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Evaluation of anthrax vaccine safety in 18 to 20 year olds: A first step towards age de-escalation studies in adolescents☆

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Abstract

Background/objectives: Anthrax vaccine adsorbed (AVA, BioThrax®) is recommended for post-exposure prophylaxis administration for the US population in response to large-scale *Bacillus anthracis* spore exposure. However, no information exists on AVA use in children and ethical barriers exist to performing pre-event pediatric AVA studies. A Presidential Ethics Commission proposed a potential pathway for such studies utilizing an age de-escalation process comparing safety and immunogenicity data from 18 to 20 year-olds to older adults and if acceptable

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Contributors' statement

Drs. James King and Eric Espeland conceptualized and designed the study, drafted the initial manuscript and approved the final manuscript as submitted.

Dr. Yonghong Gao created and wrote the statistical analyses plan at the inception, performed the analyses reported in the manuscript, reviewed and revised the manuscript and approved the final manuscript as submitted.

Drs. Conrad P. Quinn and Thomas M. Dreier were the primary subject matter experts on anthrax and anthrax vaccines, provided invaluable perspectives on the study design, reviewed the manuscript and approved the final manuscript as submitted.

Ms. Cabrini Vianney provided the background research and specific manuscript writing for the ethical issues of medical countermeasure research in children for the introduction and discussion, reviewed and commented on the multiple drafts of the manuscript and approved the final manuscript as submitted.

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proceeding to evaluations in younger adolescents. We conducted exploratory summary re-analyses of existing databases from 18 to 20 year-olds (n = 74) compared to adults aged 21 to 29 years (n = 243) who participated in four previous US government funded AVA studies.

Methods: Data extracted from studies included elicited local injection-site and systemic adverse events (AEs) following AVA doses given subcutaneously at 0, 2, and 4 weeks. Additionally, proportions of subjects with ≥4-fold antibody rises from baseline to post-second and post-third AVA doses (seroresponse) were obtained.

Results: Rates of any elicited local AEs were not significantly different between younger and older age groups for local events (79.2% vs. 83.8%, P = 0.120) or systemic events (45.4% vs. 50.5%, P = 0.188). Robust and similar proportions of seroresponses to vaccination were observed in both age groups.

Conclusions: AVA was safe and immunogenic in 18 to 20 year-olds compared to 21 to 29 year-olds. These results provide initial information to anthrax and pediatric specialists if AVA studies in adolescents are required.

Keywords

Pediatric medical countermeasure research; Anthrax vaccine; Presidential Ethics Committee; Children

1. Introduction

Bioterrorist attacks using spores derived from *Bacillus anthracis* have been identified as a high priority threat by the United States (US) Department of Homeland Security [1]. This issue was highlighted by bioterrorism-related cases of anthrax illnesses after envelopes containing spores of this organism were sent through the US mail [2]. Accordingly, the US Department of Health and Human Services (HHS) has been charged to address preparedness for such attacks. This preparedness includes providing guidance on the use of post-exposure prophylaxis (PEP) using anthrax vaccine and antibiotics [2]. Anthrax Vaccine Adsorbed (AVA, BioThrax®) manufactured by Emergent BioSolutions Incorporated, was licensed in the US in 1970 for prevention of anthrax in adults aged 18 to 65 years. However, children and pregnant women are special populations for its use [3,4]. AVA is prepared from sterile culture filtrates of the toxigenic, nonencapsulated *B. anthracis* V770-NP1-R grown in a protein-free medium. The final product formulation contains aluminum hydroxide, sodium chloride, benzethonium chloride and formaldehyde [3]. The primary immunogen in AVA is anthrax toxin protective antigen (PA). Anti-PA IgG antibodies are considered to protect against anthrax by neutralizing the *B. anthracis* toxins, inhibiting spore germination, and enhancing phagocytosis and killing of spores by macrophages [5–13].

The current US Advisory Committee on Immunization Practices recommendation for PEP use of AVA is subcutaneous (SC) administration of three doses at 0, 2 and 4 weeks to be initiated within 10 days following an anthrax event [14]. The safety profile of AVA in adults 18–65 years of age is well established [15–22]. There is however, a paucity of data on AVA safety and immunogenicity in special populations, and none in children [23].

