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Predictors of Reactogenicity for an AS03-adjuvanted Avian Influenza A (H5N1) Vaccine

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2017

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An abstract of
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Abstract

Predictors of Reactogenicity for an AS03-adjuvanted Avian Influenza A (H5N1) Vaccine
By Sabrina Rachel Williams

Objectives: To evaluate the determinants of local and systemic reactogenicity following first and second vaccination with an AS03-adjuvanted or non-adjuvanted Influenza A /Indonesia/5/2005 (H5N1) vaccine. **Methods:** The data comes from a Phase II clinical trial, Systems Biology of Influenza A (H5N1) Virus Monovalent Vaccine With and Without Adjuvant System 03 (AS03) (Clinicaltrials.gov identifier NCT01910519) conducted by Emory University and sponsored by the National Institute of Allergy and Infectious Diseases of the NIH in 2013-2014. The dataset contains data on all 50 study participants with epidemiological information collected at baseline, and clinical and immunological data collected on Days 0, 21(±3), 42 (±3) and 100 (±14). Frequency and proportion of individual reactogenicity events were compared across the AS03-adjuvanted and non-adjuvanted groups. Due to significant multicollinearity among the predictors and small sample size, associations between predictors and both local and systemic reactogenicity could not be determined. **Results:** Descriptive data suggests a strong association between H5N1 vaccination with the AS03 adjuvant and the local reactogenicity events, pain and swelling. **Conclusions:** Data from this clinical trial empirically support reactogenicity trends from other clinical trial data, however, conclusions cannot be drawn from these analyses. Further research is needed to understand the relationships between the predictors and reactogenicity outcomes, especially with relation to how an increase in reactogenicity outcomes affect vaccine response and uptake.

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Introduction

Between 2003 and 2017, there have been 858 laboratory-confirmed cases of avian influenza A (H5N1) and 453 deaths reported to the World Health Organization [1]. The majority of these cases are the result of close contact between humans and infected poultry or contaminated environments [1]. While human-to-human transmission with the current H5N1 strain has not been sustained, secondary human cases have occurred in past outbreaks and the increasing potential for reassortment affirms the potential of a pandemic resulting from these strains [2].

In the last 100 years, there have been four pandemics attributable to influenza with the most recent occurring in 2009 and infecting an estimated 60.8 million people in the U.S. alone [3]. While a vaccine was produced for the 2009 pandemic, availability in sufficiently large quantities did not occur until after the peak of viral circulation [4]. In comparing the average case fatalities between the 2009 H1N1 pandemic (~0.02%), outbreaks of H5N1 (~60%), a delay in vaccination will have serious consequences [1, 3]. Of even more concern would be a delay in uptake, even amid a pandemic, due to vaccine side effects. Currently, the U.S., the Biomedical Advanced Research and Development Authority (BARDA) maintains a stockpile and emergency preparedness plans for deployment should a pandemic emerge [5]. The stockpiled vaccines are to be used in conjunction with the AS03-adjuvant to increase vaccine immunogenicity, as a non-adjuvanted vaccine requires at least two doses and high antigenic content [6]. As the purpose of adjuvants is to provoke an immune response, changes in the reactogenicity profiles are to be expected [7].

Secondary data analysis was conducted using data from this clinical trial to examine the association between an AS03-adjuvanted H5N1 vaccine and local and systemic reactogenicity events, as well as associations with other predictors, such as age, sex, race, ethnicity, and immunogenicity.

Methods

Study Design and Participants

The study was designed and conducted as a single site, double-blinded, Phase II clinical trial, whereby healthy, non-pregnant adults, between the ages of 21 and 45, were randomized 2:1 to receive two doses intramuscularly of either an AS03-adjuvanted or a non-adjuvanted Influenza A (H5N1) monovalent vaccine. Given the association between the AS03-A adjuvant and narcolepsy, adults with a family history of sleeping disorders or received a score greater or equal to 11 on the Epworth Sleepiness Scale or had a positive result on the Narcolepsy Mini Screen Questionnaire or tested positive for one of the common allele associated with narcolepsy were excluded [8].

Randomization and appropriate labelling of vaccine doses were conducted by the Emory Investigational Drug Service. An unblinded study nurse, without responsibility relating to any other aspect of the study, administered a 0.5 mL IM dose of the vaccine into the deltoid muscle. Apart from the unblinded nurse, study staff and participants were unaware as to whether or not they received the adjuvanted or non-adjuvanted form of the vaccine.

