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Evaluation of sex, race, body mass index and pre-vaccination serum progesterone levels and post-vaccination serum anti-anthrax protective immunoglobulin G on injection site adverse events following anthrax vaccine adsorbed (AVA) in the CDC AVA human clinical trial☆

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Abstract

Background—Anthrax vaccine adsorbed (AVA) administered intramuscularly (IM) results in fewer adverse events (AEs) than subcutaneous (SQ) administration. Women experience more AEs than men. Antibody response, female hormones, race, and body mass index (BMI) may contribute to increased frequency of reported injection site AEs.

Methods—We analyzed data from the CDC AVA human clinical trial. This double blind, randomized, placebo controlled trial enrolled 1563 participants and followed them through 8 injections (AVA or placebo) over a period of 42 months. For the trial's vaccinated cohort (n = 1267), we used multivariable logistic regression to model the effects of study group (SQ or IM), sex, race, study site, BMI, age, and post-vaccination serum anti-PA IgG on occurrence of AEs of

☆The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Mention of a product or company name does not constitute endorsement by the CDC.

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Conflicts of interest statement

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any severity grade. Also, in a women-only subset (n = 227), we assessed effect of pre-vaccination serum progesterone level and menstrual phase on AEs.

Results—Participants who received SQ injections had significantly higher proportions of itching, redness, swelling, tenderness and warmth compared to the IM study group after adjusting for other risk factors. The proportions of redness, swelling, tenderness and warmth were all significantly lower in blacks vs. non-black participants. We found arm motion limitation, itching, pain, swelling and tenderness were more likely to occur in participants with the highest anti-PA IgG concentrations. In the SQ study group, redness and swelling were more common for obese participants compared to participants who were not overweight. Females had significantly higher proportions of all AEs compared to males. Menstrual phase was not associated with any AEs.

Conclusions—Female and non-black participants had a higher proportion of AVA associated AEs and higher anti-PA IgG concentrations. Antibody responses to other vaccines may also vary by sex and race. Further studies may provide better understanding for higher proportions of AEs in women and non-black participants.

Keywords

Anthrax vaccine adsorbed; Anthrax vaccines/adverse events; Sex factors; Race; Body mass index; Immunological response

1. Introduction

Anthrax vaccine absorbed (AVA, Biothrax, Emergent Bio-Solutions, Lansing, Michigan), is the only anthrax vaccine licensed in the United States. Originally licensed in 1970, the principal immunogen is protective antigen (PA) and the adjuvant is aluminum hydroxide. The original approved AVA regimen consisted of 0.5 mL SQ injections at 0, 2 and 4 weeks and 6, 12, and 18 months with annual boosters thereafter [1]. Since the introduction of the Department of Defense's (DoD) mandatory anthrax vaccination program in 1998 [2], service personnel and others have raised concerns that subsequently have been addressed in various published studies, including those related to high rates of injection site adverse events, (AEs) particularly in women [3–8]; potential reproductive toxicity [9–11]; physical disability [12–15]; and nonspecific longer term symptoms such as Gulf War and chronic fatigue syndromes [16–19]. In 1999, the US Congress directed the Centers for Disease Control and Prevention (CDC) to evaluate the safety and efficacy of AVA and in particular an apparent sex difference in the occurrence of AEs. A pivotal component of the CDC's Anthrax Vaccine Safety and Efficacy Research Program was the AVA human clinical trial to evaluate route change (SQ to IM) and dose reduction (reduced priming schedule of 0, 4 weeks and 6 months and a biannual/triannual booster) [20]. Participants (n = 1563) received a total of 8 doses of vaccine or saline placebo during 42 months. Following the trial's interim analysis, the FDA revised the licensed schedule to specify IM administration and the exclusion of the two week priming dose [21]; and the Advisory Committee for Immunization Practices (ACIP) revised its recommendations for vaccine use [22,23].

Details of the clinical trial were previously published [24,25]. In summary, changing from SQ to IM administration reduced the frequency of AEs in men and women and substantially

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diminished absolute differences in occurrence of AEs between men and women. We conducted this study to investigate potential risk factors for AEs and the role of the menstrual cycle with regard to injection site reactogenicity in women.

2. Methods

The CDC AVA human clinical trial was a randomized, double-blind, placebo-controlled, Phase 4 study conducted from 2002 to 2005 with participants enrolled and followed at 5 major U.S. vaccine research centers: Baylor College of Medicine, Houston, TX; Emory University School of Medicine, Atlanta, GA; Mayo Clinic, Rochester, MN; University of Alabama at Birmingham; and the Walter Reed Army Institute of Research, Silver Springs, MD. Eligibility requirements included being 18–61 years of age, healthy, having two intact upper arms, indicating a willingness to participate, having no history of anthrax infection or immunization against anthrax and if female, not being pregnant and not planning to be pregnant during the study period.

