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Metabolites as biomarkers of adverse reactions following vaccination: A pilot study using nuclear magnetic resonance metabolomics

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ABSTRACT

An Adverse Event Following Immunization (AEFI) is an adverse reaction to a vaccination that goes above and beyond the usual side effects associated with vaccinations. One serious AEFI related to the smallpox vaccine is myopericarditis. Metabolomics involves the study of the low molecular weight metabolite profile of cells, tissues, and biological fluids, and provides a functional readout of the phenotype. Metabolomics may help identify a particular metabolic signature in serum of subjects who are predisposed to developing AEFIs. The goal of this study was to identify metabolic markers that may predict the development of adverse events following smallpox vaccination. Serum samples were collected from military personnel prior to and following receipt of smallpox vaccine. The study population included five subjects who were clinically diagnosed with myopericarditis, 30 subjects with asymptomatic elevation of troponins, and 31 subjects with systemic symptoms following immunization, and 34 subjects with no AEFI, serving as controls. Two-hundred pre- and post-smallpox vaccination sera were analyzed by untargeted metabolomics using ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Baseline (pre-) and post-vaccination samples from individuals who experienced clinically verified myocarditis or asymptomatic elevation of troponins were more metabolically distinguishable pre- and post-vaccination compared to individuals who only experienced systemic symptoms, or controls. Metabolomics profiles pre- and post-receipt of vaccine differed substantially when an AEFI resulted. This study is the first to describe pre- and post-vaccination metabolic profiles of subjects who developed an adverse event following immunization. The study demonstrates the promise of metabolites for determining mechanisms associated with subjects who develop AEFI and the potential to develop predictive biomarkers.

1. Introduction

Vaccinations are generally regarded as one of the most important public health achievements in the last century and have dramatically reduced the morbidity and mortality from numerous infections caused by various bacteria and viruses. The literature is replete with data demonstrating vaccination safety and efficacy [1]. However, on rare occasions, immunization can be associated with an adverse event. An Adverse Event Following Immunization (AEFI) is an adverse reaction to a vaccination that goes above and beyond the usual side effects that are known to be associated with vaccinations. Some common side effects include redness at the local site of the vaccination, or rarely, a severe allergic reaction such as anaphylaxis [1]. Similarly, AEFI can vary in clinical severity

Abbreviations: AEFI, Adverse Events Following Immunizations; NMR, nuclear magnetic resonance; SSFI, systemic symptoms following immunization; CAD, coronary artery disease; DSS-D6, 4-dimethyl-4-silapentane-1-sulfonic acid; PCA, principal components analysis; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable influence on projection; ECG, electrocardiogram; +MyoC, group 1 subjects vaccine events resulting in myocarditis; +Trop–Sx, group 2 subjects vaccine events resulting in asymptomatic elevation of troponins.

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from very mild to incapacitating. One serious AEFI related to the current smallpox vaccine is myopericarditis, which refers to inflammation of the myocardium, pericardium, or both. This inflammation can clinically manifest with symptoms such as chest pain, dyspnea, and palpitations [2], and it has been estimated that myopericarditis and subclinical myopericarditis (elevated cardiac biomarkers in the absence of clinical symptoms) occurs in 4.6 and 28.7 per 1000 smallpox vaccinees, respectively [3]. Since 2002, millions of military service members have received the smallpox vaccine containing the vaccinia virus due to the potential use of smallpox as a bioterrorism agent [2]. In fact, if such an act were to occur, nearly 300 million doses of smallpox vaccine are available to immunize the US population [4]. Knowing and preparing for adverse events associated with this vaccine in a setting of a mass immunization campaign would be critical to providing quality immunization healthcare.

The ability to predict who may experience an AEFI could significantly impact immunization healthcare outcomes. Metabolomics, which is the study of the low molecular weight complement of a biological system, may be able to identify a metabolic signature or metabotype to identify individuals who may be predisposed to developing a serious AEFI [5,6]. Metabolomics provides a point-in-time functional readout of the phenotype of an individual as determined by the influence of the sum total of genetic and environmental factors such as nutrition, medications, health or disease status and treatments or exposures [7]. Identification of a predictive metabotype or selected metabolites would allow clinicians to potentially mitigate serious AEFI and ultimately improve the quality of immunization healthcare. If identified, the contributing molecules might represent novel biomarkers of risk that can supplement existing clinical decision making for risk stratification or vaccine exemptions, as well as help identify vaccine candidates that are more reactogenic before they undergo large-phased studies seeking FDA approval [3]. Leveraging the science of metabolomics in an effort to improve the benefit-to-risk ratio of an immunization given to a healthy population might improve the quality of immunization programs, which is critically important in an era of increasing vaccine hesitancy. Therefore, this exploratory study was undertaken to determine whether a) metabotypes of baseline samples could be used for predicting adverse events following smallpox immunization, and b) to reveal potential biomarkers of AEFI by comparing case and control samples. This study compared the metabolic signatures from a subset of healthy military personnel who had reported adverse events following smallpox immunization compared to individuals reporting no symptoms.