Prior to the baseline and first vaccination visit (Day 0), demographics (birthdate, sex, race, and ethnicity) were self-reported by participants and recorded by study staff. Blood was also collected at this time for immunological assays. Following vaccination, participants were observed for 15 minutes for immediate hypersensitivity. Following this initial 15 minutes, an MD or other RN examined the injection site. This procedure was repeated at the second vaccination visit, 21 days (±3 days) after the first, including blood collection prior to vaccination for the assays. Additional visits after each study product administration occurred to assess safety; Appendix 1 lists all study visits and clinic procedures according to study protocol. Participants were provided with and given instructions for a memory aid to record injection-site (local) and systemic reactogenicity events for 7 days following both first and second vaccinations. At the additional visits that occurred during this period, study staff corroborated data recorded in the

memory aid, as well as reminded participants about recording events. Local reactogenicity was defined by pain, redness and induration (swelling) at the injection site. Systemic reactogenicity was defined by fatigue, body aches (myalgia), shaking/shivering body movements, nausea, diarrhea, headache, joint pain (arthralgia), and fever. The grading scale was adapted from the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.0 [insert citation]. All measurements of the different grades for each participant for both local and systemic events was subjective, excluding fever (Appendix 2). Participants were provided with an electronic oral thermometer and instructed on best practices to record daily temperature. Serious adverse events and onset of new medical conditions were collected by study staff throughout the entire duration of the study (Appendix 1) and overall health status, especially in regard to the inclusion and exclusion criteria, were reassessed prior to second vaccination.

Study Products

Participants randomized to the adjuvated vaccine group received the GlaxoSmithKline Biologicals' (Rixensart, Belgium) Influenza A (H5N1) Virus Monovalent Vaccine with AS03 adjuvant (also referred to as adjuvanted Q-Pan H5N1), while participants randomized to the alternative group received a vaccine against the same influenza strain, without the AS03 adjuvant. Both groups received vaccine against the A/Indonesia/5/2005 strain, provided by the Biomedical Advanced Research and Development Authority (BARDA) stockpile. The vaccine and AS03 adjuvant system were provided as separate multi-dose vials, which were prepared into pre-filled syringes at the Emory Investigational Drug Service (Atlanta, Georgia).

Data Source

Data for this thesis come from the Systems Biology of Influenza A (H5N1) Virus Monovalent Vaccine with and without AS03 adjuvant (VAX-010) clinical trial (Clinicaltrials.gov identifier NCT01910519) conducted by Emory University and sponsored by The National

Institute of Allergy and Infectious Diseases (NIAID) in 2013-2014. Study staff conducted convenience sampling to screen 99 volunteers and enroll the 50 participants.

Study Variable Measurements

At enrollment, study participants reported their birthdate, gender, race and ethnicity. Age was calculated based on calendar date at first study product administration and birthdate. Blood was collected at each study visit, excluding Days 200 and 400 for immunologic assay or clinical safety testing. ELISA titers from Days 0, 21 (i.e. 21 days following first vaccination and prior to second vaccination), 42 (i.e. 21 days following second vaccination) and 100 were reported in the form of geometric mean optical density (OD), seropositivity (the percent of participants with an ELISA titer ≥ 0.5), and seroconversion (percent of participants with an increase in antibody response by a factor of four from baseline). Between Days 0 and 7, and Days 21 and 28, participants self-reported reactogenicity events (local and systemic) and their associated grades using the memory aid. Events and grades were verified by study staff at visits that fell within this time.

Data Analysis

Descriptive statistics were calculated to compare characteristics, such as age, gender, race and ethnicity of the two randomization groups. Unpaired t-test and Fisher's exact test was used to determine statistically significant differences between them. Descriptive data were produced for reactogenicity and immunogenicity. Reactogenicity data is represented according to study product administration (i.e. first and second vaccinations); study population is dependent on completion of either first dose only, or first and second dose. Immunogenicity data is also dependent on the study population's completion of dosing schedule and acquisition of blood samples. An unpaired t-test with a lognormal distribution was used to compare the OD between the adjuvanted and non-adjuvanted groups, while Fisher's exact test was used to compare the frequencies in seropositivity and seroconversion. Bivariate analyses were conducted to examine

relationships between exposure (vaccination with AS03), demographics, and immunogenicity variables with individual reactogenicity variables divided by dosing schedule (e.g. pain at first vaccination). Bivariate analyses were also conducted with combined local and systemic variables (e.g. any local reaction at first vaccination), based on previous definition of local and systemic.