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In 2011, a HHS interagency tabletop exercise, designated *Dark Zephyr*, was conducted to simulate an anthrax emergency [24]. During this exercise, it was estimated that up to 7.6 million people, of which approximately 25% would be children, could be exposed to *B. anthracis* spores [24]. If such a large-scale event actually happened, the absence of safety and immunogenicity data of AVA in pediatrics may result in concerns about the administration of this vaccine to individuals less than 18 years of age, a situation that could possibly deny children a potentially life-saving prophylactic countermeasure.

During the fall of 2011, the National Biodefense Science Board, now known as the National Preparedness and Response Science Board (NPRSB) was charged with assessing challenges in the use of AVA in the pediatric population in case of a large-scale anthrax emergency [24]. The NPRSB recognized that, in case of mass exposure of a population to *B. anthracis* spores, a FDA approved research investigational new drug protocol would allow the administration of AVA to children using a PEP regimen. However, this effort would require a research team to collect safety and immunogenicity data from these children after each AVA dose during this mass vaccination event. Consequently, the NPRSB noted that this type of post-event evaluation would pose major challenges to first responders, parents and research personnel in terms of mass vaccination of children during a large-scale anthrax spore exposure. Therefore, the NPRSB panel concluded that “HHS should develop a plan for and conduct a pre-event study of AVA in children, to include a research IND. HHS should submit a study protocol to one or more institutional review boards, and comply with the 21 CFR 50.54/45, CFR 46.407 federal review process." [24].

In response to the NPRSB report, the Secretary of HHS requested that the Chair of the Presidential Commission for the Study of Bioethical Issues convene a panel to review the ethical considerations of conducting clinical research studies of medical countermeasures in children. The Secretary went further to ask this panel to specifically include the ethics of conducting a pre-event AVA study in children. The Commission held four public forum meetings that addressed this issue directly and a summary report was issued on March of 2013 [25]. In that report, the Presidential Commission referred to the Code of Federal Regulations (CFR) regarding protections for children involved in research [26].

First, the Commission indicated that pre-event AVA studies could not be conducted in children in the US under 45 CFR 46.405, which specifies that studies above minimal risk require the possibility of direct benefit to the participating child. Second, pediatric studies may be possible using 45 CFR 46.407, which stipulates that a rarely utilized Presidential waiver could be sought if the information gained could possibly benefit children in general even if the study might not benefit the individual child. Finally, the Commission suggested a unique approach that would render a pre-event AVA study to “no more than a minor increase over minimum risk" by using a stepwise, age de-escalation approach. Specifically, the Commission suggested that a pre-event study of individuals 18 to 20 years of age might provide information to substantiate that such a study in 16 to 17 year-olds would involve no more than minimal risk. Consequently, the Commission indicated, with important caveats, that an age de-escalation pathway might be considered under the 45 CFR 46.404, as it poses no more than minimal risk.

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In light of this Presidential Commission's unique alternative pathway suggestion, we implemented a retrospective study with an exploratory objective to describe and compare safety and immunogenicity data from healthy individuals aged 18 to 20 years to the same type of data in individuals aged 21 to 29 years who participated in several HHS-sponsored AVA clinical trials. For this study the 'older' age group of 21 to 29 years, albeit somewhat arbitrary, was chosen as the most appropriate age group to compare safety and immunogenicity data to the 18 to 20 year old group for several reasons. First, including data from subjects up to age 65 years would result in a markedly larger and very unbalanced sample size compared to the 18 to 20 year old group. Also, previous data has shown a decrease in antibody responses to AVA as age increases in ten year increments from 18 to 65 year olds [27]. Finally, injection site reactions to AVA significantly decrease with advancing age [20].

2. Methods

2.1. Retrospective studies utilized

This investigation consisted of an exploratory summary reanalysis of existing electronically stored databases from final clinical study reports from four previous HHS-funded studies involving AVA conducted by the US Centers for Disease Control and Prevention (CDC) or funded by Biomedical Advanced Research and Development Authority (BARDA) that took place since the year 2000. Study AVA000, sponsored by CDC, had study arms that involved different AVA intramuscular (IM) or subcutaneous (SC) dosing regimens well beyond 4 weeks to examine issues of general use prophylaxis of AVA not relevant to PEP [27]. Of note, only AVA000 safety and immunogenicity data from subjects who received a full dose of AVA SC at 0, 2 and 4 weeks were included in the present study. In addition, three BARDA-funded AVA PEP studies were included in this report and designated AVA005, AVA006, AVA009 whereby full dose AVA was given SC at 0, 2 and 4 weeks [28–30].