At each study site, participants were randomly assigned to one of six study groups based on receiving either AVA or saline placebo, route of injection (SQ vs. IM), and full/reduced AVA schedule (full = 0.5 mL doses at 0, 2, and 4 weeks, and 6, 12, 18, 30 and 42 months vs. reduced = substituting one or more injections with placebo doses). The SQ study group, group 8-SQ, received AVA as originally licensed. Group 8-IM received 8 doses of AVA IM. Groups 7-IM, 5-IM, and 4-IM also received AVA IM with saline placebo doses at one or more time points. The sixth group received 8 IM or SQ saline placebo doses and did not receive any AVA (Table 1). We combined participants in the 8-IM, 7-IM, 5-IM, and 4-IM groups into one IM study group. All placebo doses were excluded from analysis.

At enrollment, each participant self-identified his/her age, race, and sex; and clinical staff collected their height and weight measurements. Female trial participants were separately consented to have serum progesterone measured. Each participant's age at baseline was categorized as <30, 30–39, 40–49, and 50+ years of age. Race was categorized as black, white, and other and we grouped the white and other categories as non-black for analysis. Body mass index (BMI) was computed using height and weight at enrollment and each follow up, and categorized using CDC categories: underweight (<18.5), normal (18.5–24.9), overweight (25–29.9), and obese (≥30) [26]. For analysis, the following three BMI categories were used: not overweight (BMI < 25), overweight (25–29.9), and obese (BMI ≥ 30).

Serum progesterone levels were measured for women in the SQ study group and for a subset of women in the IM study group. Since menstrual phases can more easily be determined in the absence of pharmacologically altered hormone levels, we excluded data from any doses when a woman was on pharmacological birth control. There were 227 pre-menopausal, non-pregnant females included in the women-only subset analysis. Pre-menopausal was defined as having self-reported regular menses during the 12-month period prior to enrollment.

Solicited and unsolicited AEs were actively monitored. However, for our study, we limited our analysis to only the solicited injection site AEs: arm motion limitation, bruising, itching,

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pain, redness, swelling, tenderness, and warmth recorded in a diary for up to 5 days. In addition to the diary, participants received a circular ruler to measure the widest diameter, in millimeters, of injection-site bruising, redness, or swelling. Participants were also screened for AEs during scheduled in-clinic examinations that occurred at 15 to 60 min after each injection and two days after each injection. Mild, moderate, or severe AEs were collapsed to create a binary variable indicating the presence or absence of an AE of any severity grade after a given dose.

Blood was drawn from study participants to measure pre and post-vaccination immune response. Total serum anti-protective antigen IgG antibody (anti-PA IgG) was measured using a quantitative enzyme-linked immunosorbent assay (ELISA) as described previously [27]. Concentrations of anti-PA IgG from post-vaccination blood samples, collected four weeks after the third to eighth injections, were calculated and categorized in quartiles. For the women-only subset analysis, serum progesterone levels were measured using the pre-vaccination blood draw. Serum progesterone concentration was assayed using the ADVIA Centaur® Progesterone Assay, which is a competitive immunoassay using direct chemiluminescent technologies [28]. The values of serum progesterone concentration provided by the testing laboratory were 0.2–1.4 and 3.3–26.0 ng/mL for follicular phase and luteal phase, respectively. We used the pre-vaccination progesterone concentration to assign a menstrual phase variable with three categories, follicular, luteal, or ovulation.

2.1. Statistical analysis

Our analysis included data from injections 3 to 8. One of our study objectives was to test for associations between post-injection anti-PA IgG concentrations and AEs. Doses 1 and 2 were excluded from our analysis because the anti-PA IgG concentrations, collected 4 weeks after injection, were at the lower limit of detection for about half of the study population after dose 1 and were not measured after dose 2 (occurred only 2 weeks after dose 1).

We compared the mean pre-injection progesterone concentrations and the mean post-injection anti-PA IgG concentrations, from injections 3 to 8, between study groups using the Wilcoxon rank sum test. We tested for differences in the distribution of risk factors for AEs reported in the patient diary between study groups using chi-square tests for categorical variables.

We modeled the log odds of experiencing an AE, adjusted for BMI category, race, post-injection anti-PA IgG level, sex, study site, age category, and study group using logistic regression and generalized estimating equations with an exchangeable covariance structure to account for repeated measurements within a participant. We tested all possible two way interactions and excluded risk factors and interactions from the model if they did not have a significant type 3 Wald test statistic. Relative risks (RR) were then estimated using the marginal method [29]. To calculate confidence intervals for the RR, we created 1000 bootstrapped data sets from random draws on individual injection data. Separate logistic models were run for each AE.

We performed several sensitivity analyses. We compared the results from the multivariable analysis using data from injections 3 to 8 to the results using data from all doses. We also

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substituted pre-injection IgG concentrations and the change in IgG between the pre-injection and post-injection measurements for post-injection IgG concentration in the multivariable model and compared the results. To assess the accuracy of the diary data we also modeled the risk of AEs as assessed by study nurses during the scheduled in-clinic exams.

