Association Between Immunogenicity and Reactogenicity: A Post Hoc Analysis of 2 Phase 3 Studies With the Adjuvanted Recombinant Zoster Vaccine

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A recurrent question is whether transient reactions to vaccines translate into better immune responses. Using clinical data from 2 large phase 3 studies of the recombinant zoster vaccine, we observed a small but statistically significant association between the intensity of a frequent side effect (pain) after vaccination and immune responses to vaccination. However, despite the statistical correlation, the impact on the immune response is so small, and the immune response in individuals without pain already sufficient, that pain cannot be a surrogate marker for an appropriate immune response. Reactogenicity cannot be used to predict immunity after vaccination.

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Vaccines induce transient inflammation that shapes the desired immune response while also causing short-lived local and systemic reactions called reactogenicity. The hypothesis that the magnitude of local reactions (typically pain, redness, or swelling at the injection site) or systemic reactions (eg, headache or fever) following vaccination is predictive of immunogenicity and efficacy (ie, "no pain, no gain") remains untested. The mechanisms underlying vaccine-associated reactogenicity

in individuals are complex and multifactorial [1], and evidence for potential associations between vaccine-induced immune responses and the frequency and/or severity of postvaccination reactions is inconsistent [1, 2].

We conducted a post hoc analysis using data from 2 phase 3 studies of the adjuvanted recombinant herpes zoster vaccine (RZV: Shingrix, GSK) to assess potential correlations between the antigen-specific immune responses and reactogenicity signs and symptoms in adults aged ≥50 years, with implications for the relationship between reactogenicity and vaccine efficacy [3, 4]. RZV contains the varicella zoster virus antigen glycoprotein E (gE) and the adjuvant AS01$_B$. AS01$_B$ is a liposome-based adjuvant containing 3-O-desacyl-monophosphoryl lipid A and the saponin *Quillaja saponaria* Molina, fraction 21 as immunostimulants. AS01$_B$ enhances gE-specific immune responses by stimulating innate immunity, leading to the transient production of cytokines and recruitment of activated antigen-presenting cells at the injection site and draining lymph nodes [5]. Some of these inflammatory markers (interleukin 6, interferon-γ inducible protein 10, C-reactive protein) can be detected in the peripheral blood of hepatitis B–naive individuals vaccinated with hepatitis B surface antigen (HBsAg) formulated in AS01$_B$, and were associated with immunogenicity and systemic reactogenicity [2]. However, when reactogenicity and antigen-specific antibody responses were evaluated, no direct association was observed [2].

In 2 phase 3 placebo-controlled studies, 2 RZV doses demonstrated >90% efficacy in preventing herpes zoster disease in adults aged ≥50 years and ≥70 years [3, 4]. In the reactogenicity subset of approximately 19 000 vaccinees (9500 placebo [0.9% saline], 9500 RZV), injection site pain was reported following 68% of RZV doses vs 7% of placebo doses [6]. Myalgia and fatigue were reported after approximately 32% of RZV doses vs ≤10% of placebo doses, and fever after approximately 12% and 2%, respectively [6]. RZV vaccination was accompanied by an increase in gE-specific antibody concentrations and CD4$^+$ T-cell frequencies 1 month post–dose 2 (PD2), shown to persist above baseline for at least 10 years [7].

We assessed the link between reactogenicity and immunogenicity in a subset of RZV recipients from the 2 phase 3 trials in whom both parameters were measured.