All four studies were selected because (1) enrolled subjects 18 to 20 years of age as well as older subjects; (2) administered full dose AVA (0.5 mL) subcutaneously (SC) for three doses at 0, 2, and 4 weeks; (3) had, at a minimum, individual subject safety data that included virtually identical local and systemic post-vaccination elicited adverse events for 7 days for at least one diary following an AVA administration; and 4) included US Food and Drug Administration (FDA) defined serious adverse event (SAE) [31] data collected from study participants for at least 30 days beyond each vaccination. All four studies funded by HHS were reviewed and approved by the appropriate institutional review boards and no personally identifiable information was obtained to perform the present study analyses.

2.2. Vaccine

Anthrax vaccine adsorbed used in these studies were all manufactured by Emergent Biosolutions Incorporated. The lots used in the AVA000 CDC study were FAV063, FAV074, FAV079, FAV087, FAV107 and FAV113. For the AVA005 study, the lot used was FAV159, and for both the AVA006 and AVA009 studies the lot was FAV392A.

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2.3. Data collected

Specific safety data collected from the CDC and BARDA funded studies were similar and collected on diaries that subjects completed for seven days following each of the three vaccinations. This safety data included elicited local injection site adverse events (AE) such as: warmth, tenderness, itching, pain, arm motion limitation, redness, lump, swelling, and bruising that occurred within the 7 days following any AVA dose given at 0, 2, or 4 weeks. One exception was that the CDC study diary did not include lump or swelling but instead had a category that was labeled 'induration' on the final case report forms. We chose to combine lump and induration as a similar category and keep the swelling category for the subjects from the BARDA funded studies. Also, common elicited systemic AEs were collected such as fatigue/tiredness, muscle-ache, headache and fever that occurred within 7 days following each AVA dose.

For all local and systemic elicited AEs except fever or injection site redness lump/induration and swelling, the severity grading was based upon the subject's individual assessment of the extent to which the AE interfered with his/her regular daily activities. The elicited AE scores were graded on a scale of: grade 0 or none = no symptom, grade 1 or mild = no interference with routine activities, grade 2 or moderate = interferes with routine activities and grade 3 or severe = incapacitating. For fever: mild, moderate and severe grades were defined as: 38.0–38.4 °C, 38.5–38.9 °C, and greater than 39.0 °C, respectively, for the BARDA funded studies; and 38–39 °C, 39.1–40.0 °C, and greater than 40 °C, respectively, for the CDC study. For injection site redness, induration/lump or swelling: mild, moderate and severe reactions were defined as 2.5–5 cm, 5.1–l0 cm and greater than 10 cm, respectively, for the three BARDA funded studies and 0.1–5 cm, 5.1–12.0 cm, and greater than 12.0 cm, respectively, for the CDC study. With regard to non-elicited AEs, only SAEs, which occurred within 30 days of vaccination given at 0, 2 or 4 weeks and were considered by the study investigator to be possibly or directly related to vaccine were included.

Individual subject immunogenicity data was obtained from each clinical studies report electronic database and included baseline results on sera obtained just before the first AVA dose, 14 days after the second dose given at 2 weeks and 28 days after the third dose given at 4 weeks. Individual subject data on serum effective dilution resulting in 50% neutralization (ED50) *in vitro* of a fixed concentration of anthrax lethal toxin, also known as toxin neutralizing assay were obtained during these studies [32]. Using the ED50 data, a standardized anthrax toxin 50% neutralizing factor (NF50) level was calculated as a quotient of the test sample and a common standard reference serum, which in this case was AVR801 ED50 for all four studies [33,34]. Finally, individual subject data of serum IgG antibody concentrations to PA, as measured by quantitative enzyme linked immunosorbent assay (ELISA), was extracted from the study databases.

For each individual study, if the subjects' antibody values were below the lower limit of quantification (LLOQ) of the respective assay, LLOQ/2 was imputed. Since the immunogenicity assays were performed in different laboratories and during different time periods, we chose to collect and report only the proportion of subjects with greater than or equal to four-fold rises from baseline (seroresponders) for both the NF50 and anti-PA IgG concentrations data to help mitigate potential laboratory variability between the four studies.