3. Results

3.1. Vaccinated cohort—Comparison of participants in SQ and IM study groups

There were 252 participants from the SQ study group and 1015 participants from the IM study group included in our analysis (Table 1). There was no significant difference between the two study groups in race, sex, BMI, or study site (Table 2). However, the age distribution of participants was significantly different between the study groups ($p = 0.02$). The average post-injection anti-PA IgG concentration from injections 3 to 8 was higher in the IM study group (mean = 261.01 (SQ) vs. 325.07 (IM), $p = 0.04$).

3.2. Vaccinated cohort—Multivariable models

Age group was not significantly associated with any AE and thus was not included in our multivariable models. Sex and study group were significantly associated with AEs as expected. Females had significantly higher rates for all AEs (Table 4). Participants who received SQ AVA had significantly higher rates of itching, redness, swelling, tenderness, and warmth than IM study group participants after adjusting for other risk factors.

Arm motion limitation, redness, swelling, tenderness, and warmth were all significantly less frequent in blacks vs. non-black participants. The risk of bruising was higher in blacks in the SQ study group (RR = 1.82, 95% CI = 1.42, 2.33). There was no difference in the risk of bruising between blacks and non-blacks in the IM study group.

Participants in the obese BMI category had a significantly lower rate of arm motion limitation (RR = 0.79, 95% CI = 0.71, 0.91) and a higher rate of warmth (RR = 1.16, 95% CI = 1.04, 1.29) than participants who were not overweight. We modeled the interaction between BMI and study group for five AEs: bruising, itching, redness, swelling, and tenderness. For these AEs, obesity was significantly associated with bruising and itching only in the SQ study group (Table 4). Obese participants were significantly more likely to experience redness, swelling, and tenderness in the SQ study group but significantly less likely to experience the same AE in the IM study group compared to participants who were not overweight.

The quartiles of anti-PA IgG concentration were <95, 95–204.1, 204.1–396.2 and ≥396.2 μg/mL (i.e., quartiles 1–4, respectively). Participants with post-vaccination anti-PA IgG concentrations in the top two quartiles were more likely to experience itching, pain, tenderness, and warmth than those with anti-PA IgG concentrations in the lowest quartile. We modeled an interaction between study group and anti-PA IgG level for redness and swelling. Participants in the IM study group with anti-PA IgG concentrations in the top three quartiles were more likely to experience redness and swelling than those with anti-PA IgG concentrations in the lowest quartile.

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3.3. Women only subset—Comparison of participants in SQ and IM study groups

There were 80 participants from the SQ study group and 147 participants from the IM study group included in the women-only analysis (Table 1). There was no significant difference between the proportions of participants in the two study groups by race, age category, or study site (Table 2). The proportion of participants in the three BMI categories did differ between the two study groups ($p = 0.04$). The average post-injection anti-PA IgG concentration from injections 3 to 8 was higher in the IM study group (262.29 (SQ) vs. 322.52 (IM), $p = 0.01$). The mean progesterone level from AVA injection 3 until the end of the study was borderline significantly higher in the IM study group (3.75 (SQ) vs. 4.22 (IM), $p = 0.06$).

As we found in the analysis with all participants, the proportion of women experiencing bruising, itching, redness, swelling and warmth differed significantly across the two study groups with higher proportions of AEs in the SQ study group ($p < 0.01$ for all 5 AEs). However, the proportions of women with arm motion limitation, pain, and tenderness were similar across study groups (Table 3).

3.4. Women only subset—Multivariable models

Relative risks and 95% confidence intervals for the repeated measures analysis are shown in Table 5. As before, age was not included in the multivariable models. After adjusting for other risk factors, menstrual phase was not significantly associated with any AE. Black women were significantly less likely than non-black women to experience redness, swelling, tenderness, and warmth. Interactions between study group and BMI remained significant in the models for redness, swelling, and warmth. Obese women who received SQ AVA were significantly more likely to experience redness, swelling, and warmth compared to women who were not overweight (Table 5). The quartiles of anti-PA IgG concentration in the women-only subset analysis were <122.4, 122.4–215.4, 215.4–390.5 and ≥390.5. Women with higher anti-PA IgG concentrations were more likely to experience arm motion limitation, itching, pain, swelling, and tenderness. Women in the IM group were significantly more likely to experience warmth if their anti-PA IgG level was in the highest quartile.

4. Discussion

Our results for several AEs were consistent in our overall study population and in the women-only subgroup. Redness, swelling, tenderness, and warmth were less likely for blacks compared to non-blacks in all analyses. In all analyses, arm motion limitation, itching, pain, and tenderness were more likely to occur in participants with the highest anti-PA IgG concentrations. Redness increased with increasing anti-PA IgG only in the IM group of all analyses. All analyses indicated that redness and swelling were more likely in the obese SQ study group participants. SQ injection and elevated post-injection anti-PA IgG concentration were independent risk factors for several AEs. In addition, although participants in the SQ study group had higher proportions of most AEs compared with the IM study group, they had lower anti-PA IgG concentrations after injections 3 to 8.