METHODS

In the phase 3 studies, 2 doses (0.5 mL/dose) were administered intramuscularly into the deltoid at an interval of 2 months [3, 4]. Reactogenicity was measured for 7 days after each vaccine dose. Blood samples were collected in a subset of participants before the first dose, and 1, 12, 24 and 36 months PD2 [6, 8].
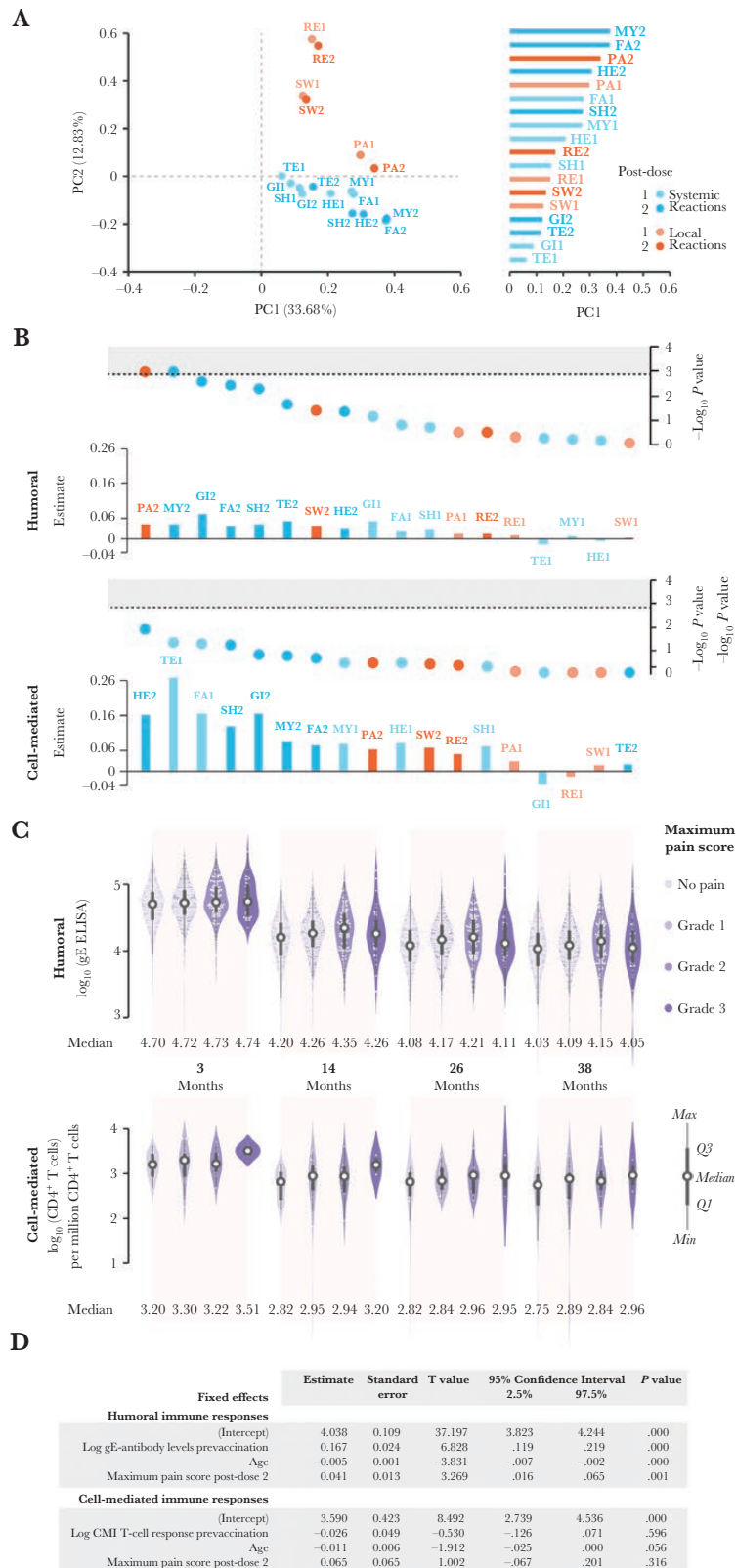
Figure 1. Correlation between maximum reactogenicity scores and immunogenicity. *A*, Principal component (PC) analysis of reactogenicity post–dose 1 (PD1) and post–dose 2 (PD2) of recombinant zoster vaccine. The right-hand graph shows the absolute loading (importance of each variable) on PC1. There is a strong correlation between PD1 and PD2 for individual symptoms; local symptoms of redness and swelling are different from all other symptoms, whereas pain is between redness and swelling and systemic variables. The most important factors on PC1 (ie, those showing the maximum variance from zero on the PC1 axis) are pain post–dose 2 (PA2), fatigue post–dose 2 (FA2), and myalgia post–dose 2 (MY2). *B*, Univariate analysis showing the correlation between the reactogenicity variables PD1 or PD2, and PD2 immunogenicity (level of anti–glycoprotein E [gE] antibodies and CD4+ T cells). Horizontal lines represent Bonferroni-corrected threshold. The figure shows that the humoral immunogenicity profile is correlated

There were 904 RZV recipients with both reactogenicity and anti-gE antibody results, and 147 with both reactogenicity and cell-mediated immunity (CMI) results (gE-specific $CD4^+$ T cells expressing 2, 3, or 4 markers per million $CD4^+$ T cells), who were included in the analysis (Supplementary Table 1). Reactogenicity and immunogenicity responses in this "immuno/reacto" subset were consistent with whole study data (Supplementary Figure 1, Supplementary Table 2) [6, 8].

We assigned a score to each symptom (pain, redness, swelling, fatigue, gastrointestinal symptoms, fever, headache, myalgia, shivering), which was the maximum grade recorded over the 7-day postvaccination period, ranging from symptom absent, redness, and swelling <20 mm, temperature <37.5°C, to grade 3 (severe; prevents normal activity, redness, and swelling >100 mm, and fever >39°C). Methods for scoring are provided in the Supplementary Data.