We assessed all reactogenicity variables independently using multivariate logistic models. Collinearity was then assessed within each model. Due to significant multicollinearity of race with AS03 vaccination, logistic models assessing each reactogenicity variable independently were not appropriate. Because of these issues, we collapsed the available data to assess a composite outcome of experiencing either any local event or any systemic event, after the first or second vaccination. All relationships between the demographic data, vaccination with or without the AS03 adjuvant, immunogenicity variables and reactogenicity variables were investigated and all attempts were made to produce methodologically valid logistic models. All statistical analyses were performed using SAS version 9.4 (Cary, NC), at an alpha level of 0.05.

Ethics

The study was approved by the Emory University Institutional Review Board (IRB) and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice.

Results

Study Subjects

Participants were enrolled, vaccinated twice and recorded their reactogenicity events from July 29, 2013 to February 3, 2014. Of the 50 participants, 35 were randomized to receive two doses of the adjuvanted Q-Pan H5N1 and 15 were randomized to receive two doses of the non-adjuvanted vaccine. Two participants within the adjuvanted group did not get the second dose and were not included in the second vaccination reactogenicity population; ELISA titers were also not collected and therefore, these participants were not included in the immunogenicity population. At a significance level of 0.05, there were not significant differences in demographic characteristics between the two vaccination groups (Table 1).

Reactogenicity Events

Following first vaccination, participants randomized to receive H5N1 vaccination with the AS03 adjuvant system were significantly more likely to experience swelling/induration (31% vs. 0%, p=0.0214) and pain (89% vs. 7%, p-value < 0.0001) compared to their non-adjuvanted counterparts (Figure 1). Of the 50 participants, only two (both randomized to the AS03-adjuvanted group) experienced moderate pain (Grade 2) following vaccination; severity of this event lasted for one day. Among the systemic reactogenicity variables, difference in frequency between the groups was not statistically significant at first vaccination and no events were more severe than a Grade 1, however, fever was more common among non-adjuvanted persons (0% vs. 13%, p-value = 0.0857).

In comparison to reactogenicity events following first vaccination, frequency and severity of both local and systemic events increased among both the adjuvanted and non-adjuvanted groups following second vaccination. Similar to first vaccination, participants randomized to receive the AS03-adjuvanted vaccine were significantly more likely to experience swelling/induration (24% vs. 0%, p = 0.0444) and pain (82% vs. 7%, p < 0.0001) when compared

to participants in the non-adjuvanted group (Figure 2). Severity of grade was more frequent and higher among adjuvanted participants; one participant experienced Grade 3 for pain, five participants experienced Grade 2 for pain, and frequency of Grade 1 fatigue increased from 10 to 15. Duration of these elevated events lasted 1-2 days. For systemic events, there were no significant differences between groups, however, frequency between first and second vaccination increased; among adjuvanted participants, frequency of a Grade 1 headache increased from 3 to 13 and increased from 0 to 4 among the non-adjuvanted group (Table 3).

Antibody Responses

Using a baseline ELISA titer value of 0.5 to indicate sufficient immunogenicity (i.e. seropositivity), participants randomized to the adjuvanted vaccine group were statistically more likely to have seropositive results (97.0%) compared to the non-adjuvanted participants (46.7%) 21 days following the second vaccination ($p = 0.0001$). Twenty-one days following first vaccination, the ELISA OD was numerically above this 0.5 baseline (0.634) for the adjuvanted group, while the geometric mean OD remained below 0.5 (0.366) for the non-adjuvanted group. By Day 100 following first vaccination, 96.8% of the adjuvanted group were seropositive in comparison to 40% of the non-adjuvanted ($p < 0.0001$).

Logistic Regression

Bivariate logistic analyses of each reactogenicity event, regardless of grade, following vaccination indicated that there were no significant associations with the predictors, excluding pain where a significant association was observed. Despite attempts to fit logistic models to the individual reactogenicity events, analysis of model assumptions demonstrated that these were heavily violated, leading to inconclusive results regarding an association between H5N1 vaccination with an adjuvant and any of the reactogenicity variables. An example of this can be seen in Appendix 3. Specifically, there was evidence of significant multicollinearity (VDP > 30 and CI > 0.5) between the adjuvanted vaccination and race and ethnicity. Multiple variations of

removing these variables were attempted, however, this assumption could not be satisfied at the individual reactogenicity event level; the one exclusion is pain, as no collinearity was seen. However, the odds ratio estimates for pain were inflated to an extreme and unlikely number (OR > 999.9, Wald $\chi^2 = 9.89$, p-value = 0.0016). This is most likely the result of the small sample sizes within each group and the small number of outcomes seen at each event following vaccination. The reactogenicity events were also condensed from their individual levels to broader dichotomous variables - any local event following each vaccination and any systemic event – in an attempt, to see if there was any possibility of fitting a model. This resolved the previous issue of multicollinearity, however, estimates of the OR continued to be inflated and not valid. When the analyses were repeated with the immunogenicity data, the same outcomes occurred and relationships between higher ELISA titers and a reactogenicity event could not be statistically assessed. Associations between the events and the predictors, age, sex, race, and ethnicity, were also not significant and similarly, effects could not be determined. Evidence of interaction and confounding could not be evaluated either as a model could not be fit to the data.