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A potential limitation of our study was the use of the diary data. This may have introduced confounding if there were differences in the likelihood of participants noting and/or reporting AE and this was associated with sex, racial category, or BMI category. Based on our comparison of diary data to in-clinic data, we do not believe that the subjective nature of diary data biased our results. We found similar results when comparing the risk of AEs that were measured in-clinic to the risk of AEs recorded in the diary data (data not shown).

A 2002 study by Zhang et al. evaluated BMI, serum progesterone and anti-PA IgG on AEs following AVA in women in the CDC Anthrax Vaccination Program (AVP) [30]. The women in that study received SQ AVA according to the original 6-dose subcutaneous schedule, also pre-vaccination blood samples for serum anti-PA IgG level and serum progesterone were tested and data on solicited AEs were obtained using a self-completed four day diary. These investigators found an elevated risk for arm soreness in obese women, an association between decreased pre-vaccination serum progesterone level and arm swelling, and an association between increased pre-vaccination anti-PA IgG and itching on the arm. There was also an association in obese women between increased pre-vaccination anti-PA IgG and redness, swelling, warmth, and presence of a “lump or knot” at the injection site. In comparison, our analysis found a significant association between increased post-vaccination anti-PA IgG and itching (RR = 1.71, 95% CI = 1.38, 2.15). We found that, in the SQ study group, obese women had a higher risk of redness, swelling, and warmth compared to women who were not overweight. Lump or knot was not an AE included in our study. Soreness was not included in our study but we used tenderness as a comparison. Although we did not find associations between tenderness and obesity in women, we did find increased tenderness in obese participants in the SQ study group of the combined analysis (RR = 1.08, 95% CI = 1.01, 1.17). We did not find associations between serum progesterone concentration and arm swelling.

Our study included 80 women who received SQ AVA compared with 128 such women studied by Zhang et al. We did see higher rates of arm swelling in women with decreased serum progesterone levels but this association was not statistically significant (RR = 1.05, 95% CI = 0.95, 1.19). The small sample of women in the SQ study group might be another limitation of our study. Moreover, our analysis only included data from doses 3 to 8. We performed a sensitivity analysis (results not shown) including doses 1 to 8. When all doses were included in the model, our results were similar for all risk factors except serum anti-PA IgG concentration. The risk of pain for participants with anti-PA IgG concentrations in the highest quartile was slightly lower than the risk in the lowest quartile but the association between pain and anti-PA IgG was not significant. The associations between anti-PA IgG and other risk factors were either more significant or unchanged.

Our repeated measures analysis included post-vaccination anti-PA IgG as a risk factor. We performed sensitivity analyses including pre-vaccination anti-PA IgG instead of post-vaccination anti-PA IgG in the multivariable model. For 7 of the 8 AEs we found weaker association between pre-vaccination anti-PA IgG and AEs than the associations between post-vaccination anti-PA IgG and AEs. However, participants with higher pre-vaccination anti-PA IgG had less pain than participants with lower concentrations (results not shown). An additional sensitivity analysis replaced post-vaccination anti-PA IgG with the difference

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between pre- and post-vaccination as a risk factor in the multivariable analysis. Our results were similar whether we included post vaccination serum anti-PA IgG or the difference between pre- and post-vaccination serum anti-PA IgG as a risk factor for AEs (results not shown).

Differences in anti-PA IgG concentrations (i.e., lower anti-PA IgG) may explain the lower rate of AEs in black participants. The trial's main analysis found that four weeks after the third injection anti-PA IgG concentrations for white participants were significantly higher than those of black participants [25]. We compared serum anti-PA IgG concentrations between black and non-black participants and found that the mean post injection anti-PA IgG concentration from injections 3 to 8 for black participants was lower than the mean concentration in non-blacks (224.2 vs. 235.2, $p < 0.01$). The mean pre-injection serum anti-PA IgG level was also significantly lower in black vs. non-black participants (24.7 vs. 34.4, $p < 0.01$). In a phase 3 trial of two yellow fever vaccines, whites had higher antibody responses than blacks and Hispanics [31]. In addition, a study of acellular and whole cell pertussis vaccines among infants found a difference but in the opposite direction. Black infants had post-immunization geometric mean titers for every antigen in these vaccines which were twice as high as for white infants. Black infants also had more pain after receiving either pertussis vaccine [32].