First, we determined the association between individually reported reactogenicity symptoms using principal component (PC) analysis. To directly assess the association between reactogenicity and immunogenicity, we computed the correlation between the immune response and reactogenicity score using random-effects models where the repeated measurements of the immunological variables were considered as response. The models included age, baseline immunological value, the log of the time of the visit (months postvaccination) when immunogenicity was measured, and the reactogenicity score after each vaccine dose as fixed effects. We considered 2 types of reactogenicity score: a global score obtained by adding each maximum severity for all reported symptoms (global reactogenicity models) and a score for each reactogenicity symptom (univariate reactogenicity models).

Ethics Approval and Consent to Participate
The trial protocols of the phase 3 studies were approved by the appropriate independent ethics committee or institutional review board at each study center and are reported in the primary publications. Written informed consent was obtained from all participants before study entry.

RESULTS

The PC analysis showed a correlation between the intensity of redness and swelling that was almost independent from the intensities of systemic symptoms (see separation on PC2) (Figure 1A). By contrast, the intensity of injection-site pain was more associated with systemic symptoms (fatigue, myalgia, and headache) than other local symptoms. The figure shows a strong correlation between post–dose 1 (PD1) and PD2 for each reactogenicity variable. The most important factors on PC1 (ie, those showing the maximum variance from zero on the PC1 axis) were pain, fatigue, and myalgia PD2. Correlation analysis confirmed that the systemic symptoms were correlated between each other and similar patterns were observed irrespective of the dose number, with stronger associations observed PD2 (Supplementary Figure 2).

More than 60% of vaccinees did not report symptoms after RZV vaccination, other than pain. Pain was reported by >60% of participants after each dose (Supplementary Table 2). In terms of immunogenicity, the magnitude of gE-specific immune responses was generally high in all participants [8]. Table 1 shows the results of the global reactogenicity models, 1 for humoral and 1 for CMI responses. The estimates refer to the estimated change in specific antibody concentration or $CD4^+$ counts with reactogenicity score. The models showed that the global reactogenicity score PD2, but not PD1, was correlated with the antibody response ($P < .0001$, estimate 0.112) and only marginally with $CD4^+$ T-cell response ($P = .073$, estimate 0.230). However, although statistically significant, the absolute increase in antibody response associated with reactogenicity was minimal; that is, when the global score of reactogenicity increased by 1, antibody levels increased PD2 by 1.29-fold. There was a strong effect of age on immunogenicity, even when the model is adjusted for reactogenicity (Table 1). Reactogenicity PD1 was not significant in the model because it does not add additional information compared to PD2, as both are highly correlated (Figure 1A).

To better understand the correlation between the immune response and each reactogenicity variable, we used univariate reactogenicity models where we analyzed the association between each reactogenicity symptom and the humoral and CMI responses after each dose (1 model for each reactogenicity variable PD1 and PD2) (Figure 1B). Only pain and myalgia PD2 showed a statistically significant association with humoral immune response when corrected for multiplicity (Bonferroni

correction), although the estimated impact on antibody levels was small, as observed for the global score of global reactogenicity model. The association with CMI response did not reach statistical significance, despite a larger estimated effect.

We next focused on the association between the immune response and pain, which is the most common symptom reported after RZV vaccination (Supplementary Table 2) and the symptom correlating the most with humoral immune response in our univariate analysis (Figure 1B). The univariate reactogenicity model described above was therefore applied to pain. There was a general trend for higher CD4$^+$ T-cell responses to be associated with more pain, irrespective of the time after vaccination (Figure 1C). However, only anti-gE antibodies, but not CD4$^+$ T cells, were significantly correlated with maximum pain PD2 (Figure 1B). Despite the statistical significance of the finding, the size effect was minimal for anti-gE antibodies. Although there was a weak correlation between severity of pain and immunogenicity, vaccinees without pain were still able to elicit strong antibody and T-cell responses [8] and are therefore likely protected by vaccination (Figure 1C).

DISCUSSION

In a population of adults aged ≥50 years who received 2 doses of RZV, we observed that the global score of reactogenicity PD2 was significantly associated with antibody response and marginally associated with CD4$^+$ T-cell response (Table 1). Additionally, there was a weak but statistically significant association between the gE-specific immune response and maximum injection site pain score PD2 (Figure 1D). However,

37% of individuals did not report pain after RZV PD2 and 5% reported grade 3 pain (Supplementary Table 2), whereas an immune response was induced in most [6, 8], and protection was approximately 90% or more in the study populations [3, 4]. This implies that the presence or intensity of reactogenicity symptoms is not a reliable marker of immunogenicity or protection. Similarly, while age and baseline anti-gE antibody level were more associated with the vaccine immune response than reactogenicity (Figure 1D), vaccine efficacy nevertheless was high across all age strata [3], and was not determined by the level of prevaccination immunity to varicella zoster virus [8].