Discussion

Although, difficulties with model fitting occurred, the results of these analyses indicate that these were due to the sample size of the initial study. Differences between the randomization groups in the demographics were not significant, as shown in Table 1, and immunogenicity data, presented in Table 2, indicate that the AS03 adjuvant boosted immune response to the influenza A/Indonesia/5/2005 (H5N1) strain. Consequently, it cannot be determined, using this data and logistic regression, that the adjuvant increases the odds of a reactogenicity event. Similarly, it also cannot be determined if higher ELISA titers are correlated with higher frequencies of reactogenicity events and more severe grades. Using the descriptive data, inferences can be made and support current literature regarding the relationship between an adjuvanted H5N1 vaccine and specific reactogenicity events, such as pain, swelling, fatigue, and headache [9-14].

Local and systemic reactogenicity events were common among participants who received the vaccine with the AS03 adjuvant in terms of count and percentage of the group. As shown in Table 3, there were 31 (86%) participants who experienced Grade 1 Pain following first vaccination and 15 (45%) participants who experience Grade 1 Fatigue following second vaccination. Swelling and redness following vaccination occurred among few participants. These results are consistent with the reactogenicity trends observed in the literature. In a 2014 article examining the immunogenicity of an AS03-adjuvanted H5N1 vaccine, the authors reported that pain was one of the most frequent local events and fatigue was one of the most frequent systemic event; differences between their adjuvanted and non-adjuvanted groups were statistically significant with more frequency occurring among those who received an adjuvanted dose [9]. Similar results and conclusions were reported by a 2006 article looking at the safety and immunogenicity of a H5N1 vaccine [10].

Strengths and Limitations

Data used in this study were collected by clinical research staff and the study participants. Randomization occurred outside of the clinic setting and study staff and participants were blinded in regard to the vaccination with or without the AS03 adjuvant, which provided a means to reduce biases in event occurrence and severity reporting. Participants had several visits in the 7 days following first and second study product administration, which allowed for study staff to review best practices for determining event grade, corroborate the appropriate grade for events, and to remind participants about recording the events in their memory aid. However, data related to the reactogenicity measurements were self-reported and subjective, despite a robust classification scheme for determining grade. The only exception to this was fever, which was objectively measured by an oral thermometer. Similarly, reactogenicity events could have been unrelated to the vaccine and adjuvant. As shown in the data analysis and results, development of any logistic model was hindered by the small sample size of the initial study (n=50) and the relatively small frequency of reactogenicity events following each dose of the vaccine in both groups. Even in events (i.e. pain following vaccination) where frequencies of events and severity of grades between groups were observably different, the small sample size caused an unusual and random relationship between the randomized exposure with race, restricting model analysis. As such, associations between AS03 vaccination and each reactogenicity event could not be statistically determined and only descriptive analyses were presented.

Conclusions

Given public health concerns about the H5N1 strain resulting in the next pandemic, use of an adjuvant increases the immunogenicity of the vaccine and leads to more available doses without needing to produce more vaccine. However, without understanding the associations between the side effects of the vaccine with an adjuvant providing more vaccine will not matter unless vaccine uptake is also high. It has been demonstrated in research conducted in the US, Greece, Mexico, and China, using the seasonal flu vaccine or the H1N1 vaccine, that side effects of vaccination are barriers to vaccine uptake [15-19]. In contrast, the increased immunogenicity of an adjuvanted vaccine could have the strength to balance against the negative perceptions of vaccination brought about by the reactogenicity events, especially in a pandemic setting. As clinical trials have demonstrated an increased efficacy due to the AS03 adjuvant, this level of immune protection could override concerns about side effects and promote vaccine uptake rather than diminish it [6, 9-14].

Of the published literature discussing the associations between both local and systemic reactogenicity events and H5N1 vaccination with an AS03 adjuvant, most research examines these as secondary endpoints with the primary research objectives focusing on the effects of an adjuvant on immunogenicity [6, 9-14]. Research on this specific relationship is also outdated with, again, most of these studies based on research from a decade or more ago. There is a gap in the literature regarding reactogenicity and adjuvants. While the results of this statistical analysis led to inconclusive results due to a small sample population, descriptive analysis indicated that there was an increased association among the adjuvanted population. Research beyond this exploratory analysis is needed.