Sex-specific differences in innate, humoral and cell-mediated immune responses to vaccination have been reported [33–35]. In addition, sex differences in the frequency and severity of adverse events (fever, pain, inflammation) following immunization have been noted for several vaccines including AVA [6,8], influenza [36,37], and measles mumps and rubella (MMR) [38,39]. In the pregnant woman, coincident with marked hormonal changes, there is a shift toward an anti-inflammatory phenotype which also likely influences cell-mediated and humoral responses to vaccines [35]. Although precise biological mechanisms underlying the sex-specific responses to vaccines are unknown, genetic and hormonal factors are considered important [35]. Sex hormones have also been associated with a higher susceptibility to autoimmune diseases in women compared with men [40–42]. We hypothesize that hormonal phase may impact the occurrence of AEs in women. Estrogen has been shown to enhance the secretion of IgG [43]. As serum progesterone levels fluctuate more widely than serum estrogen during a woman's menstrual cycle, we estimated menstrual phase based on serum progesterone levels. Because estrogen levels are highest during the follicular phase of the menstrual cycle [44], we hypothesized that we would see more AEs in women whose progesterone levels indicated that they were in the follicular phase, but we did not find this association.

In conclusion, we found female and non-black participants had higher anti-PA IgG concentrations and a higher incidence of AVA associated injection site AEs. In future analyses, we propose to study a possible effect of sex on AEs after placebo saline injections. Further studies of sex and racial differences in the occurrence of AEs with AVA and other vaccines should be considered.

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References

1. Bioport Corporation. Anthrax Vaccine Adsorbed (BIOTHRAX™) Package Insert. Lansing, MI: Bioport Corporation (Manufactured by); 2002. US Lic. No. 1260
2. Cohen, WA. Implementation of the Anthrax Vaccination Program for the Total Force. The Secretary of Defense; 1998. (http://www.vaccines.mil/documents/902implementationpolicy.pdf) (accessed June 12, 2013)
3. Sever JL, Brenner AI, Gale AD, Lyle JM, Moulton LH, Ward BJ, et al. Safety of anthrax vaccine: an expanded review and evaluation of adverse events reported to the Vaccine Adverse Event Reporting System (VAERS). Pharmacoepidemiol Drug Saf. 2004; 13(12):825–40. [PubMed: 15386719]
4. Lange JL, Lesikar SE, Rubertone MV, Brundage JF. Comprehensive systematic surveillance for adverse events of anthrax vaccine adsorbed, *US Armed Forces* 1998–2000. Vaccine. 2003; 21(15): 1620–8. [PubMed: 12639483]
5. Committee to Assess the Safety and Efficacy of the Anthrax Vaccine, Medical Follow-up Agency. The anthrax vaccine Is it safe? Does it work?. Washington, DC: Institute of Medicine, National Academy Press; 2002.
6. Pittman PR, Gibbs PH, Cannon TL, Friedlander AM. Anthrax vaccine: short-term safety experience in humans. Vaccine. 2001; 20(5–6):972–8. [PubMed: 11738765]
7. Pittman PR, Kim-Ahn G, Pifat DY, Coonan K, Gibbs P, Little S, et al. Anthrax vaccine: immunogenicity and safety of a dose-reduction, route-change comparison study in humans. Vaccine. 2002; 20(9–10):1412–20. [PubMed: 11818160]
8. Hoffman K, Costello C, Menich M, Grabenstein JD, Engler RJ. Using a structured medical note for determining the safety profile of anthrax vaccine for US soldiers in Korea. Vaccine. 2003; 21(27): 4399–409. [PubMed: 14505923]
9. Ryan MA, Smith TC, Sevick CJ, Honner WK, Loach RA, Moore CA, et al. Birth defects among infants born to women who received anthrax vaccine in pregnancy. Am J Epidemiol. 2008; 168(4): 434–42. [PubMed: 18599489]
10. Weisen AR, Littell CT. Relationship between prepregnancy anthrax vaccination and pregnancy and birth outcomes among US Army women. JAMA. 2002; 287(12):1556–60. [PubMed: 11911758]
11. Catherino WH, Levi AK, Kao TC, Leondires MP, McKeeby J, Segars JH. Anthrax vaccine does not affect semen parameters, embryo quality, or pregnancy outcome in couples with a vaccinated male military service member. Fertil Steril. 2005; 83(2):480–3. [PubMed: 15705399]
12. Sulsky SI, Grabenstein JD, Delbos RG. Disability among US Army personnel vaccinated against anthrax. J Occup Environ Med. 2004; 46(10):1065–75. [PubMed: 15602181]
13. Sulsky SI, Luippold RS, Garman P, Hughes H, Amoroso PJ. Risk of disability for US army personnel vaccinated against anthrax, 1998–2005. Vaccine. 2011; 29(35):6035–41. [PubMed: 21704102]
14. Sulsky SI, Luippold R, Garman P, Hughes H, Boyko EJ, Maynard C, et al. Disability among US Army veterans vaccinated against anthrax. Vaccine. 2012; 30(43):6150–6. [PubMed: 22874851]
15. Downing J, Greig TW, Quattlebaum MD, Valentin M, Heeren TC, Grabenstein JD. Assessing the safety of anthrax immunization in US Army aircrew members via physical examination. J Occup Environ Med. 2007; 49(10):1079–85. [PubMed: 18000413]
16. Shoenfeld Y, Agmon-Levin N. 'ASIA'—autoimmune/inflammatory syndrome induced by adjuvants. J Autoimmun. 2011; 36(1):4–8. [PubMed: 20708902]
17. Rook G, Zumla A. Gulf War syndrome: is it due to a systemic shift in cytokine balance towards a Th2 profile? Lancet. 1997; 349(9068):1831–3. [PubMed: 9269228]
18. Nicolson GL, Marwan YN, Nicolson NL, Haier J. High prevalence of mycoplasmal infection in symptomatic (chronic fatigue syndrome) family members of mycoplasma-positive Gulf War Illness patients. J Chronic Fatigue Syndr. 2003; 11(2):21–36.