2. Material & methods

2.1. Study subjects and samples

Serum samples were collected from a subset of subjects who were previously enrolled in a multi-center, prospective, active surveillance cohort study of healthy subjects receiving the smallpox vaccine (DryVax® or ACAM 2000®) whose results have been published previously [3]. Samples from 100 active-duty military personnel who had previously provided informed consent for future vaccine research were obtained for analysis. Paired samples obtained prior to and 13–28 days following smallpox immunization (with or without other concomitant vaccines) were identified for analysis. The study population was broken down into four distinct clinical groups: five subjects with clinically diagnosed myopericarditis following immunization (Group 1, +MyoC); thirty subjects with asymptomatic elevation of troponins, often referred to as subclinical myocarditis (Group 2, +Trop–Sx); thirty-one subjects with a variety of systemic symptoms following immunization (SSFI) including arthralgias, myalgias, fevers and/or headache (Group 3); and thirty-four subjects with no reported symptoms or AEFI, which served as controls for immunization response (Group 4). All samples used in this study were stored in an ultra-low, −80 °C freezer and had no previous freeze/thaw cycles prior to preparation for metabolomics analysis. The Institutional Review Board of Womack Army Medical Center approved the study and informed consent for future vaccine research was obtained from all participants during the time of enrollment when the serum samples were originally obtained.

2.2. Metabolomics sample preparation and data acquisition

Serum samples (100 pre-vaccination and 100 post-vaccination) were extracted and analyzed by broad spectrum (untargeted) metabolomics using ^{1}H NMR spectroscopy, as described previously [8–14]. Briefly, each serum sample was prepared by mixing an aliquot (400 μL) of each of the study serum samples with methanol. A 3:1 ratio of MeOH:Serum (1200:400 uL) was used for extraction to prepare the samples for metabolomics data acquisition and analysis. Samples were vortexed and centrifuged and 1000 μL of supernatant was transferred to new tubes and lyophilized to dryness. Samples were reconstituted in 700 μL of phosphate-buffered D_2O master mix containing Chenomx ISTD (0.6 mM 4,4-dimethyl-4-sila pentane-1-sulfonic acid (DSS-D_6, Chemical Shift Indicator)) and 0.2% NaN_3. The samples were vortexed and centrifuged at 16,000 rcf for 5 min, then a 600 μL aliquot of each sample supernatant was transferred into 5 mm NMR tubes (Bruker-BioSpin, Switzerland) for data acquisition. In addition, phenotypic pooled serum samples were made by combining aliquots from each of the study samples that belong to the same phenotype (Groups 1–4). A representative pooled sample was also made using aliquots from 25 randomly selected samples. Three aliquots of each of the phenotypic pooled serum samples and ten aliquots of the representative pooled sample (400 μL each) were created and prepared identical to the individual serum samples. NMR spectra were acquired for the individual study samples and the pooled samples on a Bruker Avance III 700 MHz NMR spectrometer (Bruker-Biospin, Rheinstetten, Germany) using a cryogenically cooled 5 mm ATMA probe at 25 °C and a 1D NOESY presaturation pulse sequence (noesypr1d) [9]. ^{1}H NMR spectra were preprocessed using ACD NMR Processor 12.0 software (Advanced Chemistry Development, Toronto, ON, Canada). Spectra were binned (0.07–8.50 ppm) using intelligent bucketing integration with a 0.04 ppm bucket width and a 50% looseness factor. Chemical shift regions for water (4.66–5.16 ppm) and methanol (3.30–3.37 ppm) were excluded from binning. Each of the bin integrals were normalized to the total integral of each spectrum.

2.3. Multivariate and statistical analysis

Multivariate analysis, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), using the normalized binned data that was first Pareto scaled and mean centered was performed using MKS Data Analytics Solutions' SIMCA 14.1 (Umetrics, Umeå, Sweden). The PCA scores plots were inspected to ensure that the phenotypic pooled samples were tightly clustered in the center of phenotypic groups and that the pools created from the representative sample were clustered in the center of samples used to create this pool, a quality control method that is widely used in metabolomic studies [15]. Loadings plots and variable influence on projection (VIP) plots were inspected. The VIP statistic summarizes the importance of the bin in differentiating the study groups and for pre- versus post-vaccination comparisons within a study group in multivariate