NIAID /JHU/FHI 360*

Validate an Easy to Administer Algorithm to Define Penicillin (B-lactam) Allergy Status in STD Outpatients

Initial Statistical Analysis Plan

DMID Protocol Number: 18-0023

NCT04620746

* This plan was prepared by the lead biostatistician of FHI 360. The plan received additional technical input from NIDID and Johns Hopkins University. FHI 360 will be responsible for conducting data analyses for the final clinical study report and for primary manuscripts for publication.

Contents

1. INTRODUCTION

This is a multicenter clinical study to screen and enroll eligible individuals coming for care at ambulatory STD clinics and similar venues who self-report a history of PCN or other beta-lactam antibiotic allergy. The study will screen approximately 5,000 men and women to achieve 1,000 evaluable participants from 5 study sites in different geographic areas of the US over a 14-16-month recruitment period.

Two methods of validating PCN allergy will be used:

- **Method 1 – PCN Skin Test (PST) Group:** skin testing of subjects with low-risk history followed by one-step oral challenge of subjects with negative skin tests.
- **Method 2 – Direct Oral Challenge (DOC) Group:** direct two-step oral challenge of subjects with low-risk history.

Each site will perform both allergy validation interventions, Method 1 PST Group and Method 2 DOC Group. The anticipated enrollment at each site will be approximately 200 subjects overall with 100 in each group. Since each intervention has different logistical considerations, sites will enroll subjects in sequential block groups of 25. Sites will be assigned the order of block groups randomly. Each site will plan to enroll 8 block groups, 4 in each allergy validation intervention.

The analysis plan provides additional details regarding the planned statistical analysis of the study protocol (version 2.0, 09March2020). Tables, figures and listings shells will be developed prior to the start of data cleaning and included as an appendix to a future version of the plan.

2. STUDY OBJECTIVES

The study objectives described in the protocol are as follows:

2.1 Primary Objective

- Validate an algorithm which incorporates a PCN (B-lactam) allergy history screening questionnaire followed by an allergy validation intervention — 1) skin testing and oral challenge or 2) direct two-step oral challenge — to produce a short standardized post-study questionnaire (4-6 questions) for use in ambulatory STD settings.

- Determine how many subjects who report PCN (B-lactam) allergy can be treated with PCN or B-lactam drugs.

2.2 Secondary Objectives

- Assess subject and provider acceptability of a PCN (B-lactam) allergy testing algorithm.

- Determine the feasibility of implementing PCN skin testing followed by oral challenge for those with negative skin test results, or direct two-step oral challenge in an ambulatory STD setting.

3. STUDY OUTCOME MEASURES

3.1 Primary Outcome Measures

- Algorithm / PCN (B-lactam) allergy history screening questionnaire performance in ambulatory STD populations, specifically the negative predictive value (NPV) of the algorithm on true PCN (B-lactam) allergy.
- In ambulatory STD patients who report a history of PCN (B lactam) allergy and who have low-risk histories, determine the prevalence of PCN reactivity validated either by skin test or by direct oral challenge.

3.2 Secondary Outcome measures

- Acceptability
  - Proportion of study subjects who find the testing procedures to be helpful.
  - Reasons why study subjects refuse to participate in the study.
  - Proportion of study subjects who were negative on oral challenge who now feel confident in taking PCN or similar antibiotics.
  - Proportion of study providers who will not offer PCN allergy assessment in the future.
- Feasibility
  - Proportion of study providers who will offer PCN allergy assessment in the future.
  - Reasons why study providers will not offer PCN allergy assessment in the future.
- Both acceptability and feasibility
  - Types and frequencies of elicited reactivity to PCN through skin testing and oral challenge among study subjects.

4. Analysis Populations

This analysis plan describes six different analysis populations that are used for different purposes. The final statistical report will include an accounting of all eligible persons screened and the number enrolled. The reason for excluding any data from any analysis population will be documented.

4.1 Screened Population

Approximately 300 males and females will be screened from participating STD clinics, emergency departments or ambulatory clinics in different regions of the US.

4.2 Eligible Population

This population includes screened participants who meet all inclusion/exclusion criteria in the protocol. The criteria are

- Inclusion Criteria: Subjects must meet all the following inclusion criteria to be eligible to participate in the study:
    - 18 years of age or older
    - Be able to provide informed consent
    - Report having a history of allergy to PCN or B-lactam drugs
- Exclusion Criteria: Subjects meeting any of the following criteria at baseline will be excluded from study participation:
    - Not able to stay for testing and challenge (1-3 hours) on day of clinic visit (or return within 2 weeks to complete study procedures)
    - Other exclusion criteria, per clinical judgment, which prohibits enrolling in study

4.3 Enrolled Population

This population includes all eligible participants who consent to enroll in the study. This analysis population will mainly be used for analyzing participants' baseline characteristics and allergy history.