Exact mechanisms underlying development of short-term reactions to vaccines are not known [1] but likely involve inflammatory components that are also required for induction of antigen-specific responses, as shown in humans and mice with AS01 [2, 9, 10]. This suggests that inflammatory reactions to the vaccine partially explain the global association between immunogenicity and reactogenicity. Although our model indicated a significant positive correlation between maximum pain score reported within 7 days of dose 2 and gE-specific antibody immune responses, the size effect was minimal and likely of no individual clinical significance; that is, a vaccine recipient experiencing severe pain may have a similar, higher, or lower immune response than an individual with no/mild pain, and both are likely to be protected. Our study outcome may be relevant to other vaccines and in particular, adjuvanted vaccines known to be more reactogenic than nonadjuvanted vaccines. A previous study of HBsAg combined with different adjuvants, including AS01, confirms the lack of an association between reactogenicity and immunogenicity of individual significance [11].

Table 1. Results of the Global Random Effects Model for Humoral and Cell-Mediated Immune Responses and the Global Reactogenicity Score After Each Dose of Adjuvanted Recombinant Zoster Vaccine

Fixed Effects	Estimate[a]	SE	T Value	95% CI 2.5%	95% CI 97.5%	P Value
Humoral immune responses						
(Intercept)	4.743	0.110	43.193	4.523	4.955	.000
Log gE antibody levels prevaccination	0.172	0.024	7.076	.123	.222	.000
Age	−0.005	0.001	−4.072	−.007	−.002	.000
Maximum global score PD1	−0.041	0.036	−1.120	−.114	.042	.263
Maximum global score PD2	0.112	0.028	3.928	.049	.169	.000
Time (months since vaccination)	−0.259	0.003	−88.268	−.264	−.254	.000
Cell-mediated immune responses						
(Intercept)	4.118	0.401	10.281	3.283	4.897	.000
Log gE-specific T-cell responses prevaccination	−0.039	0.050	−0.797	−.130	.063	.425
Age	−0.011	0.006	−1.988	−.022	.000	.047
Maximum global score PD1	0.097	0.181	0.535	−.242	.430	.593
Maximum global score PD2	0.230	0.129	1.790	−.016	.485	.073
Time (months since vaccination)	−0.223	0.025	−9.026	−.275	−.172	.000

Model input parameters are presented in terms of the estimate of the effect size, standard error, 95% CI, and P value. The T value is the ratio between the estimate and the standard error

Abbreviations: CI, confidence interval; gE, glycoprotein E; PD1, post–dose 1; PD2, post–dose 2; SE, standard error.

[a]The humoral and cell-mediated estimates refer to the estimated change in antibody concentration or CD4$^+$ counts with each increase in reactogenicity score.

In our correlation analysis, injection site pain was more associated with systemic symptoms than local symptoms. This was somewhat unexpected and to our knowledge, has not been reported previously. This observation does not imply a causal relationship and more research is needed to investigate possible associations between pain and systemic reactions.

Strengths of our analysis include the availability of reactogenicity and immunogenicity data from participants in 2 large phase 3 studies in whom vaccine efficacy was demonstrated. Potential limitations of our study include the relatively small sample size for the group with available results for CMI that may limit the interpretation of the association with $CD4^+$ T-cell response, and the post hoc nature of this analysis that was based on a nonrandomized subset of study participants. Additionally, reporting of pain is intrinsically subjective and may be experienced and reported differently by individuals. A study specifically designed to assess associations between reactogenicity and immunogenicity is needed to confirm the initial observations made here.

A small but statistically significant association was found between the intensity of a frequent side effect (pain) after RZV vaccination and immune responses to vaccination. However, individuals reporting no pain often mounted an immune response that was as good as responses in those reporting pain. In other words, those who do not experience a reaction to vaccination are nonetheless likely to mount a good immune response and be protected. Despite the statistical correlation between the immune response and severity of pain PD2, the impact on the immune response is so small, and the immune response in individuals without pain already sufficient, that pain cannot be a surrogate marker for an appropriate immune response.

In the case of RZV, >90% vaccine immunogenicity and effectiveness has been demonstrated in adults who received 2 doses [3, 4, 8]. Thus, it is essential to provide the second vaccine dose to achieve optimal protection, regardless of the reactions experienced after the first dose (further vaccination is only contraindicated in cases of severe allergic reaction, eg, anaphylaxis, after a previous dose). The results of this study are relevant for healthcare providers managing questions related to reactogenicity and its link with vaccine efficacy.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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