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As mentioned at the beginning of the methods section, the designs of the CDC and BARDA studies were somewhat different. The CDC study was designed as a pre-exposure general use prophylaxis investigation to examine SC and IM administration of AVA and alternate booster schedules. However, the present study included only the CDC study arm whereby vaccine was administered SC at 0, 2, and 4 weeks to collect relevant safety information that could apply to a PEP vaccination regimen. Also, it must be noted that the CDC study included additional safety assessment visits for which serum samples were not obtained. In addition, the serum antibody testing was prioritized to the anti-PA IgG concentrations. Therefore, only a subset of the subjects had paired sera evaluated for NF50. The BARDA funded studies were designed to evaluate a PEP regimen whereby AVA was administered SC at 0, 2, and 4 weeks and the priority was NF50 analyses; only a subset of sera were evaluated by ELISA. Also, for the BARDA funded study AVA009, no serologic evaluations were determined at week 4. In conclusion, although safety information was available for all subjects included in the present study, serologic data was available for only a subset of subjects.

2.4. Statistics analysis plan

The elicited AE data profiles collected from the subjects were uniform between studies in terms of utilizing a 7-day post-vaccination diary to collect similar AE symptoms. When pooling the safety data from these studies, both a mixed-effect model and a fixed-effect model were implemented to estimate the overall AE proportions after each AVA dose. Because the safety data collection methodology was so similar between studies, the estimated AE rate results under these two different models were quite similar. Therefore, overall AE proportions were estimated by simply pooling subjects from all four studies. In addition, since each subject could contribute three AE information data corresponding to the three doses, the issue of repeated measures was investigated through generalized estimating equations (GEE) methodology. The estimation results under GEE were very similar to the results obtained ignoring the feature of repeated measures, so in this paper we present the safety results analyzed by simple pooling over the three doses.

Immunogenicity results, as represented by proportions of subjects with seroresponses, were reported for individual studies and pooled values were utilized for certain comparisons between the age groups.

This investigation was exploratory and not intended to provide confirmatory evidence for a hypothesis. Therefore, we chose to present the safety data simply as proportions of subjects within each age category with an elicited local or systemic adverse event of any severity (grade 1 to 3) or of maximum severity (grade 3) after any of the three AVA doses. Proportions of seroresponders between baseline and 2 weeks following the second AVA dose; and between baseline and 4 weeks following the third AVA dose are reported for each study for 18 to 20 year-olds and 21 to 29 year-olds.

There were several types of exploratory comparisons conducted in the investigation. The primary comparisons were the proportions of subjects with any local or any systemic elicited AE between the 18 to 20 year-olds and subjects 21 to 29 years of age for all three doses combined. We also reported on the proportions of any (grade 1 to 3) or any maximum

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(grade 3) for the individual nine local or four systemic AEs following any of the three AVA doses. In addition, to examine a possible trend of the AE rates over the sequence of dosing, proportions of AEs after each of the AVA doses were calculated for any local or systemic elicited AE (grade 1 to 3) or any maximum (grade 3) but not for each individual local or systemic elicited AE.

Finally, the proportions of subjects with seroresponses (greater than or equal to 4-fold rise in antibody measurement compared to baseline) after the second and third AVA dose, as measured by NF50 or anti-PA IgG concentrations were analyzed for each age group. Proportions of AEs or seroresponses for all comparisons were analyzed using Fisher's exact test.

3. Results

For all four studies combined there were 74 subjects 18 to 20 years of age and 243 subjects 21 through 29 years of age (Table 1). Table 1 also illustrates the demographics of subjects enrolled in each of the studies. When all study groups were combined, there were no significant differences in demographic characteristics of age, race or gender between the two age categories.

Table 2 shows the proportion of subjects in each age group who experienced any grade 1 to 3, or any maximum grade 3 elicited AEs. The denominators are the sum of the numbers of diaries collected after dose 1, dose 2 and dose 3. For example, for the younger age group, there are 74, 71 and 71 diaries collected for doses 1, 2 and 3, respectively. Therefore, the denominator used for this age group was 216 (= 74 + 71 + 71). There were no significant differences in overall combined local or systemic AEs between the two age groups for any grade 1 to 3, or of grade 3 local AEs within seven days after each vaccination.