4.4 Treated Population

This population includes all enrolled participants who received Method 1 PST or Method 2 DOC. This analysis population will mainly be used for analyzing participants' baseline characteristics, primary outcome measures and secondary outcome measures.

4.5 Per-Protocol Population

This population includes all treated participants who completed the study without protocol deviation. This analysis population will also be used for analyzing participants' baseline characteristics, primary outcome measures and secondary outcome measures.

4.6 Provider Population

The Provider Population includes all clinic providers who complete the provider feasibility survey. This analysis population will mainly be used for analyzing secondary outcome measures for provider acceptability and feasibility of PCN allergy evaluation including the allergy history screening questionnaire, skin testing and oral challenge in busy ambulatory STD settings.

5. GENERAL STATISTICAL ISSUES

All confidence intervals will be two-sided with 95% coverage. Likewise, all statistical tests will be two-sided and at 0.05 significance level.

Missing data will be treated as missing at random without imputation unless available evidence indicated that missing data is informative. Data at all non-missing time points will be used and included in relevant analyses.

All comparisons between treatment groups will be made without adjustment for multiple comparisons. All statistical analyses will be done using SAS® (SAS Institute Inc., Cary) Version 9.4.

6. Sample Size Consideration

The sample size for this study was selected to have adequate study power to conclude a high NPV of the PCN (B-lactam) allergy history screening questionnaire in ruling out the presence of true PCN (B-lactam) allergy. The approach proposed by Steinberg et al. (2009) is used to obtain the minimal sample size needed to achieve a lower 95% confidence bound for NPV that exceeds the result with a useless test at a fixed level of significance with a given study power. To target at least 85% power at 5% of significant level, the listed sample sizes (see table) provide lower 95% confidence bounds for NPV under the following various assumptions:

- The true population prevalence with PCN allergy: 1%, 3%, 5%
- The observed % of PCN allergy in this study: 1%, 3%, 5%
- The PCN allergy diagnostic kit with sensitivity at least 85% and specificity 95%
- 5% of participants who report a PCN allergy history will have a high-risk allergy history and therefore will not proceed to skin testing.

Population prevalence of PCN allergy	Observed % of PCN allergy in study	Estimated NPV	The lower 95% confidence bound for NPV						
			99.0%	98.4%	97.9%	97.5%	97.3%	97.0%	95.0%
1%	1%	99.84%	1259	796	635	556	526	488	351
	3%	99.51%	420	265	212	185	175	163	117
	5%	99.18%	252	159	127	111	105	98	70
3%	1%	99.84%	8119	2964	1958	1559	1421	1259	759
	3%	99.51%	2707	**988**	653	520	474	420	253
	5%	99.18%	1624	593	392	312	284	252	152
5%	1%	99.84%	11300	9519	4770	3378	2950	2483	1259
	3%	99.51%	37547	3174	1590	1126	984	828	420

	5%	99.18%	22533	1905	954	676	590	497	252

A sample of 988 subjects who reported to have PCN allergy history would provide an estimated NPV = 99.51% with 85% power to conclude that the NPV of the study allergy history screening questionnaire is least 98.4% at 5% significant level.

7. STATISTICAL ANALYSIS

7.1 Study Participant Disposition and Follow-up

Information about study participant disposition and follow-up during the study will be provided and summarized for each treatment group as follows:

- A flowchart will document the flow of participants through the study and relate this to the different analysis populations. See Appendix A.
- Tables will summarize:
    - the number and percentage of participants included in each of the analysis populations, excluded, with high-risk history of PCN allergy, who received skin testing, who received oral challenge and who completed the study.
    - the reasons for early discontinuation from the study including the number who refused to participate in skin testing, the number who refused oral challenge etc.