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Tables and Figures

Table 1. Demographic Characteristics of Participants*

	Randomization Group			
	Vaccinated with AS03 (n = 35)	**Vaccinated without AS03 (n = 15)**	**All (n = 50)**	**p-value**
Age, years				0.2982
Mean (SD)	30.3 (6.9)	28.1 (5.5)	29.6 (6.5)	
Median	28.8	26.3	27.3	
Range	21-44	22-40	21-44	
Sex				0.5287
Male (%)	13 (37.1)	7 (46.7)	20 (40)	
Race				0.0623
Caucasian (%)	26 (74.4)	7 (46.7)	33 (66.0)	
African American (%)	7 (20.0)	3 (20.0)	10 (20.0)	
Asian (%)	1 (2.8)	3 (20.0)	4 (8.0)	
Multi-Racial (%)	1 (2.8)	2 (13.3)	3 (6.0)	
Ethnicity				0.2107
Hispanic (%)	1 (2.9)	2 (13.3)	3 (6.0)	

*Demographics were also recorded for adults who were consented, but eventually excluded based on study criteria. For the purposes of this thesis, these were not reported. P-values were calculated using unpaired t-test and Fisher's exact test.

Table 2. Geometric Mean Optical Density (OD) of Antibody against the Influenza A/Indonesia/5/2005 (H5N1) Virus in Participants Receiving Two Intramuscular Doses, as Assessed by ELISA.*

	Randomization Group		p-value
	Vaccination with AS03	Vaccination without AS03	
Before Vaccination			
No. of Subjects	33	15	
OD (95% CI)	0.158 (0.113, 0.220)	0.149 (0.104, 0.212)	0.823
Seropositivity, % (95% CI)	12.1 (0.369, 23.9)	6.67 (0, 21.0)	1.00
21 Days after First Dose			
No. of Subjects	33	15	
OD (95% CI)	0.634 (0.474, 0.848)	0.366 (0.211, 0.633)	0.049
Seropositivity, % (95% CI)	57.6 (39.8, 75.4)	40.0 (11.9, 68.1)	0.353
Seroconversion % (95%CI)	51.5 (33.5, 69.5)	26.7 (1.31, 52.0)	0.129
21 Days after Second Dose			
No. of Subjects	33	15	
OD (95% CI)	1.73 (1.47, 2.05)	0.572 (0.378, 0.865)	<0.0001
Seropositivity, % (95% CI)	97.0 (90.8, 100)	46.7 (18.1, 75.3)	0.0001
Seroconversion, % (95%CI)	84.8 (71.9, 97.8)	53.3 (24.7, 81.9)	0.0312
Day 100 after First Dose			
No. of Subjects	31	15	
OD (95% CI)	1.41 (1.16, 1.71)	0.506 (0.327, 0.784)	<0.0001
Seropositivity, % (95% CI)	96.8 (90.8, 100)	40.0 (11.9, 68.1)	<0.0001
Seroconversion, % (95%CI)	72.7 (56.7, 88.8)	40.0 (11.9, 68.1)	0.0523

