77	3.51	15.32	39	*2.19*	2.86	3.69	12.71
Dyspnoea	39	*2.01*	2.63	3.40	13.89	68	1.92	2.35	2.86	28.01	44	1.54	1.98	2.52	21.31
Feeling hot	6	1.25	2.49	4.55	1.51	19	*3.41*	5.04	7.27	2.80	9	1.70	2.99	4.95	2.08
Flank pain	5	1.99	4.93	19.47	0.26	13	***12.39***	21.88	34.88	0.53	10	**6.59**	18.59	34.53	0.42
Flushing	27	**5.70**	8.33	12.54	2.53	58	***9.13***	11.88	15.06	4.63	34	**5.41**	7.35	10.01	3.75
Muscle spasms	12	1.39	2.27	3.53	4.41	39	*3.17*	4.15	5.36	8.42	31	*3.00*	4.05	5.39	6.68
Neck pain	9	*2.28*	4.03	6.74	1.28	15	*2.83*	4.38	6.56	2.46	10	*2.04*	3.49	5.66	1.92
Oxygen saturation decreased	2	0.35	1.10	2.80	1.13	14	*2.72*	4.28	6.49	2.31	5	0.87	1.85	3.56	1.85
Pain in extremity	12	1.06	1.72	2.68	6.13	33	1.91	2.55	3.36	12.02	33	*2.45*	3.28	4.32	9.11
Pruritus[a]	31	*3.15*	4.27	5.67	6.30	22	1.18	1.69	2.36	12.19	21	1.42	2.06	2.90	9.33
Renal pain	3	0.97	2.55	5.78	0.28	6	*2.03*	4.15	8.14	0.53	9	**4.74**	14.28	31.38	0.41
Throat tightness	5	1.27	2.70	5.19	0.94	12	*2.77*	4.54	7.15	1.68	7	1.64	3.10	5.46	1.32
Unresponsive to stimuli	2	0.46	1.45	3.68	0.60	10	*2.49*	4.26	6.98	1.39	1	0.17	0.77	2.46	0.80
Urticaria[a]	53	***8.94***	11.84	15.22	4.21	46	**4.14**	5.30	6.72	7.69	50	**5.31**	6.76	8.55	6.45
Ventricular tachycardia	8	*2.42*	4.46	7.91	0.85	3	0.45	1.18	2.64	1.82	3	0.50	1.29	2.88	1.57
Vitreous hemorrhage						5	*2.26*	7.40	34.61	0.19					
Angioedema (SMQ)[a]	67	**4.43**	5.43	6.61	11.35	58	*2.06*	2.57	3.17	21.67	57	*2.53*	3.16	3.90	17.12
Hypersensitivity (SMQ)[a]	101	*2.21*	2.61	3.07	37.74	104	1.18	1.39	1.62	74.32	99	1.37	1.62	1.91	60.18
Oropharyngeal allergic conditions (SMQ)[a]	15	*2.01*	3.11	4.64	3.89	8	0.53	0.96	1.64	7.68	4	0.28	0.64	1.30	5.88
Ventricular tachyarrhythmias (SMQ)[a]	14	*2.71*	4.27	6.48	2.31	8	0.78	1.42	2.42	4.85	6	0.62	1.23	2.25	4.13

$2 < EB05 \leq 4 < \mathbf{EB05} \leq 8 < \boldsymbol{EB05} < \infty$

PT Preferred Term, *SDR* signal of disproportionate reporting, *SMQ* Standardized Medical Queries, *EB05* empirical Bayes lower bound of the 90 % credible interval, *EBGM* empirical Bayes Geometrical Mean, *EB95* empirical Bayes upper bound of the 90 % credible interval, *E* expected number

[a] Stress test subset > non-stress test subset

Importantly, this study also included patients who were elderly, had a history of myocardial infarction and heart failure, and also patients with chronic obstructive pulmonary disorder. The CaRES study, however, was not large enough to detect rare events, such as anaphylactic reactions, given the study size of ~1000 patients. We did not observe a similar differential between stress and non-stress study indications in our analysis and in fact detected a tendency for somewhat lower proportionate reporting with stress test indications.

The second study was of the effects of perflutren on systemic and pulmonary hemodynamics [13]. There were 32 patients enrolled, of which half had elevated baseline pulmonary arterial systolic pressure (PASP) established by right heart catheterization. All subjects were administered perflutren, and then had PASP monitored at frequent intervals for a total of 60 min. The study showed that there were no significant changes in PA pressure after administration of perflutren in either group.

The third study was a retrospective study looking at a US hospital service-level database, and included over 1 million critically ill patients who underwent clinically indicated echocardiogram with or without perflutren [14]. In comparing propensity-matched groups, there was no significant mortality difference between those who received perflutren and those who did not.

A relative advantage of the SRS data used in our analysis is that it originates from "real-world use" of the agent, including a diverse patient population and various clinical settings, but the extent to which this diversity "survives" to

make it into a spontaneous report is unclear. However, we acknowledge additional important limitations in our data and methods.

From a combined data and methods limitation perspective, we did not perform a case-level clinical review (case narratives are not included in FAERS extracts for public use). DA and results of any quantitative analysis of SRS data are most meaningful when correlated with case-level clinical information.