7.2 Analysis of Baseline Data

Baseline variables will be summarized for the Treated population, the Enrolled population and the Per Protocol Population. The following baseline measures collected at the screening and enrolment visits from participants will be summarized by treatment group:

- Demographic data: age, gender, ethnicity and race.
- Allergy history
- Vital signs and focused physical exam

For PST Group only:

- HIV status, last CD4 (within last 6 months) and HAART status if HIV+
- Skin conditions
- Skin testing history

For DOC Group only:

- Current pregnancy status of female participants (assigned female at birth)

The following pre-trial survey results of providers will be summarized by site:

- Practice setting
- Type of provider
- Specialty or defined area of practice

- Current approach to penicillin (PCN) allergic patients
- Access to PCN skin testing and whether PCN skin testing/oral challenge is offered.
- Barriers to offering/performing PCN skin test/oral challenge

Data will be presented in summary tables by treatment group, site and overall. Categorical variables, and continuous variables that have been categorized at discrete levels, will be summarized by frequencies and percentages. Continuous variables will be summarized in means, standard deviations, medians, minima and maxima.

7.3 Analysis of Primary Objective

The primary analysis will be performed on the Treated population and Per Protocol population. Estimates with corresponding exact (Clopper-Pearson) 95% confidence intervals for the following measures will be provided:

- The prevalence of PCN reactivity validated either by skin test or by direct oral challenge among those participants who report a history of PCN (B lactam) allergy and who have low-risk histories.
- The NPV on true PCN (B-lactam) allergy of the algorithm / PCN (B-lactam) allergy history screening questionnaire performance in the Treated population.

The NPV for each low-risk allergy item listed on the questionnaire will be estimated and will be compared with the prevalence of PCN reactivity using exact test of binomial proportion. The p-values will be adjusted with Benjamini-Hochberg procedure.

To construct a short standardized post-study questionnaire (4-6 questions) from the PCN (B-lactam) allergy history screening questionnaire, we will first use the item-characteristic curve from Item Response Theory (IRT) to evaluate each question item's discrimination ability of true PCN (B-lactam) allergy and use variable importance evaluation functions to indicate which questions are most useful for predicting the true PCN (B-lactam) allergy. Based on the results of IRT and variable importance analyses, we will then apply machine learning approaches such as random forest (Svetnik et al., 2003) via R package caret, to select a short post-study questionnaire that has the highest NPV among those possible short questionnaires with 4-6 selected questions.

7.4 Analysis of Secondary Objective

For the following measures, the frequencies and the percentages with corresponding exact 95% confidence intervals of participants will be tabulated by intervention group (if available), , study site and overall in the following measures.

7.4.1 Assessment of Subject Acceptability/Feasibility:

In the Eligible Population:

- those who refuse to participate in the study. The reasons of not interested in participating in the study will be summarized.

- For those who prefer to see an allergist for skin testing, summarize the reasons why they have not seen an allergist until now.

In the Treated Population:

- those who experience elicited reactivity to PCN allergy skin testing in the PST Group and oral challenge by dosage and group.
- those who find the testing procedures of to be helpful. The reasons of finding the PCN testing not helpful will be summarized.
- Those feeling comfortable with allergy testing conducted in settings where STD services are provided rather than by an allergist.
- chance of referring a friend or family member having a history of penicillin allergy.
- confidence in taking PCN or similar antibiotics among those who have been skin tested and are negative for PCN allergy.

7.4.2 Assessment of Provider Acceptability/Feasibility:

In the Provider Population, summarize by group (if possible) in numbers and proportion of:

- those who prefer PCN skin testing, direct oral challenge, or no preference.
- those who feel PCN allergy assessment can be performed safely and effectively in a setting which treats patients with STIs.
- those who will offer PCN allergy assessment in the future.
- reasons why study providers will not offer PCN allergy assessment in the future.

7.5 Analysis of Oral Challenge Results

Results of vital signs and focused physical exams (full dose only) after each administration of drug will be summarized in frequencies and percentages for each intervention group (test dose for DOC group; test & full dose for PST group). The type of reactions and severity will be summarized by intervention group and overall.

7.6 Analysis of Skin Testing Results

Results of skin prick testing and intradermal testing after each control and each reagent will be summarized in frequencies and percentages for those in PST group. If any result is found positive/equivocal, the size of the wheal will be summarized in means, standard deviations, medians, minima and maxima.

7.7 Analysis of Adverse Events

The Treated population and the Per Protocol population will be used for adverse event (AE) analysis. AEs will be summarized by time of onset (within/over 24 hours of study drug/test administration), relatedness to the intervention group, seriousness, severity, whether treated, outcome, and duration by intervention group, study site and overall. Categorical variables, and

continuous variables that have been categorized at discrete levels, will be summarized by frequencies and percentages. Continuous variables will be summarized by means, standard deviations, medians, minima and maxima.