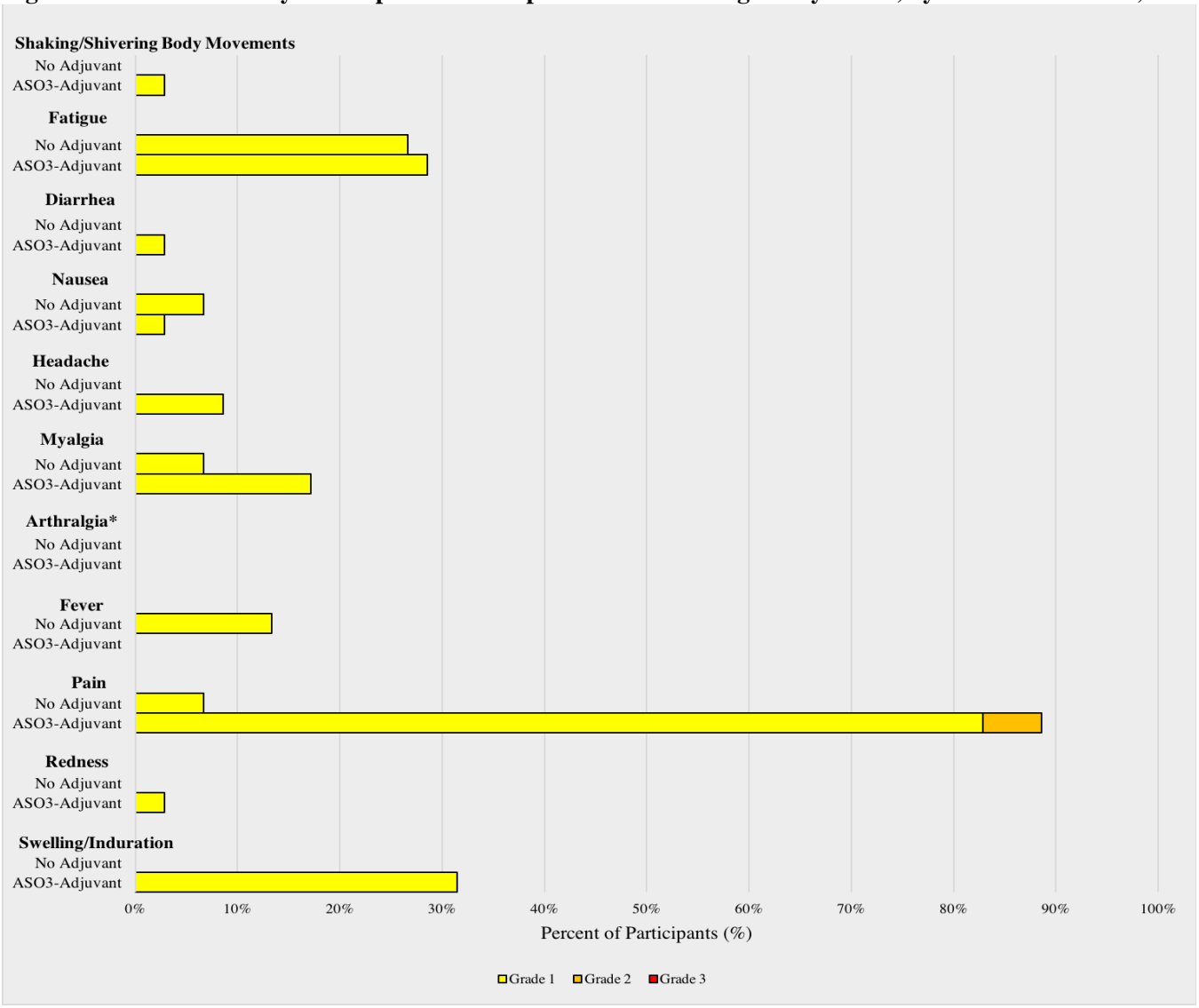
*ODs between groups were compared with the use of an unpaired t-test, using a log-normal distribution, while seropositivity and seroconversion percentages were compared with the use of Fisher's Exact test. Seropositivity was defined as an ELISA titer greater than or equal to 0.5. Seroconversion was defined as an increase in antibody titer by a factor of 4 or more, as compared with the titer before vaccination. CI denotes confidence interval.

Table 3. Reactogenicity Counts by Randomization Group and Grade*

	First Vaccination						Second Vaccination					
	AS03-adjuvanted (n = 35)			Non-adjuvanted (n = 15)			AS03-adjuvanted (n = 33)			Non-adjuvanted (n = 15)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
Induration	11	0	0	0	0	0	8	0	0	0	0	0
Redness	1	0	0	0	0	0	1	0	0	0	0	0
Pain	31	2	0	1	0	0	26	5	1	1	0	0
Fever	0	0	0	2	0	0	1	0	0	1	0	0
Joint Pain (Arthralgia)	0	0	0	0	0	0	5	0	0	0	0	0
Body ache (Myalgia)	6	0	0	1	0	0	9	1	0	2	1	0
Headache	3	0	0	0	0	0	13	1	0	4	1	0
Nausea	1	0	0	1	0	0	2	0	0	1	0	0
Diarrhea	1	0	0	0	0	0	0	0	0	0	0	0
Fatigue	10	0	0	4	0	0	15	0	0	6	2	0
Shaking/Shivering Body Movements	1	0	0	0	0	0	2	1	0	1	0	0