The usual 'warnings, precautions and indications for use' for SRS data applies, which precludes making causal inferences except in unusual circumstances [42, 43]. The data only contain adverse events, and not a total number of patients. These are also self-initiated reports, and there are likely many events that go unaccounted, and also events that were reported, but unsubstantiated. Therefore, actual risk or incidence rates cannot be determined. Furthermore, public available SRS databases are plagued by reporting artefacts, biases (e.g., notoriety), recorded and unrecorded confounders (prominently by indication) and effect modifiers, and deficits in quality and completeness of information including duplicate reporting, which may escape proprietary duplicate detection algorithms. [44–47].

DA does not neutralize the defects, which typically remain unresolved during initial signal detection and confounding may be amplified by performing subset analysis. Therefore, confounding by indication is one plausible explanation for the finding with Ventricular tachyarrhythmia SMQ [narrow] in the stress-indication-specific subset analysis, given that stress echocardiography is typically performed in patients with suspected or confirmed coronary artery disease and the use of dobutamine in pharmacological stress protocols. Further limiting the interpretation of the increased reporting of the ventricular tachyarrhythmia SMQ [narrow] in the stress indication subset is the difficulty in making "clean" subset assignment based on the reported indications, and the multiple individual PTs comprising the SMQ, some being of more clinical import than others (e.g., Ventricular extrasystoles vs Ventricular tachycardia).

The suspect drug analyzed was perflutren, without specification of the shell composition (i.e., lipid vs human albumin shells). Perflutren without further specification and perflutren albumin are recorded as suspect drugs in the FAERS database and both were used in our analysis. Therefore, our results reflect a composite analysis of both proprietary products. Therefore, the ability to detect signals of novel formulation-specific AEs may have been impaired.

DA has been the subject of heated debate with extreme viewpoints of "unbridled optimism" to "considerable skepticism" [48]. Some authorities consider such analysis a useless and potentially harmful "garbage in, garbage out" exercise, while others unrealistically maintained that such quantitative analysis, if performed with certain proprietary software, can neutralize the enormous limitations of spontaneous reports ("garbage in, gospel out"). We take a moderate position between the aforementioned extremes fully acknowledging DA's strengths and limitations. 2-D DA does not accommodate the complex multivariate drug and event relationships that are characteristic of SRS data. Masking may result in false-negative findings in DA of SRS data, though this is more likely to occur with rare events in DA of pharmaceutical company databases [36]. Some have claimed that data mining is "objective". Real-world experience indicates that such views may be unrealistic and sensitive to specific software selection and implementation [49]. Our decision rule based on the presence of non-overlapping intervals and at least one SDR for purposes of subset reporting comparisons, is conservative, in that overlapping confidence or credibility intervals up to a point, may occur despite significant differences because the rule is based on linear addition of variance rather than addition in quadrature [50].

We used one specific implementation of DA, MGPS, including a specific metric/threshold combination (EB05 >2) reported to be less sensitive than other algorithms when common implementations are compared [51]. Other DA methods exist, including another Bayesian shrinkage method, the Bayesian Confidence Propagation Neural Network, and non-Bayesian methods such as the proportional reporting ratio, reporting odds ratio, P-plots, and standardized gamma [33, 52, 53]. Bayesian approaches attempt to achieve classifications that are more accurate on average, but individual shrinkage adjusted O/E should not be regarded as "corrected", or more correct, than the same O/E ratio calculated by other methods, as some have claimed. Expressed a little differently "if you use an empirical Bayes estimate, everything gets pulled toward the central bulge. You have to grit your teeth and believe the fact that even though any one estimate may be off, overall you're getting a lot of improvement. That's what we have to get people, including ourselves, to believe" [54]. In fact, increased errors in the form of false-negative findings, either absolute or relative in terms of timing, can occur with MGPS, although as with all forms of DA it is highly contingent on the specific implementation [48, 55, 56]. Recently, it has been reported that under-reporting typical of SRS data may bias MGPS results [57]. Other methods may therefore have returned different results, especially for rare DECs. Whether a given method is preferred partly depends on the relative impacts of false-positive and false-negative classification errors, which are situation dependent. Currently, most researchers do not consider any single DA algorithm to be universally superior to others [33, 56]. Regardless of the specific algorithm

used, SDR-defining thresholds are not set in stone. Some might advocate a lower threshold (e.g., >1) for events of a sufficiently serious nature. Using a lower threshold could have changed the conclusions.

Finally, the sequence of events that stimulated our analysis represents an interesting and educational illustration of how the process of signal evaluation resulted in the reduction of labeled precautions and warnings that were originally instituted based on signals from spontaneous reports.

5 Conclusion

We performed a DA of spontaneous reports of perflutren in a large health authority database used for detecting signals of rare events, to search for signals of novel events and extend results of previous signal evaluations based on observational studies. Using general pharmacovigilance thresholds, our analysis converges with previous studies supporting the safety of perflutren, and further weakens an initial signal of major cardiopulmonary AEs identified by case-level information from spontaneous reports. DA of spontaneous reports may support a priori signal detection, and in some instances can be judiciously applied to further refine an index of suspicion by supplementing previous signal evaluation exercises.

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Compliance with Ethical Standards

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Conflict of interest Manfred Hauben, Eric Hung, and Vincenza Snow are employees and stockholders of Pfizer, Incorporated. Manfred Hauben holds stocks in General Electric, a subsidiary of which, GE Healthcare, markets the ultrasound contrast agent OPTISON™. Manfred Hauben, Eric Hung, Vincenza Snow, Kelly C. Hanretta, and Sripal Bangalore have no other conflicts of interest that are relevant to the content of this study.

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