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19. Appel S, Chapman J, Shoenfeld Y. Infection and vaccination in chronic fatigue syndrome: myth or reality? Autoimmunity. 2007; 40(1):48–53. [PubMed: 17364497]
20. Committee to Assess the Safety and Efficacy of the Anthrax Vaccine, Medical Follow-up Agency. An Assessment of the CDC Anthrax Vaccine Safety and Efficacy Research Program. Washington, DC: Institute of Medicine, National Academy Press; 2002.
21. Sun, W. Biologics license application supplement approval letter. Food and Drug Administration; 2008. (http://www.fda.gov/biologicsbloodvaccines/vaccines/approvedproducts/ucm124462.htm) (accessed June 12, 2013)
22. CDC. Advisory Committee on Immunization Practices (ACIP) meeting minutes archive. n.d. <http://www.cdc.gov/vaccines/acip/meetings/downloads/min-archive/min-feb09.pdf>.(accessed June 12, 2013)
23. CDC. Use of Anthrax Vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) 2009. MMWR Recomm Rep. 2010; 59:1–30.
24. Marano N, Plikaytis BD, Martin SW, Rose C, Semenova VA, Martin SK, et al. Effects of a reduced dose schedule and intramuscular administration of anthrax vaccine adsorbed on immunogenicity and safety at 7 months. JAMA. 2008; 300(13):1532–43. [PubMed: 18827210]
25. Wright JG, Plikaytis BD, Rose CE, Parker SD, Babcock J, Keitel W, et al. Effect of reduced dose schedules and intramuscular injection of anthrax vaccine adsorbed on immunological response and safety profile: a randomized trial. Vaccine. 2014; 32(8):1019–28. [PubMed: 24373307]
26. CDC. About BMI for Adults, Centers for Disease Control and Prevention. n.d. (http://www.cdc.gov/healthyweight/assessing/bmi/adultbmi/index.html) (accessed January 25, 2013)
27. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. Emerg Infect Dis. 2002; 8(10):1103–10. [PubMed: 12396924]
28. ADVIA. Centaur, ReadyPack and ACS 180. Malvern, PA: Siemens Medical Solutions USA, Inc; 1998.
29. Localio RA, Margolis DJ, Berlin JA. Relative risks and confidence intervals were easily computed indirectly from multivariable logistic regression. J Clin Epidemiol. 2007; 60(9):874–82. [PubMed: 17689803]
30. Zhang Y, Martin SW, Rose CE, Biagini RE, Franzke LH, Smith JP, et al. Evaluation of body mass index, pre-vaccination serum progesterone levels and anti-anthrax protective antigen immunoglobulin G on injection site adverse events following anthrax vaccination in women. Pharmacoepidemiol Drug Saf. 2008; 17(11):1060–7. [PubMed: 18781705]
31. Monath TP, Nichols R, Archambault WT, Moore L, Marchesani R, Tian J, et al. Comparative safety and immunogenicity of two yellow fever 17d vaccines (Arilvax and YF-Vax) in a phase 3 multicenter, double-blind clinical trial. Am J Trop Med Hyg. 2002; 66(5):533–41. [PubMed: 12201587]
32. Christy C, Pichichero ME, Reed GF, Decker MD, Anderson EL, Rennels MB, et al. Effect of gender, race, and parental education on immunogenicity and reported reactogenicity of acellular and whole-cell pertussis vaccines. Pediatrics. 1995; 96(3):584–7. [PubMed: 7659481]
33. Cook IF. Sexual dimorphism of humoral immunity with human vaccines. Vaccine. 2008; 26(29): 3551–5. [PubMed: 18524433]
34. Cook IF. Sex differences in injection site reactions with human vaccines. Hum Vaccin. 2009; 5(7): 441–9. [PubMed: 19377279]
35. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis. 2010; 10(5):338–49. [PubMed: 20417416]
36. Engler RJ, Nelson MR, Klote MM, VanRaden MJ, Huang CY, Cox NJ, et al. Half-vs full-dose trivalent inactivated influenza vaccine (2004–2005): age, dose, and sex effects on immune responses. Arch Intern Med. 2008; 168(22):2405–14. [PubMed: 19064822]
37. Nichol KL, Margolis KL, Lind A, Murdoch M, McFadden R, Hauge M, et al. Side effects associated with influenza vaccination in healthy working adults: a randomized, placebo-controlled trial. Arch Intern Med. 1996; 156(14):1546–50. [PubMed: 8687262]