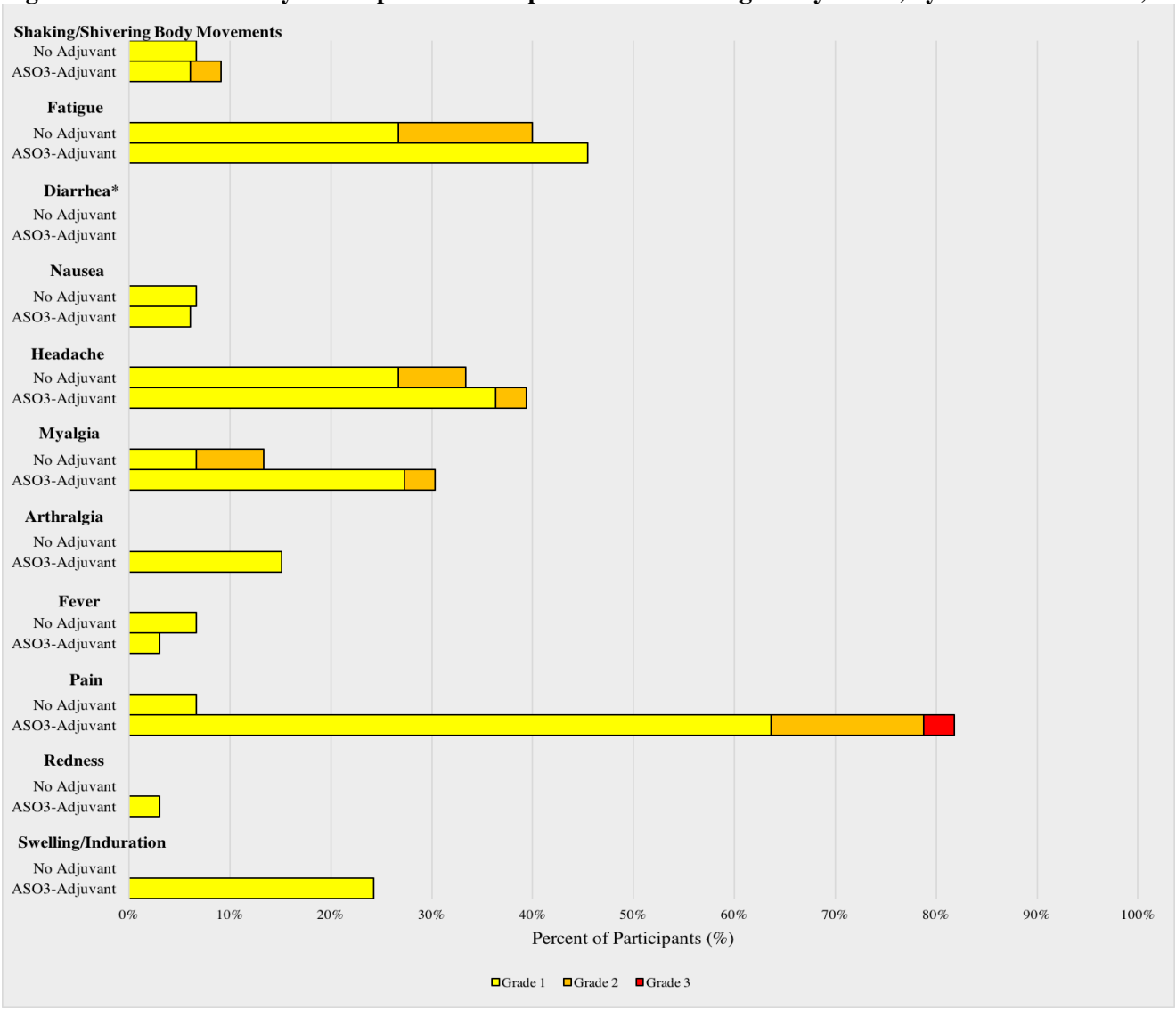
*These counts represent the number of participants who experienced each event over the 7 days following study product administration at each grade. Participants could have experienced multiple grades; each grade is recorded in this table.

Figure 1. Percent of Study Participants who Experienced a Reactogenicity Event, by Maximum Grade, Post First Vaccination



*None of the participants in either group experienced any arthralgia in the 7 days following vaccination.

Figure 2. Percent of Study Participants who Experienced a Reactogenicity Event, by Maximum Grade, Post Second Vaccination



*None of the participants in either group experienced any diarrhea in the 7 days following vaccination.

Appendices

Appendix 1: Schedule of Study Visits

	Day -42 to Day -1	**D0**	**D1**	**D3**	**D7**	**D14**	**D21**	**D22**	**D24**	**D28**	**D35**	**D42**	**D100**	**D200**	**D400**
Window in Days	-42 to -1 from D0				+/- from D0	+/- 2 from D0	+/- 3 from D0			+/- 1 from D21	+/- 2 from D21	+/- 3 from D21	+/- 14 from D0	+/- 14 from D0	+/- 14 from D0
Study Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Informed Consent and HIPAA	X														
PID Assignment	X														
Verify Eligibility	X	X					X								
Demographics/Medical History	X														
Vital Signs	X	X					X								
Targeted Physical Exam	X	X	X	X	X	X	X	X	X	X	X	X	X		
Pregnancy Test*	X	X					X								
Narcolepsy Mini Screen Questionnaire	X	X					X								
Epworth Sleepiness Scale Questionnaire	X	X					X						X		X
Randomization		X													
Vaccination		X					X								
Memory Aid†		X	X	X	X		X	X	X	X					
Injection Site Examination		X	X	X	X		X	X	X	X					
Assessment of AEs and Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Draws	X	X	X	X	X	X	X	X	X	X	X	X	X		

* For females of child-bearing age

† Memory aid was given at time of vaccination (D0 and D21) and was completed by participants. On overlapping study visit days, staff corroborated grading of events for those days.