With regard to individual local elicited AEs of any grade, Table 2 reveals that subjects aged 18 to 20 years had significantly lower rates of any local warmth (37.5% vs. 46.5%; $P<0.05$) and higher rates of maximum swelling (1.1% vs. 0%; $P< 0.05$) compared to older subjects. There were no significant differences in individual or of combined systemic elicited AEs of any or of maximum grade in this data set.

Table 3 displays the results of elicited AEs related to sequential vaccine dosing at the three time points 0 to 2 to 4 weeks. In general, there was a decline in the rates of elicited AEs with each subsequent dose. For both age groups, there were statistically significant declines in maximum local AEs and any grade systemic AEs.

No SAEs were recorded in subjects aged 18 to 29 years who received AVA by SC injection in the four combined studies within 30 days of any of the three AVA doses given at study weeks 0, 2, and 4.

Table 4 displays the seroresponse rates for the two age groups as measured by serum NF50 or anti-PA IgG concentrations. In general, the proportions of subjects with seroresponses were high after the second and third AVA doses. There were no significant differences in proportions of NF50 or anti-PA IgG antibody seroresponses after the second or third AVA

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doses between the 18 to 20 and 21 to 29 year olds. As noted in the methods, serologic data for both assays were only available on a subset of subjects included in the present study.

4. Discussion

AVA appeared tolerable in both age groups in the present study, with few maximum grade 3 elicited AEs and no SAEs attributable to AVA observed in subjects that received AVA at 0, 2 or 4 weeks. Overall, the 18 to 20 year olds had similar rates of local and systemic AEs compared to the 21 to 29 year age group.

It is important to note that the overall proportions of elicited local and systemic AEs were similar to those reported for other vaccines, including diphtheria-tetanus-acellular pertussis vaccines (Tdap and DTaP), Hepatitis A and Hepatitis B, and others [17,35–39]. Relevant to the focus on the possibility of pediatric AVA studies in the future, the present study revealed proportions of elicited AEs that were similar to those seen in published studies of adolescents given booster doses of Tdap, or of 4 to 6 year-olds given DTap [40,41].

Finally, data from Table 3 regarding frequencies of AEs in each sequential AVA dosing week are somewhat reassuring in that the proportions do not increase and in fact generally appear to decrease with each subsequent vaccine dose. This information may be comforting for parents and children if pediatric studies involving AVA are ever initiated.

The immunogenicity results demonstrated that seroresponses measured in the 18 to 20 year-old subjects were not significantly different than those observed in the older subjects after the second and third AVA doses. We chose not to include subjects older than 29 years of age because the AVA000 study revealed that antibody responses decline with age [42].

A limitation of the present investigation was the small numbers of 18 to 20 year-old subjects studied. This situation resulted in unbalanced groups when comparing results between the small numbers of 18 to 20 year olds to the larger number of older subjects. To some degree, this unbalance was mitigated by limiting the older age group to 21 to 29 years of age resulting in a ratio of about 1 to 3.3 of the younger to the older age group. The small number of young subjects led to the decision not to compare study safety or immunogenicity data in additional smaller subgroups such as differences between subjects of different gender or race.

Another limitation was that numerical scoring criteria for mild, moderate and severe (grades 1, 2, or 3) local elicited AE safety data such as fever, lump, induration and swelling differed between the CDC and BARDA studies. These differences were somewhat mitigated analyzing just two categories as either the percentage that had any grade or only grade 3 elicited AE in the present study. Also, in the present study, the safety data likely varied between studies because of factors such as the use of different clinical research organizations, clinical research associates, and geographically clinical sites. However, this limitation is somewhat mitigated by the relative uniformity of type of local and systemic elicited AEs in all four of the studies utilized in these analyses.

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Another study limitation was that the serologic data were available for only a subset of subjects in this study. Also, as previously mentioned, it was impractical to compare the actual NF50 measurements or anti-PA IgG concentrations between the four studies because of likely laboratory variability of assays that were conducted over more than a decade and tested in different laboratories. However, using the proportion of seroresponders, rather than comparing geometric mean values, helped mitigate immunogenicity variability between the studies included in this report.