8. REFERENCES

Steinberg DM, Fine J, Chappell R. Sample size for positive and negative predictive value in diagnostic research using case-control designs. Biostatistics. Jan 2009;10(1):94-105.

Svetnik, V., Liaw, A., Tong, C., Culberson, J. C., Sheridan, R. P. Feuston, B. P (2003). Random Forest: A Classification and Regression Tool for Compound Classification and QSAR Modeling, Journal of Chemical Information and Computer Sciences, Vol. 43, pg. 1947-1958.

Appendix A: Figure 1. Analysis Population Flow Diagram

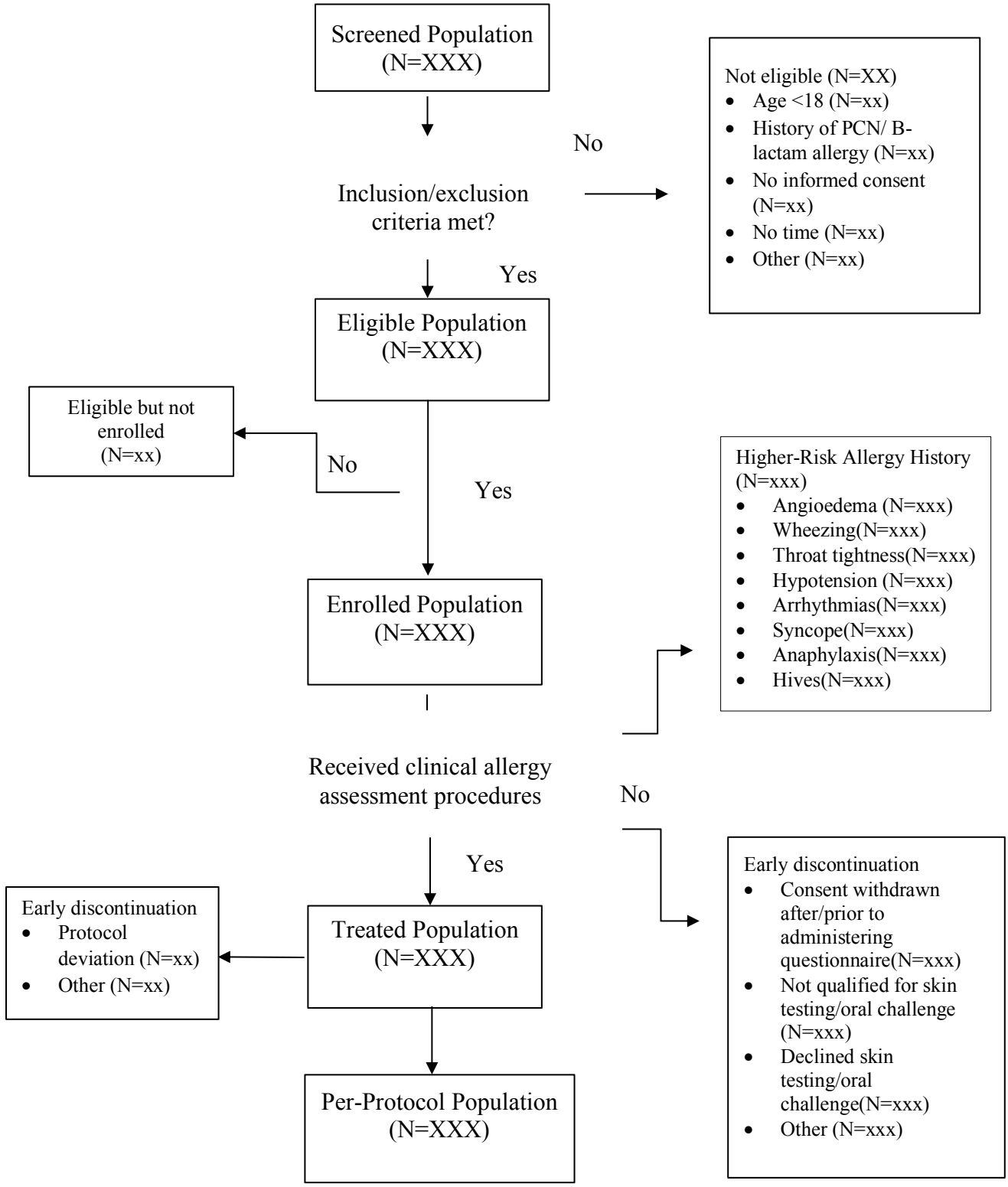

Figure 2. Per Protocol Population Flow Diagram