Appendix 2: Reactogenicity Grading: Injection Site (Local) and General (Systemic)

Injection Site Reactions:

	INJECTION SITE REACTIONS			
	Grade			
	0	**1**	**2**	**3**
Swelling/Induration	None	Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)	Moderate induration, able to slide skin, unable to pinch skin; limiting instrumental activities of daily living	Severe induration, unable to slide or pinch skin; limiting arm movement limiting self-care activities of daily living
Redness	None	Asymptomatic or mild symptoms; intervention not indicated	Moderate; minimal, local; limiting age-appropriate instrumental activities of daily living	Severe but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self-care activities of daily living
Pain	None	Mild	Moderate pain; limiting instrumental activities of daily living	Severe pain; limiting activities of daily living

General Adverse Reactions:

	GENERAL ADVERSE REACTIONS			
	Grade			
	0	**1**	**2**	**3**
Fatigue	None	Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental activities of daily living	Fatigue not relieved by rest, limiting self-care activities of daily living
Body ache (Myalgia)	None	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL
Shivering / Shaking body movements	None	Mild symptoms	Moderate symptoms; limiting instrumental activities of daily living	Severe symptoms; limiting self-care activities of daily living
Nausea	None	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated
Diarrhea	None	Increase of <4 stools per day over baseline;	Increase of 4 - 6 stools per day over baseline;	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated;
Headache	None	Mild pain	Moderate pain; limiting instrumental activities of daily living	Severe pain; limiting self-care activities of daily living
Joint pain (Arthralgia)	None	Mild pain	Moderate pain associated with signs of inflammation; redness or joint swelling; limiting instrumental activities of daily living	Severe pain associated with signs of inflammation, erythema, or joint swelling; irreversible joint damage; disabling; limiting self-care activities of daily living
Fever	None	100.4 - 102.2 degrees F	102.3 - 104.0 degrees F	>104.0 degrees F

Appendix 3. Modeling Strategy for the Relationship between Vaccination with AS03 adjuvant and Swelling after First Vaccination

Data Analysis Method	OR (95% CI)	Wald Test Statistic of β_1 (p-value)	Conclusion
1. Bivariate Analysis (Swelling* = Vaccination with AS03)	>999.9 (<0.001, > 999.9)	0.0029 (p = 0.9571)	No significant association
2. Multicollinearity Assessment a. Including all predictor variables (AS03 vaccination, age, gender, race, ethnicity, ELISA titers)	>999.9 (<0.001, > 999.9)	0.0287 (p = 0.8655)	Significant collinearity (VDP = 883) with race and ethnicity observed; Not a valid estimate
b. Dropping race as a predictor	>999.9 (<0.001, > 999.9)	0.0058 (p = 0.9392)	Significant collinearity (VDP = 1205); Not a valid estimate
c. Dropping ethnicity as a predictor	>999.9 (<0.001, > 999.9)	0.0323 (p = 0.8575)	Significant collinearity (VDP = 833); Not a valid estimate
d. Dropping both race and ethnicity	>999.9 (<0.001, > 999.9)	0.0031 (p = 0.9559)	Significant collinearity (VDP = 1830) Not a valid estimate
3. Bivariate Analysis (Local† = AS03 Vaccination)	149.3 (14.26, > 999.9)	17.45 (p < 0.0001)	Not a valid estimate
4. Multicollinearity Assessment a. Including all predictor variables	>999.9 (9.324, >999.9)	7.909 (p = 0.0049)	No collinearity observed, but not a valid estimate
b. Dropping race as a predictor	333.8 (13.73, >999.9)	12.73 (p = 0.0004)	Not a valid estimate
c. Dropping ethnicity as a predictor	>999.9 (12.44, >999.9)	8.691 (p = 0.0032)	No change in estimate
d. Dropping both race and ethnicity	380.5 (17.70, >999.9)	14.41 (p = 0.0001)	Not a valid estimate

*Swelling was dichomtized from the individual daily grade memory aid to indicate whether or not there was any swelling regardless of grade, following the first vaccination at any time during the 7 days.
† Local is a variable indicating any local reactogenicity (pain, redness, or swelling/induration) during the 7 days following vaccination.