In summary, the above limitations necessitate that well-designed prospective studies of balanced groups of 18 to 20 year-olds and older adults should be performed to validate our findings. Nevertheless, data from the present study suggests that AVA is as safe and immunogenic in the 18 to 20 year old when compared to the 21 to 29 year age group.

Current epidemiologic research does not support an association between AVA and severe, unusual, or chronic illnesses in adults [43,44]. These epidemiologic studies combined with the data from the present study may be useful in informing future discussions of conducting post-event or even pre-event AVA pediatric studies in the future. Certainly, immediate and long term safety data for AVA administered to children would be important information for medical response providers if this vaccine is to be given to this population during a large scale anthrax emergency. The ‘age de-escalation’ approach discussed in the Presidential Ethics Report raises significant ethical and regulatory issues that deserve further discussion.

As previously mentioned, this report involved only subjects that received AVA SC because this is what is currently recommended for PEP (14). However, it is likely that AVA administered SC or IM would result in similar antibody responses [45]. Additionally, it is clear that higher local reactogenicity rates have been observed when AVA is administered SC vs. IM [18,45]. Additionally, most health care personnel are more familiar with vaccine administered IM and thus this route may be preferable to use during an emergency PEP situation. Notably, pre-exposure prophylactic AVA is now recommended and approved by the FDA to be given IM [3] and this method of administration may be better tolerated in younger children with smaller limbs and less subcutaneous tissue than most adolescents and adults. It should be noted that the PEP AVA regimen for which FDA licensure is being sought involves SC administration not IM. However, it is anticipated that post-licensure studies directly comparing the safety and immunogenicity of SC vs. IM PEP regimens are planned.

In conclusion, the results of these exploratory analyses demonstrate that AVA appears to be safe and immunogenic in 18 to 20 year olds compared to individuals 21 to 29 years of age. Additionally, this investigation provides some information relevant to the ‘age de-escalation’ approach suggested by the Presidential Ethics Commission.

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Abbreviations:

AE	adverse events
AVA	anthrax vaccine adsorbed
BARDA	Biomedical Advanced Research and Development Authority
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
ED50	effective dilution resulting in 50% neutralization
ELISA	enzyme linked immunosorbent assay
HHS	Health and Human Services
IgG	immunoglobulin G
IND	investigational new drug
NF50	standardized anthrax toxin 50% neutralizing factor
NPRSB	National Preparedness and Response Science Board
PA	protective antigen
SC	subcutaneous

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Table 1

Demographics of subjects participating in four studies[1] who received anthrax vaccine adsorbed (AVA) subcutaneously at 0, 2 and 4 weeks[2].

Study & group	Number of subjects	Number (%) females	Number (%) Caucasian	Number (%) African-American	Number (%) other race
Subjects aged 18 to 20 years					
AVA000	14	6	11	3	0
AVA005	26	9	21	1	4
AVA006	17	8	16	1	0
AVA009	17	7	10	7	0
Total	74	30 (40.5)[2]	58 (78.4)[2]	12 (16.2)[2]	4 (5.4)[2]
Subjects aged 21 to 29 years					
AVA000	63	31	49	8	6
AVA005	56	24	48	4	4
AVA006	78	41	68	7	3
AVA009	46	24	26	18	2
Total	243	120 (49.4)	191 (78.6)	37 (15.2)	15 (6.2)

[1] AVA000 is a Center for Disease Control and Prevention (CDC) study (Ref. [27]) and AVA005, AVA006, and AVA009 (Refs. [28–30]) are Biomedical Advanced Research and Development Authority (BARDA) funded studies.

[2] *P*-value assessed as not statistically significant by Fisher's exact test comparing the 18 to 20 and 21 to 29 year old age groups.

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Table 2

Ratios[1] [percentages] and (95% confidence intervals) of elicited adverse events {AE} within 7 days of anthrax vaccine adsorbed (AVA) administered subcutaneously at 0, 2, or 4 weeks.