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38. Khalil MK, Al-Mazrou YY, Al-Ghamdi YS, Tumsah S, Al-Jeffri M, Meshkhas A. Effect of gender on reporting of MMR adverse events in Saudi Arabia. East Mediterr Health J. 2003; 9(1–2):152–8. [PubMed: 15562745]

39. Benjamin CM, Chew GC, Silman AJ. Joint and limb symptoms in children after immunisation with measles, mumps, and rubella vaccine. BMJ. 1992; 304(6834):1075–8. [PubMed: 1586818]

40. Pennell LM, Galligan CL, Fish EN. Sex affects immunity. J Autoimmun. 2012; 38(2):J282–91. [PubMed: 22225601]

41. Verthelyi D. Sex hormones as immunomodulators in health and disease. Int Immunopharmacol. 2001; 1(6):983–93. [PubMed: 11407317]

42. Ahmed SA, Hissong BD, Verthelyi D, Donner K, Becker K, Karpuzoglu-Sahin E. Gender and risk of autoimmune disease: possible role of estrogenic compounds. Environ Health Perspect. 1999; 107(Suppl 5):681–6.

43. Lahita RG. Sex hormones and the immune system—Part 1. Human data. Baillière's Clin Rheumatol. 1990; 4(1):1–12.

44. Stricker R, Eberhart R, Chevailler M, Quinn FA, Bischof P, Stricker R. Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT® analyzer. Clin Chem Lab Med. 2006; 44(7):883–7. [PubMed: 16776638]

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Table 1

Number of participants in vaccinated cohort and women-only subset by AVA trial study group.

Group	Vaccinated cohort		Women only subset	
	Total	Included[a]	Female	Included[a,b]
8-SQ AVA	259	252	134	80
8-IM AVA	262	256	135	71
7-IM AVA	256	250	132	76
5-IM AVA	258	249	0	0
4-IM AVA	268	260	0	0
0-SQ Placebo	127	0	64	0
0-IM Placebo	133	0	68	0
Total	1563	1267	533	227

[a] Doses 3–8.

[b] Post-menopausal, surgically sterile, and women on pharmacologic birth control were excluded.

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Table 2

Comparison of risk factors between SQ and IM study groups.

Risk factor		All participants (*n* = 1267)			Females (*n* = 227)		
		SQ (*n* = 252) (%)	IM (*n* = 1015) (%)	*p*-Value[a]	SQ (*n* = 80) (%)	IM (*n* = 147) (%)	*p*-Value[a]
Race	White	77.4	74.7	0.58	80.0	70.7	0.10
	Black	17.5	20.4		18.8	21.8	
	Other	05.2	04.9		01.2	07.5	
BMI	<25	38.1	34.4	0.50	51.3	37.4	0.04
	25–30	32.1	35.3		15.0	29.3	
	30+	29.8	30.3		33.7	33.3	
Age group (years)	<30	30.2	26.9	0.02	27.5	29.3	0.07
	30–39	15.9	24.7		16.3	30.6	
	40–49	34.9	28.6		46.2	33.3	
	50+	19.0	19.8		10.0	06.8	
Site	A	21.0	21.2	1.00	18.8	18.4	1.00
	B	13.9	13.9		16.2	15.6	
	C	19.1	19.4		22.5	21.8	
	D	21.8	21.8		13.8	15.0	
	E	24.2	23.7		28.7	29.3	
Anti-PA IgG	Mean	261.01	325.07	0.04[b]	262.29	322.52	0.01[b]
	Median	192.70	209.60		194.95	239.80	
Sex	Female	52.0	51.9	0.99			
	Male	48.0	48.1				
Progesterone	Mean				3.75	4.22	0.09[b]
	Median				0.89	1.10	

[a]Chi-squared.

[b]Wilcoxon rank sum test.

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Table 3

Proportion of participants experiencing adverse event.