Symptom	Elicited at least one AE of any severity grade 1 to 3			Elicited at least one AE of maximum severity grade 3		
	18 to 20 year-olds	21 to 29 year-olds	*P*-value[2] young vs. older group	18 to 20 year-olds	21 to 29 year-olds	*P*-value[2] young vs. older group
Local elicited adverse events[3]						
Any local	**171/216 [79.2%] (74.1, 84.4)**	**593/708 [83.8%] (80.8, 86.4)**	**0.120**	**8/216 [3.7] (1.6, 7.2)**	**24/708 [3.4%] (2.2, 5.0)**	**0.837**
Warmth	81/216 [37.5]	329/708 [46.5]	**0.021**	1/216 [0.5]	4/708 [0.6]	0.887
Tenderness	149/216 [69.0]	527/708 [74.4]	0.114	5/216 [2.3]	12/708 [1.7]	0.584
Itching	58/216 [26.9]	211/708 [29.8]	0.405	1/216 [0.5]	5/708 [0.7]	0.832
Pain	126/216 [58.3]	407/708 [57.5]	0.828	4/216 [1.9]	7/708 [1.0]	0.322
Arm motion	61/216 [28.2]	243/708 [34.3]	0.097	3/216 [1.4]	7/708 [1.0]	0.735
Redness	100/216 [46.3]	339/708 [47.9]	0.685	2/216 [0.9]	3/708 [0.4]	0.476
Induration or lump[4]	127/216 [58.8]	411/708 [58.1]	0.848	5/216 [2.3]	6/708 [0.8]	0.113
Swelling[4]	67/174 [38.5]	212/519 [40.8]	0.595	2/174 [1.1]	0/519 [0]	**0.021**
Bruise	32/216 [14.8]	105/708 [14.8]	0.998	0/216 [0]	1/708 [0.1]	0.735
Systemic elicited adverse events[3]						
Any systemic	**98/216 [45.4%] (38.6,52.3)**	**357/707 [50.5%] (46.7, 54.2)**	**0.188**	**7/216 [3.2%] (1.3, 6.6)**	**19/707 [2.7%] (1.6, 4.2)**	**0.697**
Fatigue	53/216 [24.5]	188/707 [26.6]	0.550	2/216 [0.9]	11/707 [1.6]	0.584
Muscle ache	74/216 [34.3]	246/707 [34.8]	0.887	2/216 [0.9]	7/707 [1.0]	0.972
Headache	44/216 [20.4]	166/707 [23.4]	0.346	4/216 [1.9]	10/707 [1.4]	0.735
Fever	2/216 [0.9]	6/707 [0.8]	0.935	0/216 [0]	0/707 [0]	1.000

[1]Ratios represent numbers of diaries collected with at least one grade 1, 2 or 3 (mild, moderate or severe) or maximum grade 3 (severe) elicited AE within 7 days of vaccination (numerator) and total number of diaries collected at dose 1, 2 and 3 combined (denominator).

[2]Fisher's exact test was used to calculate the *P*-values.

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[3] *Note*: Assuming three diaries per subject, a small percentage of diary information (3%) appeared not to have been available for 18 to 20 year old subjects thus the denominators are 216 instead of 222. Similarly, 3% were not collected from the older age group thus the denominators are 707 or 708 instead of 729. This calculation does not apply to the swelling category because only subjects in the BARDA funded studies recorded swelling, however the proportion of missing data points for swelling was also 3%.

[4] Note that the CDC (study AVA000) did not collect information specifically labeled lump or swelling but rather used the term induration. Therefore, we decided to combine the categories of lump and induration and report swelling as a separate category that was only collected from the BARDA funded studies.

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Table 3

Ratios[1], [percentages], and (95% confidence intervals) of local or systemic elicited adverse events {AE} of any severity (grades 1 to 3) or of maximum grade 3 severity after each of the three anthrax vaccine adsorbed dose.

Diary collected after dose given at week: ages group	Week Zero: Dose 1	Week Two: Dose 2	Week Four: Dose 3	*P*-value[2] for trend testing
Anygrade 1–3 local AE				
18 to 20 years	60/74 [81.1%] (70.3, 89.3)	59/71 [83.1] (72.3, 90.9)	52/71 [73.2%] (61.4, 83.1)	0.263
21 to 29 years	207/242 [85.5%] (80.5, 89.7)	200/236 [84.7%] (79.5, 89.1)	186/230 [80.9%] (75.2, 85.7)	0.190
Any maximum grade 3 local AE				
18 to 20 years	5/74 [6.8%] (2.2,15.1)	3/71 [4.2%] (0.9,11.9)	0/71 [0%] (0, 5.1)	**0.045**
21 to 29 years	13/242 [5.4%] (2.9, 9.0)	7/236 [3.0%] (1.2, 6.0)	4/230 [1.7%] (0.5, 4.4)	**0.030**
Anygrade 1–3 systemic AE				
18 to 20 years	42/74 [56.8%] (44.7, 68.2)	34/71 [47.9%] (35.9, 60.1)	22/71 [31.0%] (20.5, 43.1)	**0.002**
21 to 29 years	136/242 [56.2%] (49.7, 62.5)	121/235 [51.5%] (44.9, 58.0)	100/230 [43.5%] (37.0, 50.2)	**0.007**
Any maximum grade 3 systemic AE				
18 to 20 years	2/74 [2.7%] (0.3, 9.4)	5/71 [7.0%] (2.3,15.7)	0/71 [0%] (0, 5.1)	0.489
21 to 29 years	9/242 [3.7%] (1.7, 6.9)	7/235 [3.0%] (1.2, 6.0)	3/230 [1.3%] (0.2,3.8)	0.119

[1]Ratios represent numbers of diaries collected with at least one grade 1 to 3 elicited AE within 7 days of vaccination (numerator) and total number of diaries collected at dose 1, 2 or 3 (denominator).

[2]*Note:* significant *P* values (<0.05) are shown in bold text. Cochran–Armitage test was used to test the trend over the three consecutive AVA doses.

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Table 4

Ratios[1] [percentages] and (95% confidence intervals) of subjects with seroresponses {greater than or equal to 4-fold rise from pre-vaccination value}, as measured by NF50 assessment or serum anti-protective antigen (PA) IgG concentrations two weeks after the second doses (week 4) and four weeks after the third doses (week 8) of anthrax vaccine adsorbed (AVA) compared to baseline.

Age group	Proportion of week 4 seroresponders		Proportion ofweek 8 seroresponders	
	18 to 20 years	21 to 29 years	18 to 20 years	21 to 29 years
NF50 assessment				
Study				
AVA000	2/2 [100]	15/17 [88.2]	3/3 [100]	18/18 [100]
AVA005	26/26 [100]	53/55 [96.4]	26/26 [100]	54/54 [100]
AVA006	15/16 [93.8]	72/75 [96.0]	16/16 [100]	74/74 [100]
AVA009	ND[2]	ND[2]	14/14(100)[3]	37/37 [100][3]
Total	43/44 [97.7][4] (88.0, 99.9)	140/147 [95.2][4] (90.4, 98.1)	59/59 [100][5] (93.9,100)	183/183 (100][5] (98.0,100)
Serum anti-PA IgG co ncentration[6]				
AVA000[1]	13/13 [100]	60/61 [98.4]	13/13 [100]	61/61 [199]
AVA005	7/7 [100]	16/16 [100]	7/7 [100]	15/15 [100]
AVA006	5/6 [83.3]	21/21 [100]	6/6 [100]	20/20 [100]
Total	25/26 [96.2][7] (80.4, 99.9)	97/98 [99.0][7] (94.4, 100)	26/26 [100][8] (86.8,100)	96/96 [100][8] (96.2,100)

[1] Ratios represent numbers of subjects that had greater than or equal to 4-fold rises in antibody values from baseline to week 4 (two weeks after the second AVA dose) or week 8 (four weeks after the third AVA dose (numerator). The denominator of the ratio represented the number of subjects with paired sera tested at weeks 0 and 4 or weeks 0 and 8. Note, not all subjects included in the present study had paired sera available for seroresponse testing for both time-points or for both serologic assays NF50 or anti-PA IgG concentrations by ELISA.

[2] ND = not done because sera were not collected at week 4 in the AVA009 study.

[3] Sera tested at two weeks after third dose rather than four weeks (6 week blood draw).

[4] *P*-value = 0.684, Fishers Exact test between two age groups at week four.

[5] *P*-value = 1, Fishers Exact test between age groups at week eight.

[6] Study AVA009 had no anti-PA IgG concentration data

[7] *P*-value = 0.377, Fisher's Exact test between age groups at week four.

[8] *P*-value = 1.0, Fisher's Exact test between age groups at week eight.

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