AE	Subset/cohort	SQ		IM		*p*-Value[a]
		n	%	*n*	%	
Arm motion limitation	Female	39/80	48.7	78/146	53.4	0.50
	All	110/252	43.7	542/1015	53.4	0.01
	Female	33/80	41.2	30/146	20.5	<0.01
Bruising	All	110/252	43.7	203/1015	20.0	<0.01
	Female	60/80	75.0	58/146	39.7	<0.01
Itching	All	171/252	67.9	294/1015	29.0	<0.01
	Female	61/80	76.3	106/146	72.6	0.55
Pain	All	187/252	74.2	749/1015	73.8	0.89
	Female	66/80	82.5	78/146	53.4	<0.01
Redness	All	207/252	82.1	463/1015	45.6	<0.01
	Female	73/80	91.3	91/146	62.3	<0.01
Swelling	All	219/252	86.9	544/1015	53.6	<0.01
	Female	74/80	92.5	125/146	85.6	0.13
Tenderness	All	226/252	89.7	856/1015	84.3	0.03
	Female	64/80	80.0	61/146	41.8	<0.01
Warmth	All	192/252	76.2	376/1015	37.0	<0.01

[a]Chi-squared.

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Table 4

Multivariable modeling results: RR and 95% CI for all participants adjusted for BMI category, race, post-injection anti-PA IgG level, sex, study site, and study group.

AE	BMI: ≥30 vs. <25	Black vs. non-black	IgG: quartile 4 vs. 1	Female vs. male	SQ vs. IM
Arm motion limitation	0.79 (0.71,0.91)	0.86 (0.75,0.96)	1.38 (1.19,1.56)	1.69 (1.55,1.85)	0.66 (0.58,0.73)
Bruising	2.07 (1.60,2.98)[a]	1.82 (1.42,2.33)[a]	1.52 (1.20,1.96)	1.91 (1.60,2.29)	1.41 (0.96,1.89)
Itching	1.27 (1.08,1.40)[a]	1.11 (0.99,1.25)	2.55 (2.09,2.90)	1.73 (1.58,1.91)	3.14 (2.75.3.61)
Pain	0.96 (0.88,1.04)	0.96 (0.88,1.03)	1.22 (1.11,1.33)	1.27 (1.20,1.35)	0.91 (0.85,0.97)
Redness	1.37 (1.23,1.49)[a]	0.65 (0.57,0.72)	2.50 (2.05,2.90)[b]	1.52 (1.41,1.61)	2.45 (2.11,2.89)
Swelling	1.27 (1.17,1.37)[a]	0.83 (0.76,0.89)	2.30 (1.96,2.61)[b]	1.50 (1.41,1.58)	2.19 (1.92,2.52)
Tenderness	1.08 (1.01,1.17)[a]	0.72 (0.68,0.76)	1.13 (1.06,1.19)	1.26 (1.21,1.30)	1.08 (1.01,1.15)
Warmth	1.16 (1.04,1.29)	0.67 (0.59,0.75)	1.84 (1.58,2.05)	1.61(1.49,1.73)	2.70 (2.52,2.88)

RR were calculated from logistic regression models. Log binomial models would not converge for several AEs.

[a]Significant interaction between study group and risk factor. Results shown are for SQ study group only.

[b]Significant interaction between study group and risk factor. Results shown are for IM study group only.

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Table 5

Multivariable modelling results: RR and 95% CI for women-only subset adjusted for BMI category, race, post-injection anti-PA IgG level, menstrual phase, study site, and study group.

AE	BMI: ≥30 vs. <25	Black vs. non-black	IgG: quartile 4 vs. 1	Follicular vs. luteal	SQ vs. IM
Arm motion limitation	0.85 (0.65,1.12)	1.07 (0.81,1.34)	1.53 (1.10,2.12)	1.16 (0.94,1.45)	0.85 (0.69,1.04)
Bruising	0.98 (0.60,1.64)	2.85 (1.79,4.50)[a]	1.30 (0.83,2.17)	1.03 (0.76,1.68)	1.92 (1.26,2.92)
Itching	1.29 (1.00,1.51)	0.41 (0.19,0.69)[b]	1.71 (1.38,2.15)	1.11 (0.97,1.34)	2.72 (2.25.3.31)
Pain	1.01 (0.83,1.21)	0.95 (0.77,1.12)	1.46 (1.22,1.93)	1.08 (0.92,1.27)	1.08 (0.93,1.24)
Redness	1.38 (1.22,1.58)[a]	0.55 (0.43,0.68)	1.64 (1.24,2.31)[b]	0.95 (0.84,1.11)	2.57 (1.95,3.51)
Swelling	1.36 (1.22,1.54)[a]	0.79 (0.68,0.93)	1.34 (1.13,1.55)	1.05 (0.95,1.19)	1.75 (1.50,2.05)
Tenderness	0.96 (0.88,1.07)	0.80 (0.70,0.89)	1.21 (1.06,1.33)	1.00 (0.93,1.08)	1.25 (1.16,1.33)
Warmth	1.22 (1.06,1.38)[a]	0.62 (0.48,0.78)	1.68 (1.07,2.64)[b]	1.00 (0.86,1.16)	3.28 (2.41,4.22)

RR were calculated from logistic regression models. Log binomial models would not converge for several AEs.

[a]Significant interaction between study group and risk factor. Results shown are for SQ study group only.

[b]Significant interaction between study group and risk factor. Results shown are for IM study group only.